EFFECTIVENESS OF *Bacillus Clausii* IN REDUCING DURATION OF ILLNESS IN ACUTE DIARRHOEA IN CHILDREN 6-59 MONTHS OF AGE ADMITTED WITH SEVERE DEHYDRATION

A dissertation submitted in partial fulfilment of a Masters of Medicine Degree in Paediatrics and Child Health, University of Nairobi

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DECLARATION

This dissertation is my original work and has not been presented for the award of a degree in any other university.

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LIST OF ABBREVIATIONS

WHO- World Health Organisation
ORS- Oral Rehydration Salt
HIV/AIDS- Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome
FAO- Food and Agriculture Organisation
ESPHGAN- European Society for Pediatric Gastroenterology, Hepatology and Nutrition
ESPID- European Society for Pediatric Infectious Disease
KDHS- Kenya Demographic Health Survey
KNH- Kenyatta National Hospital
CRF- Case Report Form
IMCI- Integrated Management of Childhood Illness
**Définition of Terms**

Diarrhoea- Passage of 3 or more loose or liquid stools per day, or more frequently than is normal for the individual.

Probiotics- live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.
ABSTRACT

Background
Acute infectious diarrhoea is the second most common cause of under-five mortality in the world. It has also been associated with an increased morbidity and high rate of admission into hospital due to severe dehydration. Multiple studies document that probiotics are effective in treating infectious diarrhoea in children. The effectiveness of *B. clausii*, a probiotic, in shortening the duration of diarrhoeal illness in African children admitted with severe dehydration has not been examined.

Aim
To determine whether *B. clausii* is effective in shortening acute diarrhoeal illness in under-five population with severe dehydration.

Methods
In a randomized, double-blind, placebo-controlled trial, children (age range: 6 months to 5 years) with acute diarrhoea and WHO criteria of severe dehydration were administered *B. clausii* at 2×10⁹ CFUs/5ml vial or placebo, twice daily, for 5 days. Routine standard care and WHO protocols of managing diarrhea and dehydration were followed in both groups. The primary outcome measure was the duration of diarrhoea. The secondary measures were the diarrhoeal stool output per day and the duration of stay in hospital.

Results
In a per-protocol analysis of 90 children, the mean duration of diarrhoea in the *B. clausii* group (n=44) was insignificantly shorter (77.59 ± 34.10 hours) than the placebo group (n=46) (86.74 ± 40.16 hours) There was a mean difference between the groups of 9.15 hours (*t* (88) = 1.163, *P* = 0.248, 95% C.I -6.88 – 24.79). There was a significant decrease in the mean number of diarrheal motions on day 3 *B. clausii* group 2.74±1.81 motions vs. Placebo group 3.80±2.70 motions; mean difference=1.05 motions; (*t* (88) = 2.169, *P* = 0.033, 95% C.I 0.09 – 2.02]) and day 4 *B. clausii* group 1.45±1.13 motions vs. Placebo group 2.35± 2.19 motions; mean difference = 0.893; (*t* (88) = 2.412, *P* = 0.018, 95% C.I 0.157 – 1.629]). There was no significant difference in the duration of stay in hospital between the groups *B. clausii* group (4.14 ± 0.93 days) vs. Placebo group (4.50 ± 1.43 days) There was a mean difference between the groups of 0.37 days (*t* (88) = 1.426, *P* = 0.157, 95% C.I -0.143 – 0.873).
Conclusion

In children admitted with acute diarrhea and severe dehydration, no significant difference in reducing the duration of diarrhoea and duration of hospital stay in the two groups. However significant decrease in the number of stools was seen on day 3 and 4 of treatment.
1. INTRODUCTION AND LITERATURE REVIEW

Diarrhoea is defined as the passage of 3 or more loose or liquid stools per day, or more frequently than is normal for the individual.

Each year an estimated 2.5 billion cases of diarrhoea occur among children under five years of age, and estimates suggest that the overall incidence has remained stable over the past two decades. Mortality from diarrhoea has declined over the past two decades from an estimated 5 million deaths among children under five to 1.5 million deaths in 2004.

Despite the decline, diarrhoea remains the second most common cause of death among children under five globally, following closely behind pneumonia (nearly one in five deaths is due to diarrhoea). The toll is greater than that caused by HIV/AIDS, malaria and measles combined. Africa and South Asia are home to more than 80% of all child deaths due to diarrhoea. Just 15 countries including Kenya account for almost three quarters of all deaths from diarrhoea among children less than five years of age annually.

The World Gastroenterology Organisation in their practice guideline of 2008, report that diarrhoea is an important cause of morbidity and also countries incur substantial health costs. In the United States of America, a total of 900,000 admissions occur annually due to acute diarrhoea.

Most episodes of infectious diarrhoea resolve in less than 7 days, however, a small, but significant portion persists beyond 1 or 2 weeks or longer. These ‘prolonged’ or ‘persistent’ episodes are less common, but are important contributors to the global burden of diarrheal disease. Moreover, these prolonged episodes are increasingly implicated in childhood under nutrition, micronutrient deficiencies higher morbidity and mortality from other diseases and adverse neurodevelopment. In their 2008 review of persistent diarrhoea, Bhutta et al. highlight the need for a greater understanding of the continuum of acute diarrhoea to persistent diarrhoea. They emphasised that waiting until an episode of diarrhoea reaches 14 days in duration to intensify care is ill-advised and that closer attention should be given to patients whose acute diarrhoea is ‘prolonged’, that is, 5–7 days in duration and not yet resolved.
Rotavirus is the leading cause of acute diarrhoea and is responsible for about 40% of all hospital admissions due to diarrhoea among children under five worldwide. Other major pathogens include E. coli, Shigella, Campylobacter and Salmonella along with V.cholerae during epidemics.

Various modalities of interventions have been used in different parts of the world to improve diarrhoeal mortality and morbidity which includes oral rehydration salt (ORS), cereal based ORS, antibiotics, antispasmodics and antiemetics. Some of these modalities later proved to have variable harmful effects. These include worsening of diarrhoea, increased duration of diarrhoea, paralytic ileus, and systemic untoward effects. Many antidiarrhoeal drugs have serious and occasional fatal side effects e.g. intestinal obstruction (loperamide drops and syrups) and neurological disorders such as coma (diphenoxylate).

Currently the latest recommendations for treating childhood diarrhoea in the developing world are set out in a UNICEF and WHO joint statement issued in 2004. These recommendations include the use of reduced osmolarity ORS and Zinc supplementation. These have been shown to reduce morbidity and mortality due to diarrhoea by mainly reducing the duration of diarrhoea.

ORS has had a dramatic impact on acute gastroenteritis in children since its worldwide recommendation. Stool output and vomiting has decreased in children by about 20% and 30% respectively while unscheduled intravenous fluid therapy has also declined by 33% among children using ORS.

Clinical studies have shown that a 10-14 day treatment course with zinc effectively reduces the duration and severity of both persistent and acute diarrhoea. Zinc has been associated with a 25% reduction in the duration of acute diarrhoea.

In addition to the treatment recommendations, WHO has put up a prevention strategy to combat acute gastroenteritis in children. The prevention strategy includes: rotavirus and measles vaccinations, promotion of early and exclusive breastfeeding and vitamin A supplementation, promotion of hand washing with soap, improved water supply quantity and quality, including treatment and safe storage of household water, and community-wide sanitation promotion.
In trying to manage common gastrointestinal diseases including acute gastroenteritis, there has been an increasing interest by scientists in the concept of modulating bacterial activities directed towards improving gut microbial activity. This has been brought about by a greater understanding of the human intestinal normal flora and its function.

THE NORMAL FLORA
The intestinal habitat of an individual contains 300–500 different species of bacteria. The stomach and small intestine contain only a few species of bacteria adhering to the epithelia and some other bacteria in transit. This low number may be due to the composition of the luminal medium (acid, bile, pancreatic secretion), which kills most ingested microorganisms, and because of peristalsis which impedes stable colonisation of bacteria in the lumen. By contrast, the large intestine contains a complex and dynamic microbial ecosystem with high densities of living bacteria, which achieve concentrations of up to $10^{11}$ or $10^{12}$ cells/g of luminal contents.\(^\text{13}\)

Colonisation of the gastrointestinal tract of newborn infants starts immediately after birth. Initially, the type of delivery (passage through the birth canal versus caesarean section) and the type of diet (breast versus formula feeding) might affect the colonisation pattern.\(^\text{14}\) Other environmental factors also have a major role since differences exist between infants born in developed countries and those born in developing countries, and between infants from different hospital wards.\(^\text{15}\)

Anaerobic bacteria outnumber aerobic bacteria by a factor of 100–1000. The genera bacteroides, bifidobacterium, eubacterium, clostridium, peptococcus, peptostreptococcus, and ruminococcus are predominant in human beings, whereas aerobes (facultative anaerobes) such as escherichia, enterobacter, enterococcus, klebsiella, lactobacillus, proteus, etc are among the subdominant genera.\(^\text{13}\)

Evidence obtained from various studies\(^\text{16}\) suggests that microflora have important and specific metabolic, trophic, and protective functions.

**Metabolic functions**
A major metabolic function of colonic microflora is the fermentation of non-digestible dietary residue and endogenous mucus produced by the epithelia. The overall
outcomes of this complex metabolic activity are recovery of metabolic energy and absorbable substrates for the host, and supply of energy and nutritive products for bacterial growth and proliferation. Fermentation of carbohydrates is a major source of energy in the colon. Non-digestible carbohydrates include large polysaccharides (resistant starches, cellulose, hemicellulose, pectins, and gums), some oligosaccharides that escape digestion, and unabsorbed sugars and alcohols. The metabolic endpoint is generation of short-chain fatty acids.

The anaerobic metabolism of peptides and proteins (putrefaction) by the microflora also produces short-chain fatty acids but, at the same time, it generates a series of potentially toxic substances including ammonia, amines, phenols, thiols, and indols. Available proteins include elastin and collagen from dietary sources, pancreatic enzymes, sloughed epithelial cells and lysed bacteria.

The absorption of ions in the caecum is improved by carbohydrate fermentation and production of short-chain fatty acids, especially acetate, propionate and butyrate. All of these fatty acids have important functions in host physiology. Butyrate is almost completely consumed by the colonic epithelium, and it is a major source of energy for colonic cells. Acetate and propionate are found in portal blood and are eventually metabolised by the liver (propionate) or peripheral tissues, particularly muscle (acetate). Acetate and propionate might also have a role as modulators of glucose metabolism.

**Trophic functions**

*Epithelial cell growth and differentiation*—Possibly, the most important role of short-chain fatty acids on colonic physiology is their trophic effect on the intestinal epithelium. All three major short-chain fatty acids stimulate epithelial cell proliferation and differentiation in the large and small bowel in vivo.

*Interactions between gut bacteria and host immunity*—The intestinal mucosa is the main interface between the immune system and the external environment. Gut-associated lymphoid tissues (GALT) found in the Peyers patches, contains the largest pool of immunocompetent cells in the human body. The dialogue between host and bacteria at the mucosal interface seems to play a part in the development of a competent immune system.
Protective functions

Several mechanisms have been implicated in the barrier effect. In vitro, bacteria compete for attachment sites in the brush border of intestinal epithelial cells. Adherent non-pathogenic bacteria can prevent attachment and subsequent entry of pathogen enteroinvasive bacteria into the epithelial cells. Furthermore, bacteria compete for nutrient availability in ecological niches and maintain their collective habitat by administering and consuming all resources. Finally, bacteria can inhibit the growth of their competitors by producing antimicrobial substances called bacteriocins.

Gastrointestinal disease is often a consequence of a myriad of factors, which disturbs the bowels complex ecosystem.

PROBIOTICS

With this information on the normal flora, scientists have introduced the concept of using probiotics in the management of gastrointestinal disease. The term probiotic is derived from Greek and it means “for life”. Probiotics are defined as living organisms (bacteria and yeast) administered to promote health of the host by treating or preventing infections owing to strains of pathogens. FAO/WHO defined the term probiotics as “live microorganisms administered in an adequate amount which confer a health benefit on the host.”

The clinical benefits observed with probiotic use are mainly attributed to the antimicrobial substances produced by probiotic strains and to their immunomodulatory effects. To exert their beneficial effects, probiotics must be able to overcome the obstacle of gastric acidity, to proliferate and to colonize the intestine even in the presence of biliary acids and of drugs such as antibiotics. They must adhere to intestinal walls, reducing their permeability and potentiate the local immune response, especially through secretory immunoglobulin IgA.

Probiotics have been investigated in various areas in association with diarrhoea in children and are listed in Table 1.
TABLE 1. Areas of investigation of Probiotics in Childhood Diarrhoea

Prevention of

- Acute infectious diarrhoea
- Nosocomial diarrhoea
- Antibiotic-associated diarrhoea

Treatment of

- Acute infectious diarrhoea
- Protracted infectious diarrhoea

The results coming out of research on probiotics have been fruitful. Recent set of recommendations set forth by a joint committee from the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Pediatric Infectious Disease (ESPID) state that: "Probiotics may be an effective adjunct to the management of diarrhea. However, because there is no evidence of efficacy for many preparations, we suggest the use of probiotic strains with proven efficacy and in appropriate doses for the management of children with acute gastroenteritis as an adjunct to rehydration therapy."

The majority of probiotics available are bacterial in nature with *Sacharomyces boulardii* standing out as the only yeast probiotic. Table 2 lists the probiotics used in experiments relating to diarrhoea in children.

The best-documented probiotic bacteria used in human therapy are lactic acid bacteria such as *Lactobacillus* GG, which have been shown in several studies to have a beneficial effect in children with acute diarrhoea.
TABLE 2. Probiotics Tested for Use in Diarrhoea in Children\textsuperscript{27} (modified)

Bacteria

- \textit{Lactobacillus GG}
- \textit{Lactobacillus reuteri}
- \textit{Lactophilus acidophilus}
- \textit{Lactobacillus acidophilus LB} (killed)
- \textit{Lactobacillus bulgaricus}
- \textit{Bifidobacterium bifidum} (now "lactis")
- \textit{Bifidobacterium breve}
- \textit{Streptococcus thermophilus}
- \textit{Enterococcus}
- \textit{Escherichia coli Nissle 1917}
- \textit{Bacillus clausii}

Yeast

- \textit{Saccharomyces boulardii}

In a study done by Guarino et al in 1997 involving 100 children, the probiotic \textit{Lactobacillus GG} was found to reduce the duration of diarrhoea by 24 hours.\textsuperscript{29}

In 2007, a meta-analysis by Sjaweska H et al showed that in otherwise healthy infants and children with acute infectious gastroenteritis, the use of \textit{Lactobacillus GG} was associated with a reduction in the duration of diarrhoea, particularly of rotaviral etiology.\textsuperscript{30}

\textit{Saccharomyces boulardii} has also had promising results. In a recent meta-analysis in 2007 by Szajewska H et al,\textsuperscript{31} 5 RCTs were accepted for a total of 619 patients. Combined data from four RCTs showed that \textit{S. boulardii} significantly reduced the duration of diarrhoea compared to the control. The pooled weighted mean difference was -1.1 days (95% CI -1.3 to -0.8). \textit{S. boulardii} significantly reduced the risk of diarrhoea on days 3, 6 and 7. In addition, the risk of diarrhoea lasting >7 days was significantly reduced in the \textit{S. boulardii} group vs. control (one RCT, n=88, RR=0.25, 95% CI 0.08-0.88).
**BACILLUS CLAUSII**

The use of *B. clausii* as a probiotic species has been based on over more than 40 years of clinical usage in Italy with excellent tolerability and no report of side-effects.

The polyantibiotic-resistant spores of *Bacillus clausii* are currently available in the form of a suspension containing 2 billion spores per 5 ml (Enterogermina™). The spores belong to 4 strains (O/C, T, N/R, and SIN) that are resistant to different classes of antibiotics.

*B. clausii* is an aerobic, spore-forming bacterium that is able to inhibit the growth of pathogens in the gastrointestinal tract via three distinct mechanisms:

- colonization of free ecological niches, which are no longer available for the growth of other microorganisms;
- competition for epithelial cell adhesion, which is particularly relevant for spores in the initial or intermediate germination phase;
- production of antibiotics and/or enzymes secreted into the intestinal environment, especially peptide antibiotics, which are mainly active on Gram-positive bacteria, but also enzymes that exhibit lytic activity against *Pseudomonas aeruginosa*.

The polyantibiotic-resistant *B. clausii* has proven to be highly resistant to gastric acidity and most antibiotics, with the exception of sulfonamides and trimethoprim, a few aminoglycosides and nitrofurans, combinations of penicillins with beta-lactamase inhibitors and vancomycin. Hence, *B. clausii* can be used concurrently with antibiotics without impacting on its efficacy.

Few RCTs have been conducted on *B. clausii* as a probiotic. These RCTs mainly involve prevention of antibiotic associated diarrhea, prevention of side effects of anti-*H. pylori* treatment, prevention of recurrent respiratory illness and treatment of acute diarrhoeal disease in children.

**Diarrhoeal conditions**

Italian investigators, Canani RB, Cirillo P, Terrin GA et al (2007) conducted a multicenter randomized clinical trial. It was a single blinded randomised control trial involving 571 children. Children were randomised into five groups- oral rehydration...
alone: *Lactobacillus* GG; *S. boulardii*; *Bacillus clausii*; mix of *L delbrueckii var bulgaricus*, *Streptococcus thermophilus*, *L acidophilus*, and *Bifidobacterium bifidum*; or *E. faecium* strain SF68. The primary outcomes assessed were duration of diarrhoea and the daily number and consistency of stools while the secondary outcomes were the duration of vomiting and fever and rate of admission to hospital. The study found no difference between use of ORS only and ORS with *B. clausii* in any of the outcomes.

Recently in 2007, the efficacy of *B. clausii* in reducing the duration of diarrhea was assessed in a multicentre study involving Indian children with acute infectious diarrhea. The study enrolled 255 children aged between 6 months to 5 years and suffering from acute diarrhea of less than 48 hours duration attending the out-patient departments. It was an open label randomised control trial where the treatment arm was given oral rehydration therapy and *B. clausii* twice daily for 5 days and the control arm oral rehydration therapy only. The study findings favoured the patients on *B. clausii* in comparison with the control. There was a reduction in the mean duration of diarrhea by 7.5hrs and also a reduction in the mean number of stools.35

In 2004, Nista et al in a randomised, double blind, placebo controlled trial one hundred and twenty *H. pylori*-positive patients were randomly screened to receive a standard 7 days triple therapy with rabeprazole 20 mg twice daily, clarithromycin 500 mg twice daily, and amoxicillin 1 g twice daily. and *B. clausii* thrice daily, for 14 days starting from the first day of treatment. The control group received the same 7 days triple therapy and placebo thrice daily for 14 days starting from the first day of treatment. *B. clausii* was found to reduce the incidence of most common side effects related to anti-*H. pylori* antibiotic therapy e.g. antibiotic related diarrhoea.34

**Non-diarrhoeal conditions**

In a pilot study by Mesarglia et al,35 *Bacillus clausii* was found to reduce the duration of respiratory illness. Eighty children with RRI were studied: 40 of them were randomly treated with *B. clausii* for 3 months, and followed up for further 3 months; 40 were included in the control group during the same period. Children treated with *B. clausii* had a shorter duration of respiratory illness in comparison with the control
In 2004, Ciprandi et al evaluated the cytokines levels in nasal lavage of allergic children after administration of *B. clausii*. Ten allergic children attending nursery school were evaluated. *B. clausii* treatment induced a significant decrease of IL-4 levels and a significant increase of IFN-γ, IL-12, TGF-β, and IL-10 levels. In conclusion, this study showed that the *B. clausii* may exert immunomodulating activity by affecting cytokine pattern at nasal level in allergic children with recurrent respiratory infections.37

In a follow up study in 2005, Ciprandi et al conducted a pilot study to investigate the potential effects exerted by *B. clausii* on nasal symptoms, eosinophils, and the symptomatic use of antihistamines in children with allergic rhinitis. Twenty allergic children, 13 males and seven females, with an average age of 13.4 years (range 12–15) were consecutively evaluated. Symptomatic use of levocetirizine (5 mg tablets) was prescribed for all children. Ten of them were randomly treated with oral *B. clausii*. Nasal total symptoms score (TSS) and nasal eosinophils were evaluated. Children treated with *B. clausii* spores, showed significant reduction of TSS without any side-effect. Nasal eosinophils were also significantly diminished after treatment. In conclusion, *B. clausii* may have a role in controlling allergic rhinitis symptoms.38

Small intestinal bacterial overgrowth (SIBO) is a common clinical condition due to an increase in the level of microorganisms, in excess of $10^6$ colony-forming units / milliliter of intestinal aspirate, and / or of colonic-type bacteria within the small intestine. Empirical courses of broad-spectrum antibiotics are the treatment of choice for SIBO decontamination at present. In 2009, Gabrielli et al assessed both the efficacy and tolerability of *B. clausii* for SIBO decontamination.

Participants in the study underwent treatment with *B. clausii* one vial three times a day for 1 month. *B. clausii* was found to be 47% effective in SIBO decontamination.39

The role of probiotics on modern medicine cannot be overlooked but there is need for more research especially in gastrointestinal diseases. A Cochrane Review in 2003 by Allen SJ et al concluded that probiotics appeared to be a useful adjunct to rehydration
therapy in treating acute, infectious diarrhoea in adults and children. More research is needed to inform the use of particular probiotic regimens in specific patient groups.\textsuperscript{40}

In a meta-analysis by Sunil S et al., they found there was insufficient evidence for extrapolation of current study results for global recommendations, since trials among children in community settings in the developing countries are lacking. They concluded that trials need to be carried out in these settings before further conclusions could be draw.\textsuperscript{41} Currently, no local studies have been carried out on probiotics.

There is worry about the adverse effects of probiotics due to anecdotal evidence and rare case reports. The ESPGHAN committee on nutrition recently summarised its approach to probiotics as follows, “probiotics so far used in clinical trials can be generally considered as safe. However, surveillance for possible side effects, such as infection in high-risk groups, is lacking and is needed”\textsuperscript{42}

In conclusion, this study is the first randomised trial looking at the effect of \textit{B. clausii} on the duration of acute diarrhoea in children admitted with severe dehydration in the local setting.
2. STUDY JUSTIFICATION

Acute infectious diarrhoea still remains one of the major causes of under-five mortality. This is despite efforts worldwide towards education and use of low osmolarity ORS and zinc sulphate. Other measures like access to clean water, sanitation and good nutrition still have not been achieved especially in the developing world.

Currently the Kenyan situation is still grim. Kenya is ranked as tenth out of the fifteen countries with the highest mortality due to diarrhoea. In the Kenya Demographic and Health Survey of 2008-2009, almost half of the children with diarrhoea were taken to a health care provider, 72% of children with diarrhoea were treated with some form of ORT, and 39% were given a solution prepared using standard ORS.

Scientists are now looking at other ways to reduce morbidity and mortality in children. The introduction of the rotavirus vaccine though still inaccessible to the poor Kenyan population is a step forward. The jury is still out on the use of probiotics but reports coming out of recent studies are promising.

This study therefore was aimed at assessing the effectiveness of a probiotic B. clausii in reducing the duration of diarrhoeal disease in children 6-59 months admitted with severe dehydration.

The findings of this research will help in increasing the data on probiotics in management of acute diarrhoea. It will also assist in future decisions on use of probiotics in management of acute diarrhoeal disease. If found to be effective it may help end the vicious cycle of acute diarrhoea leading to childhood undernutrition, micronutrient deficiencies higher morbidity and mortality from other diseases and adverse neurodevelopment.
3. OBJECTIVES

3.1 Primary Objective

- To assess the effectiveness of *B. clausii* in reducing the length of diarrhoeal disease in children aged 6-59 months admitted with acute diarrhoea and severe dehydration at Kenyatta National Hospital.

3.2 Secondary Objective

- To demonstrate the effect of *B. clausii* on the mean number of diarrhoeal episodes per day in children admitted with acute diarrhoea and severe dehydration.
- To assess the effect of *B. clausii* on the duration of stay in hospital of children admitted with acute diarrhoea and severe dehydration.
4. METHODOLOGY

4.1 Study design
This was a double blind placebo controlled randomised controlled trial evaluating the effectiveness of *B. clausii* in shortening the duration of acute diarrhoea.

The eligible patients will be randomly assigned to receive oral *B. clausii* plus the WHO protocol for management of diarrhoea or placebo plus the WHO protocol only.

4.2 Study Population
The study population included children aged 6 months- 59 months and involved in-patients admitted into the paediatric wards with severe dehydration.

4.3 Study Location
The study was conducted in the Paediatric Wards at the Kenyatta National Hospital (KNH) in Nairobi, Kenya. It is the largest referral hospital in East and Central Africa.

4.4 Study Period
Individual participants were enrolled in the study for a period of 1 week. The study was carried out over a period of 11 months between July 2010 and May 2011

4.5 Sample size estimation
Using the formula for calculating sample size in RCTs:

\[ n = \frac{2[(a + b)^2 \sigma^2]}{(\mu_1 - \mu_2)^2} \]

Where:
- \( n \) = the sample size in each of the groups
- \( \mu_1 \) = population mean in treatment Group 1
- \( \mu_2 \) = population mean in treatment Group 2
- \( \mu_1 - \mu_2 \) = the difference the investigator wishes to detect
- \( \sigma^2 \) = population variance (SD)
- \( a \) = conventional multiplier for alpha
- \( b \) = conventional multiplier for power

Using a preliminary study by Guarino (1997)\(^{36}\)
\[ \mu_1 = 120 \text{ hrs} \quad \text{Study drop-out rate estimated at } 20\% = 1.56 \]
\[ \mu_2 = 96 \text{ hrs} \]
\[ \mu_1 - \mu_2 = 24\text{hrs} \]
\[ \sigma_2 = 30 \]
\[ a = \alpha = 0.05 = 1.96 \]
\[ b = \beta = 0.80 = 1.28 \]

Therefore,

\[ n = \frac{2[(1.96 + 1.28)^2 30^2]}{(120 - 96)^2} \times 1.56 \]

\[ n = 51 \text{ in each group.} \]

4.6 Primary and Secondary outcomes

The primary outcome of this study that will be assessed is the duration of diarrhoea i.e. the time from the start of the treatment until the appearance of the first normal stool.

The secondary outcome was the reduction of the number of diarrhoeal episodes per day.

Duration of stay in hospital was defined as the day of admission to the day of discharge by the doctor.
5. SELECTION AND ENROLMENT OF PARTICIPANTS

5.1 Recruitment
Patient admissions into the Paediatric wards in KNH follow a rotation system involving the four wards (A, B, C and D). A ward admits on every fourth day – this is a safeguard measure to allow equal distribution of new patients in all wards and to prevent overcrowding in one ward. In this study, recruitment followed this rotation system as recruitment was aimed at capturing participants from newly admitted patients.

5.2 Consent administration
Prior to performing any study specific procedure, a signed consent form was obtained from the parent/legal guardian of the child.

The consent form described the purpose of the study, the procedure to be followed and the risks and benefits of the participation. The investigator/research assistant conducted the consent discussion and checked that the parent/legal guardian comprehended the information provided and answered any question about the study. Consent was voluntary and free from coercion. The patient could withdraw from the study at their own pleasure.

When all the inclusion exclusion criteria have been addressed and the eligibility of the participant confirmed, the subject was assigned to a randomisation treatment in the study.

5.3 Inclusion criteria
Each participant met all of the following criteria to be enrolled in this study:

- Age between 6 months and 59 months.
- Written informed consent by parent/legal guardian to participate in study.
- Patient with acute diarrhoea; defined in this study as presence of ≥ 3 liquid or loose stools in the preceding 24hrs but for less than 7 days.
- Patient with severe dehydration as defined by WHO (Appendix A)
5.4 Exclusion criteria

Participants who met the following criteria were excluded from the study:

- Patients with non severe dehydration
- Use of probiotics in the preceding 7 days
- Chronic intestinal disorders.
- Patient with severe malnutrition
- Known immunodeficiency
- Patient on immunosuppressive therapy

5.5 Randomisation and Blinding Procedure

All participants were randomised into two groups - treatment group and placebo group. Randomisation was done using permuted random blocks generated by a statistician.

The knowledge of the randomisation list sequence was only known by the study pharmacist (a selected research assistant) who also packed the study drugs into tamper proof brown envelopes that had randomisation codes.

Prior to allocating a patient to a study group, the investigator / research assistant called the study pharmacist to get the randomisation code of the envelope which would be given to the participant.

Both the investigator/ research assistants and the participant were blinded in this study.

Randomisation codes were only available once all data collected had been entered into the study database for every participant and the database had been finalised.
6. STUDY INTERVENTION

6.1 Clinical procedures

After randomisation, participant’s clinical data was recorded into a data sheet which included age, sex, weight and area of residence. Other information recorded included duration of diarrhoea, number of loose stools per day and healthcare sought prior to study.

The participants underwent a general medical examination whereby the temperature, state of dehydration, nutritional status were recorded.

6.2 Intervention

The *B. clausii* (treatment) group were managed using the WHO protocol plus *B. clausii* administered orally at 1 vial/ twice daily for 5 days. The placebo (control) group were managed using the placebo at 1 vial/ twice daily for 5 days and WHO protocol only. The participants in all groups received zinc sulphate. This interventions were started in the wards.

*B. clausii* was contained in the product Enterogermina™ which is manufactured by Sanofi- Aventis. The product comes as an oral suspension, which is odourless and tasteless packed in easy-to- open plastic vials. Each vial has a volume of 5mls containing 2 billion spores of poly-antibiotic resistant *B. clausii* spores.

The placebo used in this study was packaged in identical looking vials containing sterile water.

Clear instructions were issued to the parent/ legal guardian on how to administer the *B. clausii* preparation and how to give the zinc sulphate. The hydration status was managed using ORS or parenteral fluids using the WHO protocol. The parent / legal guardian was also given clear instructions on how to fill the daily record of loose stools or diarrhoeal motions.
7. **DATA MANAGEMENT AND STATISTICAL CONSIDERATIONS**

7.1 **Data collection**

Source data was mainly the data sheet collected on day 0, the daily diary given to the parent/guardian.

The participant's parent/guardian was issued with a daily diary which contained information on the number of diarrhoeal episodes per day. The Investigator/research assistants visited the participant daily in the ward to record the data in the daily diary as until death or discharge.

If the participant was discharged from the ward prior to the end of the study period, the parent/guardian was contacted on a daily basis by the investigator by phone call and the data on the daily diary was recorded.

7.2 **Data analysis**

Source data were collected by the study Investigator and filled into CRFs after the study. The data on the CRFs were entered into the computer database.

Data was coded, cleaned, verified and analyzed using SPSS (Statistical Package for Social Sciences) computer version 17.0 and Microsoft Excel 2007.

To assess the difference between the two groups, the $\chi^2$ test was used to analyse categorical variables and Students $t$ test for analyzing continuous variables

Data is presented using bar graphs and tables.
8. ETHICAL CONSIDERATIONS

8.1 Confidentiality

Subject confidentiality was strictly held in trust by the participating investigators, research staff, and the sponsoring institution. The study protocol, documentation, data and all other information generated were held in strict confidence. No information concerning the study or the data was released to any unauthorized third party, without prior written approval of the sponsoring institution.

8.2 Ethics, Research and Standards Committee approval

This protocol and the informed consent document and any subsequent modifications was reviewed and approved by the Ethics, Research and Standards Committee. A letter of protocol approval by Ethics, Research and Standards Committee was obtained prior to the commencement of the study.
9. RESULTS

From July 2010 to May 2011, a total of 127 patients with acute diarrhoea and severe dehydration were assessed for eligibility. A total of 25 were excluded and 102 were randomised to receive intervention and contributed data. Their median age was 9.5 months and the range was between 6-30 months.

**Figure 1: Flow diagram of the subjects' progression through the study.**

There was a drop-out rate of 11.765% due to either loss to follow up or non-compliance to the treatment regime prescribed.
9.1. CHARACTERISTICS OF STUDY PARTICIPANTS

TABLE 3: Baseline features of study participants

<table>
<thead>
<tr>
<th></th>
<th>B. clausii group</th>
<th>Placebo group</th>
<th>P/test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N= 44</td>
<td>N= 46</td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>11.3±5.3</td>
<td>11.9±6.4</td>
<td>0.626/t</td>
</tr>
<tr>
<td>Sex ( m/f)</td>
<td>20/24</td>
<td>26/20</td>
<td>0.294/χ²</td>
</tr>
<tr>
<td>Mean Weight (kgs)</td>
<td>8.05±1.77</td>
<td>8.05±1.82</td>
<td>0.995/t</td>
</tr>
<tr>
<td>Mean Temperature (°C)</td>
<td>37.74±0.92</td>
<td>37.54±0.88</td>
<td>0.280/t</td>
</tr>
<tr>
<td>Mean duration of exclusive breast feeding (months)</td>
<td>3.84±1.36</td>
<td>3.46±0.82</td>
<td>0.112/t</td>
</tr>
<tr>
<td>Mean duration of diarrhoea before admission (days)</td>
<td>3.95±1.28</td>
<td>3.61±1.29</td>
<td>0.205/t</td>
</tr>
<tr>
<td>Mean number of stools in last 24hrs before admission</td>
<td>8.14±2.23</td>
<td>8.13±1.71</td>
<td>0.989/t</td>
</tr>
</tbody>
</table>

Prior history of diarrhoeal illness

<table>
<thead>
<tr>
<th></th>
<th>B. clausii group</th>
<th>Placebo group</th>
<th>P/test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>32 (72.7%)</td>
<td>30 (65.2%)</td>
<td>0.442/χ²</td>
</tr>
<tr>
<td>No</td>
<td>12 (27.3%)</td>
<td>16 (34.8%)</td>
<td></td>
</tr>
</tbody>
</table>

History of contact with person with diarrhoea

<table>
<thead>
<tr>
<th></th>
<th>B. clausii group</th>
<th>Placebo group</th>
<th>P/test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>17 (38.6%)</td>
<td>16 (34.8%)</td>
<td>0.705/χ²</td>
</tr>
<tr>
<td>No</td>
<td>27 (61.4%)</td>
<td>30 (65.2%)</td>
<td></td>
</tr>
</tbody>
</table>

Type of fluid given in last 24hours

<table>
<thead>
<tr>
<th></th>
<th>B. clausii group</th>
<th>Placebo group</th>
<th>P/test</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORS</td>
<td>10 (22.7%)</td>
<td>9 (19.7%)</td>
<td>0.708/χ²</td>
</tr>
<tr>
<td>Plain Water</td>
<td>10 (22.7%)</td>
<td>15 (32.6%)</td>
<td></td>
</tr>
<tr>
<td>Milk Porridge</td>
<td>7 (15.9%)</td>
<td>5 (10.9%)</td>
<td></td>
</tr>
<tr>
<td>Home-made solutions</td>
<td>4 (9.1%)</td>
<td>2 (4.3%)</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>13 (29.6%)</td>
<td>15 (32.6%)</td>
<td></td>
</tr>
</tbody>
</table>

A total of 90 participants were analysed (B.clausii group n= 44 and Placebo group n=46).
The mean age of the participants in the *B. clausii* group was 11.3±5.3 months while that in the Placebo group was 11.9±6.4 months. There was no significant difference in the ages ($p=0.626$).

The study recruited slightly more males than females in the study (male=46 vs. female= 44) The *B. clausii* group had 20 males and 26 females while the Placebo group had 24 males ad 20 females. There was no significant difference between the two groups ($p=0.294$).

The mean weight of the participants was 8.05 ± 1.77 kilograms while the Placebo group had a mean weight of 8.05 ± 1.82 kilograms. There was no significant difference in the weight of the participants ($p =0.995$)

Most of the participants were febrile at the day of recruitment into the study. The mean temperature for the *B. clausii* group was 37.74 ± 0.92 °C while for the Placebo group was 37.54 ± 0.88 °C. There was no significant difference in the temperatures between the two groups ($p=0.280$)

Majority of the participants involved in the study had been exclusively breastfed for between 2-5 months (the *B. clausii* group 3.84 ± 1.36 months vs. the Placebo group 3.46 ± 0.82 months ) there was no significant difference in the age between the two groups ($p=0.112$)

The mean number of diarrhoeal motions 24 hours prior to study was 8.14 ± 2.23 motions in the *B. clausii* group while in the Placebo group it was 8.13 ± 1.71 motions. There was no significant difference in the two groups ($p = 0.989$)

The *B. clausii* participants had a mean duration of diarrhoea prior to the study of 3.95 ± 1.28 days while the Placebo group had 3.61 ± 1.29 days this difference was not significant ($p=0.205$)

32 (72.7%) of the participants in the *B. clausii* group had a past history of diarrhoeal illness while the Placebo group had 30 (65.2%). A majority of the participants had a history of contact with person with diarrhoea [B.clausii group 27(61.4%) vs. the Placebo group 30(65.2%)]
The participants were exposed to various types of fluids 24 hours prior to recruitment in the study. This included oral rehydration solution [B.clausii group 10 (22.7%) vs. Placebo group 9 (19.7%)], Plain water [B.clausii group 10 (22.7%) vs. Placebo group 15 (32.6%)], Milk/porridge [B.clausii group 7 (15.9%) vs. Placebo group 5 (5%)], Homemade solutions [B.clausii group 4 (9.1%) vs. Placebo group 2 (4.3%)], and others had various/mixed solutions [B.clausii group 13 (29.6%) vs. Placebo group 15 (32.6%)].
TABLE 4: Primary Caregiver Characteristics and Health Care Seeking Behaviour

<table>
<thead>
<tr>
<th></th>
<th>B. clausii group</th>
<th>Placebo group</th>
<th>P/test</th>
</tr>
</thead>
<tbody>
<tr>
<td>N= 44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary care-giver age distribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-19 years</td>
<td>18 (40.9%)</td>
<td>10 (21.7%)</td>
<td>0.133/ $\chi^2$</td>
</tr>
<tr>
<td>20-30 years</td>
<td>23 (52.3%)</td>
<td>33 (71.8%)</td>
<td></td>
</tr>
<tr>
<td>31-40 years</td>
<td>3 (6.8%)</td>
<td>3 (6.5%)</td>
<td></td>
</tr>
<tr>
<td>Primary care giver education status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>3 (6.8%)</td>
<td>0 (0%)</td>
<td>0.088/ $\chi^2$</td>
</tr>
<tr>
<td>Primary education</td>
<td>30 (68.2%)</td>
<td>25 (54.4%)</td>
<td></td>
</tr>
<tr>
<td>Secondary education</td>
<td>10 (22.7%)</td>
<td>19 (41.3%)</td>
<td></td>
</tr>
<tr>
<td>Tertiary education</td>
<td>1 (2.3%)</td>
<td>2 (4.3%)</td>
<td></td>
</tr>
<tr>
<td>Prior care sought prior to study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbalist/traditional healer</td>
<td>3 (6.8%)</td>
<td>2 (4.5%)</td>
<td>0.870/ $\chi^2$</td>
</tr>
<tr>
<td>Religious leader</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Over the counter medication</td>
<td>7 (15.9%)</td>
<td>8 (17.4%)</td>
<td></td>
</tr>
<tr>
<td>Health Facility</td>
<td>34 (77.3%)</td>
<td>36 (78.3%)</td>
<td></td>
</tr>
<tr>
<td>Referring health facility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private facility</td>
<td>26 (59.1%)</td>
<td>26 (56.5%)</td>
<td>0.407/ $\chi^2$</td>
</tr>
<tr>
<td>Council/dispensary</td>
<td>9 (20.5%)</td>
<td>5 (10.9%)</td>
<td></td>
</tr>
<tr>
<td>District hospital</td>
<td>1 (2.3%)</td>
<td>3 (6.52%)</td>
<td></td>
</tr>
<tr>
<td>Self Referral</td>
<td>8 (18.1%)</td>
<td>12 (26.1%)</td>
<td></td>
</tr>
</tbody>
</table>

There were also no major differences in the primary care giver and health seeking behaviours in the two groups.

Majority of the primary caregivers were aged between 20-30 years [B.clausii group 23 (52.3%) vs Placebo 33 (71.8%)] of age with a minimum of primary school education. [B.clausii group 30 (68.2%) vs. placebo group 25 (54.4%)]
Most of the participants' caregivers favoured to seek care at health facilities [B. clausii 34 (77.3%) vs. Placebo group 36 (78.3%)] with a preference of private facilities [B. clausii 26 (59.1%) vs. Placebo group 26 (56.5%)]

9.2 EFFECT OF B. CLAUSII ON THE DURATION OF DIARRHOEA

This study found that the B. clausii group (n=44) had a shorter duration of diarrhoeal illness (77.59 ± 34.10 hours) than the placebo group (n=46) (86.74 ± 40.16 hours) There was a mean difference between the groups of 9.15 hours. This difference was not statistically significant (t (88) = 1.163, P = 0.248, 95% C.I -6.88 - 24.79).
In a sub analysis of the primary outcome, we looked at the duration of diarrhoea according to age and according to duration of diarrhoea prior to starting intervention.

According to age groups (fig 3), in those < 12 months of age (n=57), this study found that the B.clausii group (n=29) had a shorter duration of diarrhoeal illness (72.48 ± 38.88 hours) than the placebo group (n=28) (88.15 ± 38.23 hours) There was a mean difference between the groups of 15.67 hours. This difference was not statistically significant (t (55) = 1.639, P = 0.107, 95% C.I -3.49 - 34.83).

In those ≥12 months of age (n=33), this study found that the placebo group (n=18) had a shorter duration of diarrhoeal illness (84.54 ± 44.05 hours) than the B.clausii group (n=15) (87.46 ± 33.43 hours) There was a mean difference between the groups of 2.91 hours. This difference was not statistically significant (t (31) = 0.210, P = 0.835, 95% C.I -25.33 - 31.15)
9.4 THE EFFECT OF *B. clausii* ON THE DURATION OF DIARRHOEA IN RELATION TO THE TIME OF STARTING THE INTERVENTION.

Figure 4: Duration of diarrhoea in relation to time of starting intervention

![Bar chart showing duration of diarrhoea in relation to time of starting intervention](image)

According to duration of illness prior to starting of intervention (fig 4), in those who started intervention ≤ 72 hours (n=37), this study found that the *B. clausii* group (n=16) had a shorter duration of diarrhoeal illness (83.46 ± 42.51 hours) than the placebo group (n=21) (93.91 ± 46.83 hours). There was a mean difference between the groups of 10.45 hours. The difference was not statistically significant (*t* (35) = 0.699, *P* = 0.489, 95% C.I -19.89 - 40.78).

In those who started intervention >72 hours (n=53), this study found that the *B. clausii* group (n=28) had a shorter duration of diarrhoeal illness (74.23 ± 28.56 hours) than the placebo group (n=25) (80.72 ± 33.38 hours). There was a mean difference between the groups of 6.49 hours. The difference was not statistically significant (*t* (51) = 0.762, *P* = 0.449, 95% C.I -10.59 - 23.57).

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On evaluation of the mean daily diarrhoeal output (Fig 5), there was a decrease in the mean number of diarrhoeal motions on day 3 [B. clausii group 2.74±1.81 motions vs. Placebo group 3.80±2.70 motions; the mean difference mean difference was 1.05 motions. This was a statistically significant difference \((t (88) = 2.169, P = 0.033, 95\% \text{ C.I} 0.09 - 2.02)\)]

On day 4, there was a decrease in the mean number of diarrhoeal motions in the B. clausii group [B. clausii group 1.45±1.13 motions vs. Placebo group 2.35± 2.19 motions] there was a mean difference of 0.893 motions. This was a statistically significant difference \((t (88) = 2.412, P = 0.018, 95\% \text{ C.I} 0.157 - 1.629)\)]

On Day 1, there was a higher mean number of diarrhoeal motions in the B. clausii group [B. clausii group 7.68±2.53 motions vs. Placebo group 7.65± 2.19 motions] there was a mean difference of 0.30 motions. This was a statistically insignificant difference \((t (88) = 2.412, P = 0.018, 95\% \text{ C.I} 0.157 - 1.629)\)]

On day 2, there was a decrease in the mean number of diarrhoeal motions in the B. clausii group [B. clausii group 4.75 ± 2.49 motions vs. Placebo group 5.67 ± 2.82
motions] there was a mean difference of 0.924 motions. This was not a statistically significant difference ($t(88) = 1.645, P = 0.104, 95\% \text{ C.I} -0.192 - 2.040$).

On day 5, there was a decrease in the mean number of diarrhoeal motions in the B.clausii group [B.clausii group 0.86 ± 1.07 motions vs. Placebo group 1.24 ± 1.37 motions] there was a mean difference of 0.375 motions. This was not a statistically significant difference ($t(88) = 1.445, P = 0.152, 95\% \text{ C.I} -0.141 - 0.892$).

On day 6, there was a decrease in the mean number of diarrhoeal motions in the B.clausii group [B.clausii group 0.41 ± 0.67 motions vs. Placebo group 0.54 ± 0.94 motions] there was a mean difference of 0.893 motions. This was not a statistically significant difference ($t(88) = 0.785, P = 0.435, 95\% \text{ C.I} -0.206 - 0.475$).

On day 7, there was a decrease in the mean number of diarrhoeal motions in the B.clausii group [B.clausii group 0.23 ± 0.48 motions vs. Placebo group 0.37± 0.53 motions] there was a mean difference of 0.893 motions. This was not a statistically significant difference ($t (88) = 1.336, P = 0.185, 95\% \text{ C.I} -0.069- 0.354$).

9.6 DURATION OF STAY IN HOSPITAL

![Figure 6: Mean duration of stay](image-url)
We further looked at the duration of stay in the ward. This study found that the *B. clausii* group (4.14 ± 0.93 days) had a shorter duration of stay in hospital than with the placebo group (4.50 ± 1.43 days). There was a mean difference between the groups of 0.37 days. The difference was not statistically significant (*t* (88) = 1.426, *P* = 0.157, 95% CI -0.143 – 0.873).

### 9.7 MORTALITY OUTCOMES

There was no participant who died during the study in both study groups.
10. DISCUSSION

Probiotics administered in addition to rehydration therapy has shown reductions in the duration and severity of diarrhoea, and have not been associated with adverse effects. A Cochrane review on the same supports the use of probiotics in acute, infectious diarrhoea. However, marked clinical variability between studies resulted in insufficient studies of specific probiotic regimens in defined groups of children or adults to inform the development of evidence-based treatment guidelines.43

*Bacillus clausii* (an example of a probiotic) is an aerobic, spore-forming bacterium that is able to inhibit the growth of pathogens in the gastrointestinal tract via three distinct mechanisms: colonization of free ecological niches, competition for epithelial cell adhesion, production of antibiotics and/or lytic enzymes21. Few studies have been done on this probiotic but it has widespread use in several studied conditions.

The aim of this study was to look at this in relation to *B.clausii* in a selected population of 6-59 months old admitted with severe dehydration.

In this study, the duration of diarrhoea was not significantly reduced by *B. clausii* preparation treatment [(77.59 ± 34.10 hours) vs. placebo (86.74 ± 40.16 hours)]. These findings were replicated in an Italian study which looked at five probiotic products commonly found in the Italian market. In the study by Canani et al, there was an estimated difference of 1 hour between the placebo and *B.clausii*. In our study, we found an estimated difference of 9.15 hours in the two groups. This may be attributed to the difference in the probiotic amount in the vials. The Italian study had $1\times10^9$ CFUs/5ml versus $2\times10^9$CFUs/5ml in our study. This requires further studies to prove this improvement in the efficacy.35

The findings are quite different from those seen in *Lactobacillus* and *Saccharomyces boulardii*. In a study done by Guarino et al in 1997 the probiotic *Lactobacillus GG* was found to significantly reduce the duration of diarrhoea by 24 hours.29 A recent meta-analysis in 2007 by Szajewska H et al31, *S.boulardii*, had a pooled weighted mean difference of 1.1 days.

Although the probiotic preparations were found to be effective in reducing diarrhoea, to better inform clinical practice studies of specific probiotic regimens in large
numbers of participants with well-defined diarrhoeal illness are needed. Trials also need to use standardized definitions for acute diarrhoea and the resolution of the illness.

Further sub-analysis on the data showed that both age and timing of the onset of the intervention had no significant impact on the duration of illness. Little data is available on this but if compared with an efficacious probiotic like Lactobacillus the findings are quite different.

The study found age of participant to have no impact on the effectiveness of B. clausii in reducing the duration of illness. The mean duration of illness in participants more than 12 months was actually longer in the patients on B. clausii group (treatment 87.46±33.43hrs vs. placebo 84.54± 44.05 hrs p=0.835). This was not statistically significant. For younger participants less than 12 months there was an insignificant decrease in duration of illness in the B. clausii group (treatment 72.48±33.88 hrs vs. Placebo 88.15 ± 38.23 hrs p=0.107). In a study by Szymanski et al44, they looked at the Lactobacillus rhamnosus probiotic, found no significant effect of age on the probiotic effect on the duration of illness both in the under 12 months (treatment 116±82.7 hrs vs. placebo 158± 90.1 p=0.30) and over 12 months( treatment 86.3±62.1 hrs vs. placebo 110± 71.5 p=0.16). The study had similar participant distribution as our study. The reason for these findings could be due to different organisms that may cause diarrhoea in older children. Several studies on the extensively studied probiotics Lactobacillus and S. boulardii have shown significant reduction in duration of diarrhoea in rotavirus positive disease. Our study did not look into the aetiological cause of the diarrhoea.

We looked at the duration of diarrhoea in relation to the duration of illness prior to starting of intervention. Our assumption was that the earlier one starts the probiotic, the shorter the duration of illness. In our study no significant differences were appreciated in both those who started the intervention ≤72hrs (treatment 83.46 ±42.51hrs vs. Placebo 93.91± 46.83 hrs p= 0.489) and those > 72hrs (treatment 74.23 ±28.56 hrs vs. Placebo 80.71 ± 33.38 p=0.449). In the same study on Lactobacillus rhamnosus by Szymanski et al44, there was no significant decrease in duration of diarrhoea in participants who started the intervention within 72 hours of the illness (treatment 88.8±59 vs. Placebo 102± 72.8 p=0.40) For those who started it later than
72 hours there was no significant decrease in the treatment group (treatment 64.8±37.8 vs. Placebo 42.5±17.5 p=0.29). These findings may be due to the mechanism of action of the probiotic which involve colonisation and displacement of harmful organisms. This may take some period of time and by that time the short natural course of the disease has already take place before the probiotic has exerted its effect.

In our study we further looked at the effect of *B.clausii* on the number of diarrhoeal episodes per day. In spite of the lack of significance in the mean duration of diarrhoeal illness, there was a significant reduction in the mean diarrhoeal episodes per day on Day 3 (treatment 2.75±1.80 vs. Placebo 3.80± 2.70 p=0.033) and on Day 4 (treatment 1.45±1.13 vs. Placebo 2.35± 2.19 p=0.018). The other days of the week showed no significant reduction in the diarrhoeal stool output. These findings are consistent with findings *Lactobacillus* and *S.boulardii*. In a study on *S.boulardii* done in Pakistan by Billoo et al\(^4\), it showed a significant reduction in mean number of stools per day on day 3 (treatment group 2.8 vs. control 4.4 p=0.01) and on day 6 (treatment group 1.6 vs. control 3.3 p=0.001).

Severe dehydration usually leads to hospitalization to receive hydration. We decided to look at the effect of *B.clausii* on the duration of stay in hospital. We found that both groups had similar length of stay in the hospital [*B. clausii* group (4.14 ± 0.93 days) vs. placebo group (4.50 ± 1.43days) *P* = 0.157] Chen et al in Taiwan, found that Bio-three (a mixture of *Bacillus mesentericus*, *Enterococcus faecalis*, and *Clostridium butyricum*) reduced the severity of diarrhoea and length of hospital stay in children with acute diarrhoea. The study involved 304 children aged 3 months to 6 years hospitalized for acute diarrhoea. The mean duration of diarrhoea after start of therapy was 60.1 hours in the probiotics group versus 86.3 hours in the placebo group (*P* = 0.003). Hospital stay was shorter in the probiotics group (2.9 ±0.8 days) than in the placebo group (4.2 ± 2.1 days) (*P* = 0.009). The later study was done in a controlled environment unlike our study. Similar reductions in hospital stay have been seen with *Lactobacillus* and *Saccharomyces boulardii*. 
II. STUDY LIMITATIONS

The study was limited mainly by financial constraints. The availability of funds would have enabled us to have more control over the study environment. We would have been able to admit the patient for the whole study period and able to monitor the patients more closely.

Majority of the data collected depended on what the parent / caregiver reported or recorded, this could have exposed the study to measurement bias. We couldn’t ignore the possibility of wrong data input into the daily diary given to the participant.

The sample size was not large enough to fully assess the effectiveness of the study on the community hence impacting on the external validity of the study.

We were not able also to assess the relationship between the probiotic and the actual cause of the acute diarrhoea i.e. rotavirus.
12. CONCLUSION

*Bacillus clausii* had no effect on shortening the duration of diarrhoeal illness in children admitted with severe dehydration but has shown some effect in reducing the diarrhoeal stool output per day.
13. RECOMMENDATIONS

Further efficacy studies on *B. clausii* should be carried out using large sample studies and in more controlled environment.

The efficacy of other probiotics in severe dehydration should be investigated in larger studies.
14. REFERENCES


12. Bhandari et al. Effectiveness of Zinc supplementation plus oral rehydration salts compared with oral rehydration salts alone as a treatment for acute diarrhoea in primary care setting; a cluster randomised trial; Paediatrics vol 121 No.5 2008 pg e1279-e1285.


23. Stefano Guandalini, MD, Probiotics for children with diarrhoea; an Update : J. Gastroenerol 2008; 42: 553-557


32. Maria C. Urdaci, PhD, Philippe Bressollier, PhD, and Irina Pinchuk, PhD. *Bacillus clausii* Probiotic Strains Antimicrobial and Immunomodulatory Activities; *J Clin Gastroenterol* 2004;38:S86–S90.


36. A phase III, controlled, open-label, randomized, parallel group, multicentric, comparative study to assess the efficacy and safety of oral rehydration therapy (ORT) in combination with spores of *Bacillus clausii* (Enterogermina™) versus ORT alone, administered for 5 days in the treatment of acute diarrhea in children. Sanofi Aventis (31 December 2008).


43. Allen SJ, Okoko B, Martinez EG, Gregorio GV, Dans LF Probiotics for treating infectious diarrhoea (Review) 2009


45. AG Billoo. MA Memon, SA Khashkeli, G Murtaza, Khalid Iqbal, M Saeed Shekhani, Ahson Q Siddiqi Role of a probiotic (Saccharomyces boulardii) in management and prevention of diarrhea World J Gastroenterol 2006 July 28; 12(28): 4557-4560

APPENDIX A

CLASSIFICATION TABLE FOR DEHYDRATION

<table>
<thead>
<tr>
<th>SIGNS</th>
<th>CLASSIFY AS</th>
<th>IDENTIFY TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two of the following signs:</td>
<td></td>
<td>▶ If child has no other severe classification:</td>
</tr>
<tr>
<td>• Lethargic or unconscious</td>
<td>SEVERE DEHYDRATION</td>
<td>— Give fluid for severe dehydration (Plan C).</td>
</tr>
<tr>
<td>• Sunken eyes</td>
<td></td>
<td>OR</td>
</tr>
<tr>
<td>• Not able to drink or drinking poorly</td>
<td></td>
<td>▶ If child also has another severe classification:</td>
</tr>
<tr>
<td>• Skin pinch goes back very slowly</td>
<td></td>
<td>— Refer URGENTLY to hospital with mother giving frequent sips of ORS on the way.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>— Advise the mother to continue breastfeeding</td>
</tr>
<tr>
<td></td>
<td></td>
<td>▶ If child is 2 years or older and there is cholera in your area, give antibiotic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>for cholera.</td>
</tr>
<tr>
<td>Two of the following signs:</td>
<td>SOME DEHYDRATION</td>
<td>▶ Give fluid and food for some dehydration (Plan B).</td>
</tr>
<tr>
<td>• Restless, irritable</td>
<td></td>
<td>▶ If child also has a severe classification:</td>
</tr>
<tr>
<td>• Sunken eyes</td>
<td></td>
<td>— Refer URGENTLY to hospital with mother giving frequent sips of ORS on the way.</td>
</tr>
<tr>
<td>• Drinks eagerly, thirsty</td>
<td></td>
<td>— Advise the mother to continue breastfeeding</td>
</tr>
<tr>
<td>• Skin pinch goes back slowly</td>
<td></td>
<td>▶ Advise mother when to return immediately.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>▶ Follow-up in 5 days if not improving.</td>
</tr>
<tr>
<td>Not enough signs to classify as some or</td>
<td>NO DEHYDRATION</td>
<td>▶ Give fluid and food to treat diarrhoea at home (Plan A).</td>
</tr>
<tr>
<td>severe dehydration.</td>
<td></td>
<td>▶ Advise mother when to return immediately.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>▶ Follow-up in 5 days if not improving.</td>
</tr>
</tbody>
</table>

Adapted from Handbook: IMCI integrated management of childhood illness. (2005)
APPENDIX B
TREATMENT PLAN C FOR SEVERE DEHYDRATION

CHART 13. Diarrhoea Treatment Plan C:
Treat severe dehydration quickly

Follow the arrows. If answer is YES go across. If NO go down.

START HERE
Can you give intravenous (IV) fluid immediately?

YES

Start IV fluid immediately. If the child can drink, give ORS by mouth while the drip is set up. Give 100 ml/kg Ringer’s lactate solution (or, if not available, normal saline), divided as follows:

| AGE                      | First give  | Then give
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(under 12 months) Infants</td>
<td>30 ml/kg in:</td>
<td>70 ml/kg in:</td>
</tr>
<tr>
<td>Children (12 months up to 5 years)</td>
<td>30 minutes*</td>
<td>2 1/2 hours</td>
</tr>
</tbody>
</table>

* Repeat once if radial pulse is still very weak or not detectable.

- Reassess the child every 15-30 minutes. If hydration status is not improving, give the IV drip more rapidly.
- Also give ORS (about 5 ml/kg/hour) as soon as the child can drink; usually after 3-4 hours (infants) or 1-2 hours (children).
- Reassess an infant after 6 hours and a child after 3 hours. Classify dehydration. Then choose the appropriate plan (A, B, or C) to continue treatment.

YES

Refer URGENTLY to hospital for IV treatment.

NO

Is IV treatment available nearby (within 30 minutes)?

YES

Are you trained to use a nasogastric (NG) tube for rehydration?

YES

Start rehydration by tube (or mouth) with ORS solution: give 20 ml/kg/hour for 6 hours (total of 120 ml/kg).

NO

Are you trained to use a nasogastric (NG) tube for rehydration?

YES

Can the child drink?

YES

Refer URGENTLY to hospital for IV or NG treatment.

NO

Can you give intravenous (IV) fluid immediately?

NO

Can the child drink?

NO

Refer URGENTLY to hospital for IV or NG treatment.

Note: If possible, observe the child for at least 6 hours after rehydration to be sure the mother can maintain hydration giving the child ORS solution by mouth.

Adapted from Pocket book of hospital care for children: guidelines for the management of common illnesses with limited resources. (WHO 2005)
This Informed Consent Form is for children admitted at Kenyatta National Hospital, and who we are inviting to participate in the research... The title of our research project is “Effectiveness of *Bacillus clausii* in reducing duration of acute diarrhea in children”

I am Dr Brian Maugo, a postgraduate student at the University of Nairobi pursuing studies leading to specialisation in Paediatrics and Child Health. I am doing a research on acute diarrhoeal disease in children, which is very common in this country. I am going to give you information and invite you to be part of this research.

Acute diarrhoea is one of the most common and dangerous diseases in this region. The drugs that are currently used to help in treating acute diarrhea include Oral rehydration salt and Zinc supplementation. There are new drugs called probiotics including *Bacillus clausii* which may help in treatment of acute diarrhoea. The reason we are doing this research is to find out if *Bacillus clausii* can help reduce the duration of diarrhea.

This research will involve oral intake of *Bacillus clausii* for 5 days as well as daily follow-up via direct interview and phone call and one follow up visit to the clinic.

We are inviting all children with acute diarrhoea and severe dehydration admitted in the Paediatric wards to participate in the research.

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this hospital will continue and nothing will change.

Because we do not know if Enterogermina reduces the duration of diarrhoea, we need to compare the current treatment and a combination of current treatment with *Bacillus*
To do this, we will put people taking part in this research into two groups. The groups are selected by chance, as if by tossing a coin.

The research takes place over 7 days. During that time I will review your child daily while in the ward. If discharged earlier, I will contact you via mobile phone daily.

*Bacillus clausii* has been used in other studies and has no known side effects. It is safe for use in children.

We will not be sharing the identity of those participating in the research. The information that we collect from this research project will be kept confidential.

The knowledge that we get from doing this research will be shared with the public through publishing of the results in journals. Confidential information will not be shared.

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following: 0737150536/0771016263

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant ____________________

Signature of Participant ____________________

Date __________________________

Day/month/year
I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this consent form has been provided to the participant.

Print Name of Researcher/person taking the consent _________________________

Signature of Researcher/person taking the consent _________________________

Date _____________________________

Day/month/year
FOMU YA KUPATA KIBALI CHA WAZAZI / WALEZI WA WASHIRIKI

Hii fomu ya kupata idhini ni kwa ajili ya watoto waliolazwa katika Hospitali ya Taifa ya Kenyatta, ambao tunuwakaribisha kushiriki katika utafiti. Jina la mradi wa utafiti wetu ni "Ufanisi wa *Bacillus clausii* katika kupunguza muda wa kuharisha katika watoto"


Kunaweza kuwa na baadhi ya maneno ambayo huelewi. Fafadhali uliza na mimi itachukua muda kueleza. Kama una maswali baadaye, unaweza bado niuliza kuharisha ni moja ya magonjwa ya kawaida na hatari ya kuharisha papo ni pamoja na *Bacillus clausii* ambayo inaweza kusaidia kuharisha. Sababu ya sisi kufanya utafiti huu ni ili kujua kama *Bacillus clausii* inaweza kusaidia kupunguza muda wa kuharisha.

Utafiti huu utahusu kukunya *Bacillus clausii* kwa muda wa siku 5, pamoja na kukfuatilia kupitia mahojiano ya moja kwa moja na simu.


Kwa sababu hatujui kama *Bacillus clausii* inapunguza muda wa kuhara, tunahitaji kulinganisha matibabu ya sasa na ile inayouhusisha *Bacillus clausii*. Ilu kuweza kufanya hivyo tutaweka washiriki wa utafiti huu katika makundi mawili.

*Bacillus clausii* imetumika katika utafiti nyingine na haina madhara maalumu. Nisalamu kwa matumizi ya watoto.

Habari kukuhusu ambayo tutakusanya kutokana mradi wa utafiti huu utakuwa siri.

Kama una maswali yoyote unaweza kuuliza hivi sasa au baadaye, hata pia baada ya utafiti imeanza. Kama unataka kuuliza maswali baadaye, unaweza kuwasiliana name kupitia nambari hizi: 0737150536/0771016263

Nimesoma/ Nimesomewa maelezo haya na nipepewa nafasi ya kuuliza maswali kukuhusu hayo maelezo. Nimeidhini kwa hiari kushiriki katika utafiti huu.

Jina la lako____________________

Sahihii ya Mshiriki__________________

Tarehe____________________

Nina uhakika kuwa nimemsomea mwakilishi fomu hii, na kwa kadri ya uwezo wangu nihilakikisha kwamba mshiriki ameelewa.

Niluthibitisha kuwa mshiriki alipewe nafasi ya kuuliza maswali kukuhusu utafiti nakuyajibu vema kwa kadri ya uwezo wangu. Mimi nathibitisha kwamba mwakilishi hakulazimishwa kutoa kibali

Jina la Mtafiti/ Mtu kuchukua kibali____________________

Sahihii ya Mtufiti/ mtu kuchukua kibali____________________

Tarehe____________________
### APPENDIX D

**CASE ASSESSMENT OF GASTROENTERITIS**

Subject Identification Number ____________  Date ____________

1. **Participant details**

<table>
<thead>
<tr>
<th>Age of Participant in Months</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male = 1  Female = 2</td>
</tr>
<tr>
<td>Weight in grams</td>
<td></td>
</tr>
<tr>
<td>Length in centimeters</td>
<td></td>
</tr>
<tr>
<td>Axillary temperature (°C)</td>
<td></td>
</tr>
<tr>
<td>Is child breastfeeding? Yes = 1 No = 2</td>
<td></td>
</tr>
<tr>
<td>Period of exclusive breastfeeding in months</td>
<td></td>
</tr>
</tbody>
</table>

2. **Parent / Guardian**

<table>
<thead>
<tr>
<th>Age in years</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Education status</td>
<td></td>
</tr>
<tr>
<td>None = 1</td>
<td></td>
</tr>
<tr>
<td>Primary = 2</td>
<td></td>
</tr>
<tr>
<td>Secondary = 3</td>
<td></td>
</tr>
<tr>
<td>Tertiary = 4</td>
<td></td>
</tr>
</tbody>
</table>

| Number of household members with gastroenteritis |  |
| Contact with person outside household with gastroenteritis |  |
| Yes = 1  No = 2  Don’t Know = 3 |  |
| Has Participant had diarrhea before |  |
Yes = 1  No = 2  If yes, how many times

Type of fluids given at home in the last 24 hours (Yes = 1  No = 2)

ORS

Plain water

Milk/ Porridge

Homemade solutions

3. Prior care sought before the participant was brought to KNH.

Code Yes = 1  No = 2

Herbalist/ Traditional healer

Religious Healer

Bought over the counter medication

4. Referring health facility

Private facility =1

Council clinic/ dispensary =2

District hospital =3

Self referral =4

5. Assessment of diarrhoea

Frequency of diarrhoea / day
Frequency of vomiting/ day

Duration of diarrhoea (days)

Duration of vomiting (days)

Blood in stool  Yes = 1  No = 2

6. Clinical assessment of severity of diarrhoea

Respiration Rate/ min

Cold extremities Yes = 1  No = 2

Capillary refill in seconds

Heart rate / min

Peripheral pulse Normal strength = 1  Weak = 2 Impalpable = 3

Level of consciousness Alert = 1 Verbal = 2 Pain = 3 Unconscious=4

Eye balls Not Sunken =1  Sunken = 2

Skin Turgor

Slightly decreased = 1  Decreased= 2  Marked decreased= 3

7. Hydration

No dehydration = 1  some dehydration = 2  Severe dehydration/ shock=3

8. Other associated problems  Yes= 1  No = 2

Distended abdomen

Convulsion
Respiratory distress

9. Nutrition Status  Yes = 1  No = 2

Normal

Visible wasting

Bilateral pedal oedema
<table>
<thead>
<tr>
<th>DAY</th>
<th>DIARRHOEA</th>
<th>VOMITING</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Subject Identification Number ______________ Date ____________

**KEY:**
- DIARRHOEA ×
- FORMED STOOL √
- VOMITING ×
- NO VOMITING √
r. Maugo Brian Maugo
Dept of Paediatrics & Child Health
School of Medicine
University of Nairobi

Dear Dr. Maugo:

RESEARCH PROPOSAL: “EFFICACY OF BACILLUS CLAUSII IN REDUCING DURATION OF ILLNESS IN ACUTE DIARRHOEA IN CHILDREN” (P150/5/2010)

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and approved your above cited research proposal for the period 19th July 2010 to 18th July 2011. You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely,

[Signature]

PROF A N GUANTAI
SECRETARY, KNH/UON-ERC

Prof. K. M. Bhatt, Chairperson, KNH/UON-ERC
The Deputy Director CS, KNH
The Dean, School of Medicine, UON
The HOD, Records, KNH
The Chairman, Dept. of Paediatrics & Child Health, UON
Supervisors: Dr. Jowi Yuko, Dept. of Paediatrics & Child Health, UON
Dr. Murla F., Dept. of Paediatrics & Child Health, UON
Dr. Laving A., Dept. of Paediatrics & Child Health, UON