A systematic review of the efficacy and safety of *Saccharomyces boulardii* in the treatment of acute gastroenteritis in the pediatric population

by
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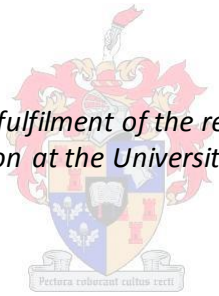

Thesis presented in partial fulfilment of the requirements for the degree Master of Nutrition at the University of Stellenbosch

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December 2016

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ABSTRACT

Background: The yeast *Saccharomyces boulardii* has been classed a probiotic because it is a live microorganism known to confer a health benefit to its host, with one such benefit being in the management of gastrointestinal disturbances like gastroenteritis. Gastroenteritis is known to be the second leading cause of death in the world's most vulnerable populations, with Rotavirus being the most common causative agent, responsible for 215,000 global child deaths during 2013. Together with a few other probiotics, *Saccharomyces boulardii* has been considered a potentially viable treatment option having been associated with a decreased duration of diarrhea, decreased number of days to the first formed stool, and decreased duration of hospital stay in individual studies. This systematic review was therefore designed to specifically investigate the effects of *Saccharomyces boulardii* on acute gastroenteritis caused by Rotavirus in the pediatric hospitalized patient.

Objectives: To assess the efficacy and safety of *Saccharomyces boulardii* in the treatment of acute gastroenteritis in the pediatric population. Secondary objectives of cost-effectiveness in terms of length of hospital stay; optimal dosing and administration routes were also investigated.

Methods: Data sources included Medline, CINAHL, Scopus and The Cochrane Library up to and including August 2015. Only randomized controlled trials in a hospital setting and involving subjects less than 16 years were included. Two reviewers independently evaluated studies for eligibility, quality and extracted the data. Data were analyzed using Review Manager 5 (RevMan 2008) software. A random effects model of meta-analysis was used due to the presence of heterogeneity of treatment effects between studies.

Results: Out of a pool of 190 articles, 10 studies were selected for final inclusion and analysis. A meta-analysis involving five of the ten included studies showed that *Saccharomyces boulardii* significantly shortened the duration of diarrhea (in days), compared to the control/placebo group (MD -0.57, 95%CI: -0.83 to -0.30, P < 0.0001). Also, participants were passing solid stools in the *Saccharomyces boulardii* group compared to the control group on Day 2 (RR 3.00; 95% CI: 0.32 to 27.87), Day 3 (RR 3.17; 95% CI: 1.89 to 5.31), Day 4 (RR 1.63; 95% CI: 1.30 to 2.06) and Day 5 (RR 1.25; 95% CI: 1.08 to 1.44), (P = 0.06). Other outcomes like number of participants having less than three stools per day during the intervention and duration of hospital stay did not produce any statistically significant results. No studies reported on any significant adverse effects associated with the use of *Saccharomyces boulardii*.

Conclusion: The results of the current systematic review appear to indicate there's a *potential benefit* with using *Saccharomyces boulardii* to treat acute gastroenteritis in the pediatric patient. Offering this unique yeast probiotic at a dose of 250mg once to twice per day for up to five days has shown some benefit and appears to be safe. However, larger and more rigorous controlled trials are needed to further investigate the efficacy and safety of individual probiotics, like *Saccharomyces boulardii*, in order to offer specific treatment guidelines.

OPSOMMING

Agtergrond: Die gis *Saccharomyces boulardii* word beskou as 'n probiotika aangesien dit 'n lewendige mikroorganisme is wat gesondheidsvoordele inhou vir die gasheer. Een van die voordele is in die behandeling van gastrointestinale afwykings soos gastroenteritis. Alhoewel gastroenteritis slegs 'n simptoom is van 'n toestand, is dit bekend om die tweede mees algemene oorsaak van sterfte te wees onder vatbare populasies in die wêreld. Rotavirus, die mees algemene oorsaak van gastroenteritis, was verantwoordelik vir 215,000 kindersterftes wêreldwyd tydens 2013. Tesame met 'n paar ander probiotika word *Saccharomyces boulardii* beskou as 'n potensiële lewensvatbare behandelingsopsie. Hierdie gis probiotika word in individuele studies geassosieer met 'n verkorte duurte van diaree, verminderde aantal dae tot die eerste gevormde stoelgang en 'n verkorte duurte van hospitaal verblyf. Hierdie sistematiese literatuuroorsaak was dus beplan om die effek van *Saccharomyces boulardii* op akute gastroenteritis veroorsaak deur Rotavirus in pediatriese gehospitaliseerde pasiënte te ondersoek.

Doelwitte: Om die effektiwiteit en veiligheid van *Saccharomyces boulardii* in die behandeling van akute gastroenteritis in die pediatrie populasie te bepaal. Sekondêre doelwitte was om die koste-effektiwiteit in terme van duurte van hospital verblyf, optimale dosering en administrasie roetes te ondersoek.

Metodes: Data bronne het Medline, CINAHL, Scopus en die Cochrane Biblioteek tot en met Augustus 2015 ingesluit. Slegs verewekansigde gekontrolleerde proewe in 'n hospitaal omgewing gedoen op persone jonger as 16 jaar is ingesluit. Twee evalueerders het die studies onafhanklik evalueer vir geskiktheid, kwaliteit en was betrokke by data ekstraksie. Data was ge-analiseer deur gebruik te maak van *Review Manager 5* (RevMan 2008) sagteware. Die stogastiese-effekte model van meta-analise was gebruik as gevolg van die teenwoodigheid van heterogeniteit van behandelings-effekte tussen studies.

Resultate: Uit 'n poel van 190 potensiële artikels is 10 studies geselekteer vir finale insluiting en analise. 'n Meta-analise op vyf van die tien studies het getoon dat *Saccharomyces boulardii* verantwoordelik was vir 'n beduidende verkorte duurte van diaree (in dae), vergeleke met die kontrole of plasebo groepe (MD -0.57, 95%CI: -0.83 to -0.30, P < 0.0001). Resultate het ook gedui dat meer deelnemers in die *Saccharomyces boulardii* groep soliede stoelgane gehad het vergeleke met die kontrole groep op Dag 2 (RR 3.00; 95% CI: 0.32 to 27.87), Dag 3 (RR 3.17; 95% CI: 1.89 to 5.31), Dag 4 (RR 1.63; 95% CI: 1.30 to 2.06) en Dag 5 (RR 1.25; 95% CI: 1.08 to 1.44), (P = 0.06). Ander uitkomste soos die aantal deelnemers met minder as drie stoelgange per dag gedurende intervensie en duurte van hospital verblyf het nie beduidende resultate gelewer nie. Geen studies het enige newe-effekte geassosieer met die gebruik van *Saccharomyces boulardii* gerapporteer nie.

Gevolgtrekking: Die resultate van die huidige sistematiese literatuuroorsig dui op 'n *potensiële voordeel* met die gebruik van *Saccharomyces boulardii* vir die behandeling van akute gastroenteritis in die pediatriese groep. Die inname van hierdie unieke gis probiotika teen 'n dosis van 250mg een tot twee maal per dag vir tot vyf dae het op sommige voordele gewys en blyk om veilig te wees. Groter en strenger gekontrolleerde proewe word egter

aanbeveel om die effektiwiteit en veiligheid van individuele probiotika soos *Saccharomyces boulardii* verder te ondersoek ten einde spesifieke behandelingsriglyne te kan voorstel.

ACKNOWLEDGEMENTS

I would like to thank the following individuals for their contributions in helping me complete this thesis:

- Professor Renée Blaauw (Supervisor) – who provided encouragement to stay motivated and who eagerly, offered her wealth of experience.
- Mrs. Janicke Visser (Co-Supervisor) - who provided ongoing guidance and motivation to complete this research project.
- Mrs. Estelle Viljoen (Registered Dietitian, Independent Reviewer) – who drew from her experiences of completing a Master's degree by offering guidance and suggestions; for the many hours spent reviewing articles that were used in this systematic review.
- Mr. Alfred Musekiwa (MSc Mathematical Statistician) – for his expertise in analyzing the data collated and for his commitment to the statistical analysis used in this systematic review.
- Mrs. Wilhelmine Pool (Medical Librarian) – for her excellent work conducting thorough electronic searches of studies on the subject matter.

CONTRIBUTIONS BY PRINCIPAL RESEARCHER AND FELLOW RESEARCHERS

The principal researcher (Morgambal Padayachee) was responsible for the following:

- developed the idea and the protocol
- planned the systematic review
- conducted a literature search with the assistance of a Medical Librarian (Mrs. Wilhelmine Pool)
- selection of appropriate literature sources together with an Independent Reviewer (Mrs. Estelle Viljoen)
- data collection and extraction in collaboration with an Independent Reviewer (Mrs. Estelle Viljoen)
- data capturing for analysis with the assistance of a statistician (Mr. Alfred Musekiwa)
- analyzing the data with the assistance of a statistician (Mr. Alfred Musekiwa)
- interpreting the data and drafting the statistical analysis with the assistance of a statistician (Mr. Alfred Musekiwa)
- compiling the thesis

The Supervisor (Prof. Renée Blaauw) and the Co-Supervisor (Mrs. Janicke Visser) provided guidance and input at all stages, and revised both the protocol and the thesis.

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LIST OF DEFINITIONS

Adverse event[1,2,3]**:** An adverse outcome that occurs during or after the use of a drug or other intervention but is not necessarily caused by it.

Bias[1,2]**:** Bias is a systematic error, or deviation from the truth, which can lead to an underestimation or an overestimation of the true intervention effect. Biases can vary in magnitude – some are small and trivial compared to the observed effect, and some are substantial, so that an apparent finding may be entirely due to bias.

Blinding[1,2]**:** [In controlled trials:] The process of preventing those involved in a trial from knowing to which comparison group a particular participant belongs. The risk-of-bias is minimized when as few people as possible know who is receiving the experimental intervention and who the control intervention. Participants, caregivers, outcome assessors, and analysts are all candidates for being blinded.

Chi-squared test[1,2]**:** A statistical test based on comparison of a test statistic to a chi-squared distribution. Used in RevMan analyses to test the statistical significance of the heterogeneity statistic.

Clinically significant[1,2]**:** A result (e.g. a treatment effect) that is large enough to be of practical importance to patients and healthcare providers.

Confidence Interval (CI)[1,2]**:** A measure of the uncertainty around the main finding of a statistical analysis. Estimates of unknown quantities are usually presented as a point estimate and a 95% confidence interval. This means that if someone were to keep repeating a study in other samples from the same population, 95% of the confidence intervals from those studies would contain the true value of the unknown quantity. Wider intervals indicate lower precision; narrow intervals, greater precision.

Fixed-effect model[1,2]**:** [In meta-analysis:] A model that calculates a pooled effect using the assumption that all observed variation between studies is caused by the play of chance. Studies are assumed to be measuring the same overall effect.

Forest plot[1,2]**:** A graphical representation of the individual results of each study included in a meta-analysis together with the combined meta-analysis result. The plot also allows readers to see the heterogeneity among the results of the studies. The results of individual studies are shown as squares centered on each study's point estimate. A horizontal line runs through each square to show each study's confidence interval – usually, but not always, a 95% confidence interval. The overall estimate from the meta-analysis and its confidence interval are shown at the bottom, represented as a diamond. The center of the diamond represents the pooled point estimate, and its horizontal tips represent the confidence interval.

Gastroenteritis (GE)[3,4]: Gastroenteritis refers to a wide variety of conditions characterized by infection or irritation of the digestive tract, particularly the stomach and intestine. Symptoms include nausea and vomiting, diarrhea and/or abdominal cramps. These symptoms sometimes include fever and weakness. The condition can be grouped according to length of episode i.e. a) "Acute" GE (AGE) if it lasts for less than 14 days, b) "Persistent" GE (PGE) if it lasts between 2 and 4 weeks, and c) "Chronic" GE (CGE) if it lasts for longer than 4 weeks.

Heterogeneity[1,2]: Used in a general sense to describe the variation in, or diversity of, participants, interventions, and measurement of outcomes across a set of studies, or the variation in internal validity of those studies. Used specifically, as statistical heterogeneity, to describe the degree of variation in effect estimates from a set of studies. It is also used to indicate the presence of variability among studies beyond the amount expected due solely to the play of chance.

Intention-to-treat analysis (ITT)[1,2]: A strategy for analyzing data from a randomized controlled trial. All participants are included in the arm to which they were allocated, whether or not they received (or completed) the intervention given to that arm. ITT analysis prevents bias caused by the loss of participants.

Mean difference[1,2]: The mean difference (more correctly 'difference in means') is a standard statistic which measures the absolute difference between the mean value in two groups in a clinical trial. It estimates the amount by which the experimental intervention changes the outcome on average compared with the control.

Meta-analysis[1,2,5]: Meta-analysis is the use of statistical methods to summarize or combine the results of two or more independent studies. It can be used to combine the numerical results of all or some of the studies included in a systematic review. This yields an overall statistic, together with its confidence interval, that summarizes the effectiveness of the experimental intervention compared with the control intervention. The combination of intervention effects estimates across studies may incorporate an assumption that the studies are not all estimating the same intervention effect, but rather estimate intervention effects that follow a distribution across studies. This is then a **random-effects meta-analysis**. Alternatively, if it is assumed that each study is estimating exactly the same quantity, a **fixed-effect meta-analysis** is performed.

***P*-value[1,2,5]:** The probability (ranging from zero to one) that the results observed in a study (or results more extreme) could have occurred by chance if in reality the null hypothesis was true. In a meta-analysis, the *P* value for the overall effect assesses the overall statistical significance of the difference between the intervention groups, whilst the *P* value for the heterogeneity statistic assesses the statistical significance of differences between the effects observed in each study.

Placebo[1,2]**:** An inactive substance or procedure administered to a participant, usually to compare its effects with those of a real drug or other intervention, but sometimes for the psychological benefit to the participant through a belief that s/he is receiving treatment. Placebos are used in clinical trials to blind people to their treatment allocation. Placebos should be indistinguishable from the active intervention to ensure adequate blinding.

Random effects model[1,2]**:** A statistical model in which both within-study sampling error (variance) and between-studies variation are included in the assessment of the uncertainty (confidence interval) of the results of a meta-analysis. When there is heterogeneity among the results of the included studies beyond chance, random-effects models will give wider confidence intervals than fixed-effect models.

Randomization[1,2]**:** The process of randomly allocating participants into one of the arms of a controlled trial. There are two components to randomization: the generation of a random sequence, and its implementation, ideally in a way so that those entering participants into a study are not aware of the sequence (concealment of allocation).

Randomized controlled trial (RCT)[1,2]**:** An experiment in which two or more interventions, possibly including a control intervention or no intervention, are compared by being randomly allocated to participants.

Review Manager version 5 (RevMan5)[1,2]**:** Software developed for The Cochrane Collaboration to assist reviewers in preparing Cochrane Reviews. Reviewers enter their protocols and reviews into RevMan, from which they are exported and sent to a Managing Editor to be considered for inclusion in the Cochrane Database of Systematic Reviews.

Risk ratio (RR)[1,2]**:** The ratio of risk in two groups. In intervention studies, it is the ratio of the risk in the intervention group to the risk in the control group. A risk ratio of one indicates no difference between comparison groups. For undesirable outcomes, a risk ratio that is less than one indicates that the intervention was effective in reducing the risk of that outcome.

Saccharomyces boulardii[6,7]**:** A strain of yeast that has been investigated for its ability to mediate the response of gut protection, which is usually displayed by normal healthy gut flora. It has displayed the ability to resist the action of gastric acid and bile, thereby making it a therapeutic agent for possibly the prevention and treatment of disorders affecting the gastrointestinal tract.

Selective outcome reporting[1,2]**:** The selection of a subset of the original variables recorded, on the basis of the results, for inclusion in publication of trials. The particular concern is that statistically non-significant results might be selectively withheld from publication.

Sequence generation[1,2]**:** This principle addresses the allocation process in a randomized controlled trial (RCT). The starting point for an unbiased intervention study is the use of a mechanism that ensures that the same kinds of participants receive each intervention.

Standard Deviation[1,2]**:** A measure of the spread or dispersion of a set of observations, calculated as the average difference from the mean value in the sample.

Standard Mean Difference (SMD)[1,2]**:** The difference between two estimated means divided by an estimate of the standard deviation. It is used to combine results from studies using different ways of measuring the same concept. By expressing the effects as a standardized value, the results can be combined since they have no units. Standardized mean differences are sometimes referred to as a d index.

Statistically significant[1,2]**:** A result that is unlikely to have happened by chance. The usual threshold for this judgment is that the results, or more extreme results, would occur by chance with a probability of less than 0.05 if the null hypothesis was true. Statistical tests produce a *P* value used to assess this.

Systematic Review[1,2]**:** A systematic review attempts to collate all empirical evidence that fits pre-specified eligibility criteria in order to answer a specific research question. It uses explicit, systematic methods that are selected with a view of minimizing bias, thus producing more reliable findings from which conclusions can be drawn and decisions made. The key characteristics of a systematic review are: (i) a clearly stated set of objectives with pre-defined eligibility criteria for studies; (ii) reproducible methodology; (iii) a systematic search to identify all studies that would meet the eligibility criteria; (iv) an assessment of the validity of findings of included studies; (v) a systematic presentation and synthesis of the characteristics and findings of the included studies. Many systematic reviews contain meta-analyses (see meta-analysis).

LIST OF ABBREVIATIONS

AAGE: Antibiotic-Associated Gastroenteritis

AGE: Acute Gastroenteritis

AIDS: Acquired Immune Deficiency Virus

CFUs: Colony Forming Units

CI: Confidence Interval

DHAKA: Dehydration: Assessing Kids Accurately

GE: Gastroenteritis

GRADE: Grades of Recommendation, Assessment, Development and Evaluation

HIV: Human Immuno-Deficiency Virus

ITT: Intention-To-Treat

MCEE: Maternal and Child Epidemiology Estimation Group

MD: Mean Difference

MDGs: Millennium Development Goals

NNT: Numbers Needed to Treat

OR: Odds Ratio

ORS: Oral Rehydration Solution

ORT: Oral Rehydration Therapy

PGE: Persistent Gastroenteritis

PROSPERO: Prospective Register of Ongoing Systematic Reviews

RCTs: Randomized Controlled Trials

RevMan5: Review Manager version 5

RR: Risk Ratio, Relative Risk

SCFAs: Short Chain Fatty Acids

SD: Standard Deviation

SDGs: Sustainable Development Goals

SMD: Standard Mean Difference

UNICEF: United Nations International Children's Fund

WMD: Weighted Mean Difference

WGO: World Gastroenterology Organization

WHO: World Health Organization

CHAPTER 1

BACKGROUND AND MOTIVATION FOR THE STUDY

1.1 INTRODUCTION

In September 2000, 189 countries, including South Africa, signed the United Nations Millennium Declaration, in an effort to address health concerns plaguing each of their populations, with a special focus on women and children.[8,9] This declaration highlighted 8 goals called Millennium Development Goals (MDGs), each of which had country-specific tracking-of-progress for the period 1990 to 2015.[8,9] One of the 8 goals, goal 4, was aimed at reducing child mortality by two-thirds, particularly the under-five mortality rate.[8,9]

In 2015, the United Nations International Children's Emergency Fund (UNICEF) reported a 53 percent decrease in the global under-five mortality rate, i.e. from 91 deaths-per-1000-live-births in 1990 to an estimated 43 deaths-per-1000-live-births in 2015.[8,9] Country-specific data was available and South Africa's successful efforts to decrease under-five mortality rate were clearly shown. Figure 1.1 below shows that this African state started off in 1990 with 61 deaths-per-1000-live-births, peaked in 2004 with 81 deaths-per-1000-live-births, followed by a dramatic drop to the current 44 deaths-per-1000-live-births.[8]

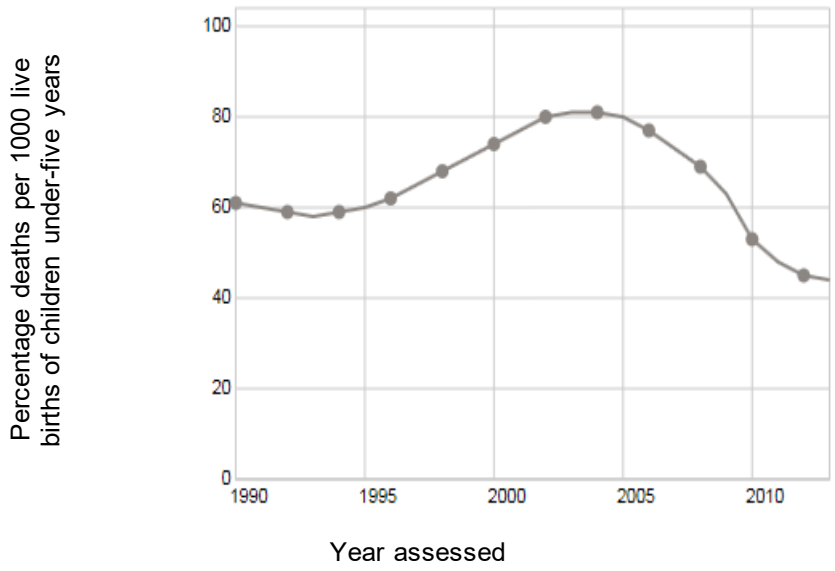

Figure 1.1: South Africa's children under-five mortality rate for the period 1990 to 2015, reflected as deaths-per-1000-live-births[8]

Although globally, fewer children under-five (12.7 million) have died since 1990, the 5.9 million children under-five reported dead in 2015, equivalent to one child dying every 11 minutes, remains unacceptable.[8,9,10] Identifying the factors responsible for causing such a high mortality rate would therefore be paramount for achieving the MDGs of reducing the 1990 under-five mortality rate by two thirds by the year 2015.[8,9,10]

Although the successes of the MDGs resulted in progress in areas of poverty, access to improved sources of water, primary school enrollment and child mortality, other larger-scale challenges remain on the agenda of the world's populations.[8,11] Some of these challenges are not related to the current systematic review (e.g. addressing concerns around gender equality and getting every child into school), but others are directly related to the incidence of infectious diseases, i.e. ending world hunger and improving health services. The approach that would best help with addressing these challenges has to be one that favors sustainability. As a result, the idea of the 2015 Sustainable Development Goals (SDGs) was born.[8,11]

The SDGs are described as new targets to replace the MDGs, with a target deadline for completion by the year 2030.[8,11] These SDGs were described as "a set of universally applicable goals that balance the three dimensions of sustainable development, i.e. environmental, social and economic". Specific to this review, the SDGs aim to end preventable deaths of newborns and children under-five years of age, reduce neonatal mortality to at least as low as 12 deaths-per-1000-live-births and under-five mortality to at least as low as 25 deaths-per-1000-live-births.[8,11]

To date and as reported by the World Health Organization (WHO) and the Maternal and Child Epidemiology Estimation Group (MCEE), the main causes of death in the under-five age group was found to be infectious diseases, prematurity and complications during labor and delivery.[8,9,10] Globally, 45 percent of under-five deaths were during the neonatal period. The remaining almost 50 percent of deaths in the under-five age group were attributable to the impact of infectious diseases.[8,9,10]

A recent 2015 report issued by UNICEF also identified infectious diseases as the main culprits for causing disease and therefore death, amongst the under-five population.[10] The top eight culprits in order of significance were identified as pneumonia, diarrhea/gastroenteritis (GE), sepsis, malaria, pertussis, measles, acquired immune deficiency virus (AIDS) and other causes. In addition, these infectious diseases were found to have a lesser impact in low-mortality risk regions versus high-mortality risk regions, i.e. infectious diseases were responsible for 39 percent, 54 percent and 47 percent of all under-five deaths in South Asia, West and Central Africa, and Eastern and Southern Africa respectively.[8,9,10] The leading cause of under-five deaths was attributed to pneumonia, with death due to GE being the second leading cause of death in this age group, contributing to 9 percent, 10 percent and 10 percent of all under-five deaths in South Asia, Eastern and Southern Africa and West and Central Africa respectively.[8,9,10] Despite being a symptom known to be both preventable and treatable, GE was still found to contribute between 5 to 10 percent of the total deaths in the under-five age group.[8,9,10]

Initiatives aimed at improving drinking water, access to safe water, sanitation and hygiene, and access to vaccines and oral rehydration solutions (ORSs) have contributed positively to curbing the effects of this symptom. However, GE still remains the second leading cause of death in the most vulnerable population.[8,9,10]

1.2 GASTROENTERITIS AND ITS MANY FORMS

GE is not a disease/condition but rather a symptom of a disease/condition, resulting in it being difficult to formulate a description without being subjective. As mentioned earlier, 2015 data released by UNICEF revealed that following pneumonia, GE was the second major cause of death, particularly in high-risk mortality regions and in the most vulnerable of groups.[9] It was therefore imperative that a clear yet concise definition of GE be identified and universally accepted. The WHO defined diarrhea/GE as "the passage of three or more loose or liquid stools per day, or more frequent passage than is normal for the individual".[4] It is important to mention that the consistency of stools and not so much the number, is also important in diagnosing GE.[4,6,7,12,13]

1.2.1 Causes of Gastroenteritis

There are numerous causative agents and accompanying mechanisms via which GE can be caused.[4,12,13] According to the WHO[4], there are four notable causes of GE which are briefly described below.

1.2.1.1 Gastrointestinal infections

Gastrointestinal infections may be caused by one of three organisms, i.e. bacterial, viral or parasitic.[12,13] Ideally, identifying the likely cause of the infection on the basis of history and clinical findings is encouraged. However, with more than twenty causative agents being associated with the development of GE (see Table 1.1 below), the cause of the GE is more than likely to be treated without the causative agent being identified.[12]

Table 1.1: Microbial agents commonly responsible for causing Gastroenteritis[12]

Bacteria	Diarrheagenic *Escherichia coli, Campylobacter jejuni,* Vibrio cholera O1, V. cholera O139, Shigella species, *V. parahaemolyticus*, *Bacteroides fragilis*, *C. coli, C. upsaliensis*, Nontyphoidal Salmonellae, *Clostridium difficile, Yersinia enterocolitica*, *Y. pseudotuberculosis*
Viruses	Rotavirus, Norovirus (calicivirus), Adenovirus (serotype 40/41), Astrovirus, Cytomegalovirus
Parasites	**Protozoans:** *Cryptosporidium parvum, Giardia intestinalis, Microsporidia, Entamoeba histolytica, Isospora belli, Cyclospora cayetanensis, Dientamoeba fragilis, Blastocystis hominis* **Helminths:** *Strongyloides stercoralis, Angiostrongylus costaricensis, Schistosoma mansoni, S. japonicum*

Specific to developing regions, both Rotavirus and *Escherichia coli* were found to be the two most common causative agents adding to the rate of occurrence of GE.[4,9] However, the WHO reported that owing to the ease in which it can be transmitted (i.e. person-to-person contact and airborne droplet transmission), Rotavirus was found to be disproportionately implicated in severe cases that frequently needed hospitalization. As illustrated in Figure 1.2 below, GE due to Rotavirus was found to be responsible for 215,000 (197,000 – 233,000) child deaths during 2013.[4]

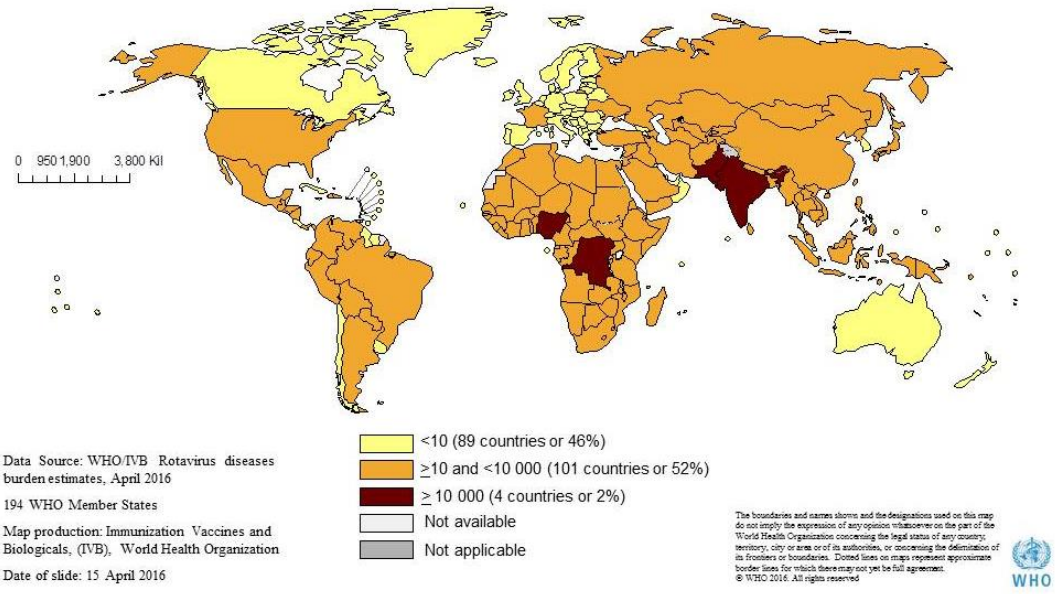

Figure 1.2: 215,000 global child Rotavirus deaths[4]

1.2.1.2 Malnutrition and contributing factors

As defined by both the WHO and UNICEF, malnutrition refers to both under-nutrition and over-nutrition.[4,9,10] However, in the context of this review, it is a term used to refer to a state of nutrient deficiency. The factors that result in malnutrition are predominantly related to the family and situation into which a child is born. By way of example, violence, political instability and volatile economic conditions like that currently being experienced in some parts of the world, has resulted in that specific regions health systems being crippled and therefore unable to offer medical support to the population.[4,9,10] Combined with poor diet, decreased accessibility/availability of food and an increased vulnerability to diseases/infections, the population's risk of malnutrition steadily worsens. In such a scenario, the most at-risk population is the under-five age group.[4,9,10]

According to the WHO and UNICEF data released in 2015, children from the poorest households are nearly two times as likely to die before the age of five as compared to their counterparts in richer households.[4,9,10] Data released by UNICEF describes the vast differences that exist in mortality rates in the under-five age group based on income levels.[8-10] Strong emphasis is being placed by UNICEF and WHO that despite the progress shown by most countries in achieving the MDGs, there remains a huge disparity amongst the low-mortality and high-mortality risk regions.[4,8-10] These organizations report that a child born in a low-income country is, on average, 11 times more likely to die before the age of five as a child in a high-income country.[8,9,10] Malnutrition and its contributing factors were associated with 54 percent of child deaths (10.8 million children) and therefore remains a huge morbidity and mortality risk for the world's youngest population.[4,8-10]

1.2.1.3 Infant feeding practices

Whether it is in a resource-rich or resource-restricted environment, breast-milk has the greatest impact on child mortality.[8,9,13-16] Breast-milk is known to consist of essential and irreplaceable nutrition to support a child's normal growth and development.[9,15,16] Mechanisms that have been proposed as responsible for the positive protective effect of breastfeeding against GE and other infections include its antimicrobial or immunological properties – it contains hormones, anti-inflammatory factors, digestive enzymes and growth modulators which all help with protecting against infections.[13-16] In addition, it can also protect an infant from the development of obesity and other non-communicable diseases.[8,9,13-16]

A 2012 report released by UNICEF indicated that worldwide, developing countries only achieved a 37 percent exclusive breastfeeding rate amongst infants less than six months of age.[8,9] Figure 1.3 below illustrates this and that fewer than half of newborns in developing countries benefitted from early initiation of breastfeeding (i.e. within the first hour of life).[8,9]

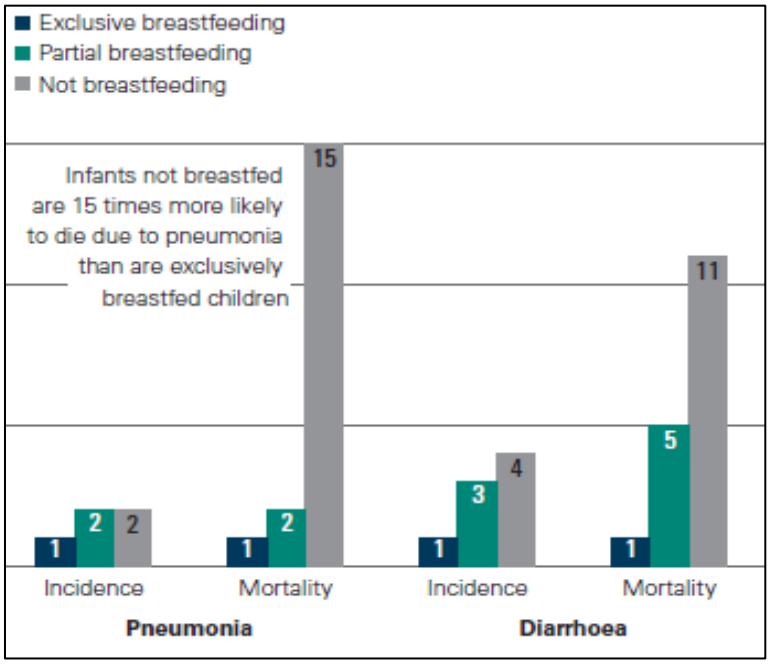

Figure 1.3: Relative risk of pneumonia and diarrhea incidence and mortality for partial breastfeeding and not breastfeeding compared with that from exclusive breastfeeding among infants' ages 0-5 months[8,9]

Even though it has been clearly documented that exclusive breastfeeding for the first six months of life offers maximum benefit, too few infants in developing countries are being exclusively breastfed for this period.[8,9,13-16] In September 2015, UNICEF released a publication highlighting key findings with regard to the MDGs and again found that only two out of five infants were put to the breast within an hour of birth, and only two out of five infants worldwide were exclusively breastfed for six months.[8,9] Sub-Saharan Africa was only able to record a 34 percent exclusively-breastfeeding rate during the period 2006 to 2012 (see Figure 1.4 below).[8,9]

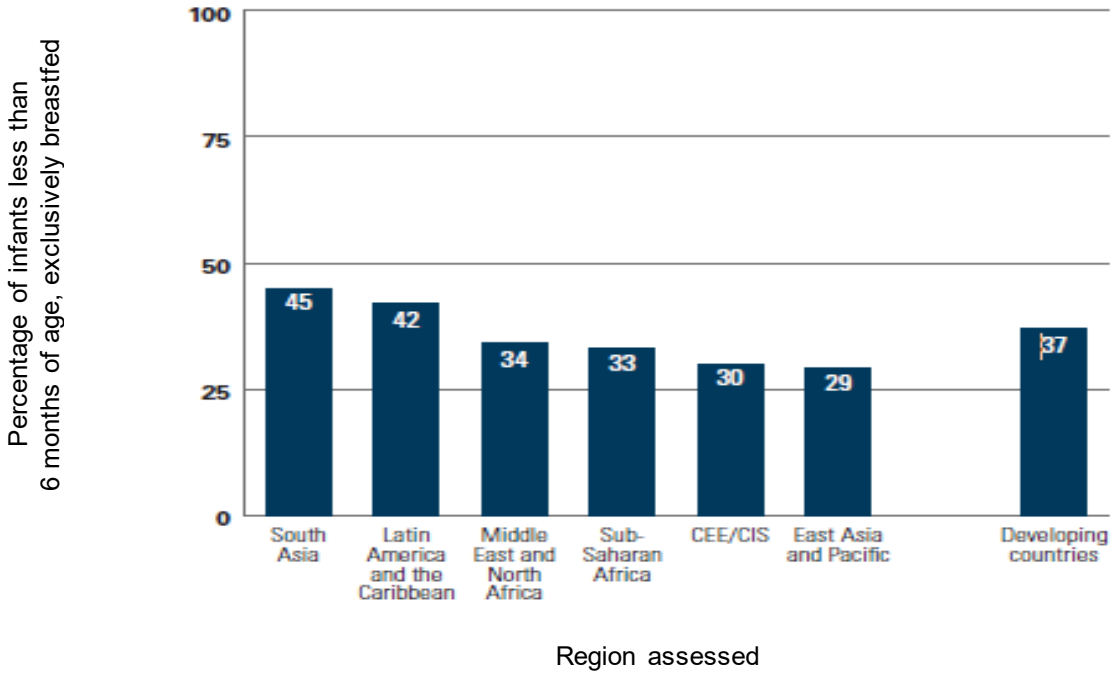

Figure 1.4: Share of infants under six months of age who were exclusively breastfed, by region, 2006-2010[8,9]

1.2.1.4 Compromised access to clean water and amenities

One other MDG was to halve the proportion of the world's population who did not have sustainable access to safe drinking water over the period 1990 to 2015.[4,8-10] Although the 2015 WHO World Statistics Report revealed that an improved proportion of the global population has been afforded access to improved drinking-water sources, a MDG met globally in 2010, there were still 748 million people without improved access to clean sources of drinking water. In addition, 14 percent of the world populations have no access to toilets, latrines or any form of sanitation, resulting in open defecation being practiced.[4,8-10]

This is of particular importance as high levels of environmental contamination and pollution will result in increased exposure to numerous diseases and infections, which would inevitably result in the symptom of GE. Populations residing in low-income regions, which are already vulnerable to disease and infection, were found to also be the population without an improved sanitation facility.[4,8-10]

1.2.1.5 Other factors

Factors like person-to-person transmission, food contamination during preparation and storage under unhygienic conditions, contaminated water sources and contaminated fresh foods have been identified as possible causes of GE, although with a much smaller contributory role.[4,8-10]

1.2.2 Categories of Gastroenteritis

According to the WHO[4,10], GE can be broken down into four different clinical types based on duration and/or other distinguishing features, i.e.

(1) acute GE (AGE) is GE that lasts for several hours or days with the main danger being dehydration;
(2) acute bloody GE or dysentery which can result in damage to the intestinal mucosa, with accompanying sepsis, dehydration and malnutrition;
(3) persistent GE (PGE) which is GE that lasts for 14 days or longer, with the main danger being malnutrition, dehydration and serious non-intestinal infection; and
(4) GE with severe malnutrition with the main danger being severe systemic infection, dehydration, heart failure and vitamin/mineral deficiencies.[4,10]

It is important to note that with each of these four clinical types of GE, dehydration is acknowledged as a common symptom. The WHO defines dehydration as "a condition that results from excessive loss of body water".[4,9] This loss of body water is also accompanied by the loss of electrolytes like sodium, potassium, chloride and bicarbonate, a combination of which can be life-threatening if not corrected, especially in the vulnerable younger populations.

Two of the earliest signs of an individual being dehydrated are the passage of dark-colored urine and ongoing thirst, signifying the body's attempts to bring about hemodynamic stability by increasing water intake and decreasing water losses. However, the longer the GE persists and the longer it is left untreated, the more significant the water and electrolyte losses and the more significant (and dangerous) the degree of dehydration.[9] This is especially true for the younger population.

1.2.3 Prevention of Gastroenteritis

The common goal of all healthcare groups, be it a community primary healthcare center or a global body like the WHO or UNICEF, would be to put in place measures to prevent a disease from occurring, as opposed to dealing with its management and associated complications.[4] In addition to the enormous country saving-of-funds that could possibly be used towards other initiatives, prevention of a disease/condition would more importantly, save the patient and his/her family from the individual, health-associated and financial challenges that accompany a sick episode.[9,10]

1.2.3.1 Increasing the coverage of Rotavirus vaccines

In support of the impact of GE caused by the Rotavirus, 2012 UNICEF report indicated that Rotavirus remained the leading cause of severe under-five childhood GE.[4,8-10] It was found to be responsible for an estimated 40 percent of all hospital admissions due to GE and caused between 420,000 and 494,000 child deaths in 2008, predominantly in low-income regions, where the vaccine was mostly unavailable (see Figure 1.5 below).[9]

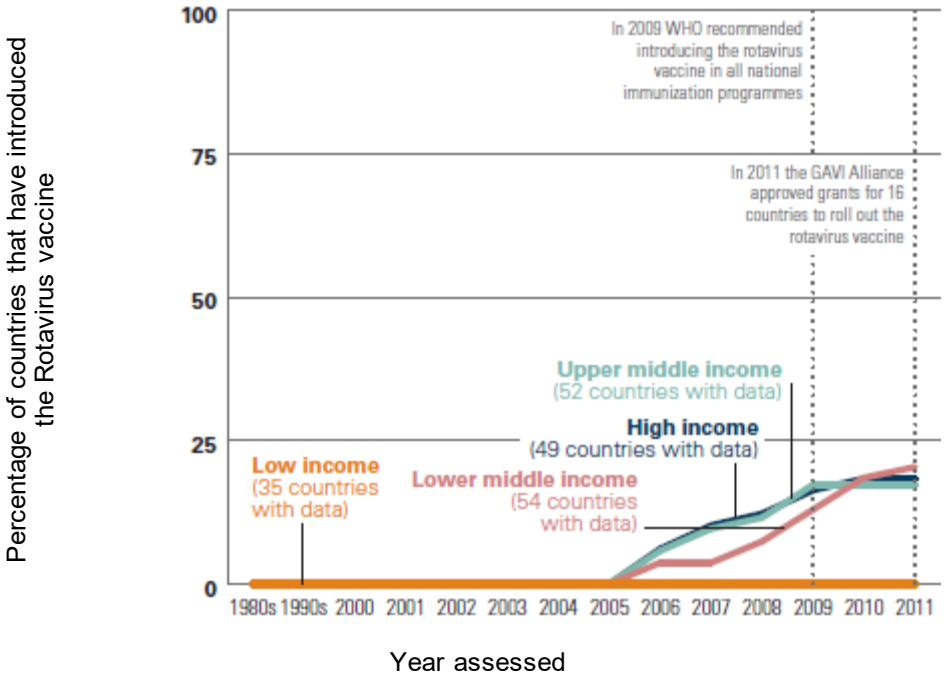

Figure 1.5: Share of countries that have introduced the Rotavirus vaccine into the entire country, by income group (per cent)[9]

It is clear that the Rotavirus vaccine is effective in reducing under-five mortality by effectively reducing the risk of contracting the virus and therefore associated GE.[9,10] However, concerted efforts need to be made so that the Rotavirus vaccine is made accessible to those groups most vulnerable (i.e. low-income countries). In addition to the malnutrition associated with food insecurity that is experienced by children in low-income households, these children also have increased mortality-risk due to infections like pneumonia and GE.[9,10]

1.2.3.2 Encouraging early and exclusive breastfeeding

It is known that early and exclusive breastfeeding imparts numerous benefits to the infant and mother.[13-18] A study that is particularly relevant to this systematic review is that conducted by Plenge-Bönig *et al* in 2010.[17] These authors aimed to investigate the effect, if at all protective, of breastfeeding against AGE caused by the Rotavirus infection. The study took the form of a case-control design and extrapolated data of children ranging between 0 and 12 months from 30 pediatric practices across Europe, namely Germany, Switzerland and Austria. The case-control design was achieved by using Rotavirus-positive cases and Rotavirus-negative controls. This resulted in the collection of 1256 stool samples, with 25 percent Rotavirus-positive and 75 percent Rotavirus-negative. These authors reported that being breastfed resulted in a reduced risk of AGE due to being Rotavirus-positive (OR 0.53; 95% CI: 0.37 to 0.76). Furthermore, younger infants (0 to 6 months of age) showed a stronger protective benefit (OR 0.33; 95% CI: 0.19 to 0.55) than the older group of infants (7 to 12 months of age).[17] One might argue that even though the study by Plenge-Bönig *et al* (2010) produced results in support of breastfeeding for decreasing the risk of AGE caused by the Rotavirus, the study was conducted in a "privileged"

environment and used a "low-risk" sample population (i.e. infants residing in a first-world country, with access to a relatively acceptable standard of healthcare).[17] As a result, can these findings be applied to populations who are deemed "higher-risk"?

In contrast to the study above, Lamberti *et al* (2011) investigated the benefits of breastfeeding infants to decrease their risk of GE, but in countries considered developing nations.[18] These reviewers conducted a systematic review to evaluate the association between the incidence of GE mortality and exclusive breastfeeding among children aged 6 to 23 months. A large body of literature was found in support of the protective effects of breastfeeding against GE incidence, prevalence, hospitalizations, mortality and all-cause morbidity. A random-effects meta-analyses was applied to data from eighteen studies indicating varying degrees of protection across levels of breastfeeding exposure, with exclusive breastfeeding (from birth to 5 months) and breastfeeding (from 6 to 23 months) offering the most benefit. Not breastfeeding resulted in a 10.5 times higher risk of dying in the 0 to 6 months age group, as compared to those exclusively breastfed. Similarly, a statistically significant finding was found for breastfeeding protecting against GE in the 6 to 23 month age group who were breastfed versus those not (RR 2.18).[18]

1.2.3.3 Promoting safe and hygienic practices and improving access to clean water

The goal of hygiene promotion is to empower people with knowledge so that they understand the role hygienic practices play in disease- and infection-prevention. According to a combined report by three global healthcare bodies, an estimated 663 million people across the globe, do not have access to an improved drinking-water source.[10,19] In addition, about 1.9 billion people still retrieve their water supplies from sources contaminated by feces.

By improving the access people have to water that is both safe for consumption and for supporting sanitation and hygiene, the mortality rate of the world's most vulnerable populations can be drastically reduced. Programs like WHOs "WASH" aims to achieve just this, i.e. improve access to safe drinking-water, sanitation and hygiene services to these populations.[19]

1.2.3.4 Promoting community-wide sanitation

Simple but effective interventions that resulted in a decrease in GE incidence included: promoting safe disposal of human waste, hand-washing with soap, increasing access to safe water, improving water quality, advancing household water treatment and safe storage.[10,19]

1.2.4 Treatment of Acute Gastroenteritis

In addition to the provision of vaccinations (e.g. Rotavirus), the aim of treatment of AGE should include (a) preventing and reversing dehydration, (b) shortening the duration of the illness; and (c) reducing the period for which the patient is infectious.[9,12,19]

1.2.4.1 Prevent and reverse dehydration

Dehydration not identified early and not treated timeously is known to have devastating consequences, especially in the most vulnerable younger age groups.[9,12,19] Accurately assessing dehydration status is critical to determining the most appropriate treatment course. However, no clinical diagnostic model/s for dehydration have been empirically derived and validated for use in resource-limited settings. Global health bodies like the WHO and the World Gastroenterology Organization (WGO) have designed algorithms to aid with identifying and treating dehydration (see Table 1.2 below).[12] However, the algorithm shown below was based largely on WHO expert opinion.

Table 1.2: Assessment of degree of dehydration in patients with Gastroenteritis[12]

	A	B	C
Condition[a]	Well, alert	Restless, irritable	Lethargic / unconscious
Eyes[b]	Normal	Sunken	Sunken
Thirst	Normal, not thirsty	Thirsty, drinks eagerly	Poorly / not able to drink
Skin pinch[c]	Goes back quickly	Goes back slowly	Goes back very slowly
Decide:	**No signs of dehydration.**	≥2 signs in B means **some dehydration**.	≥2 signs in C means **severe dehydration**.
Treat:	Use treatment Plan A.	Weigh the patient; Use Treatment Plan B.	Weigh the patient; Use Treatment Plan C **URGENTLY**.

[a] *Lethargy and sleepy are not synonymous. With lethargy, the child's mental state is dull and the child cannot be fully awakened; the child may appear to be drifting into unconsciousness.*

[b] *In some infants and children the eyes normally appear somewhat sunken. It is helpful to ask the mother if the child's eyes are normal or more sunken than usual.*

[c] *The skin pinch is less useful in infants or children with marasmus or kwashiorkor, or obese children. Other signs that may be altered in children with severe malnutrition may include cool and moist extremities, weak/absent radial pulse and reduced/absent urine flow).*

Up until recently, there has been no study to derive stable clinical diagnostic models for dehydration in children with GE. In 2015, a group of researchers conducted a prospective cohort study in Dhaka, to validate a dehydration scoring and decision tree model for children with GE.[20] The study was referred to as the "Dehydration: Assessing Kids Accurately (DHAKA)" prospective cohort study, and randomly sampled children under-five with AGE. A total 1025 children were eligible for inclusion, of which 850 were enrolled and 771 were included in the final analysis. For each child, trained nursing staff assessed children for clinical signs of dehydration on the initial assessment, followed by serial weights as the children were rehydrated.[20]

The authors determined the percent weight change with rehydration and used this to classify children with severe dehydration (>9 percent weight change), some dehydration (3 to 9 percent), or no dehydration (<3 percent). Using these clinical variables and logistic regression models, these authors developed the DHAKA dehydration tree model (see Figure 1.6 below) and the Dehydration Score (see Table 1.3 below).[20]

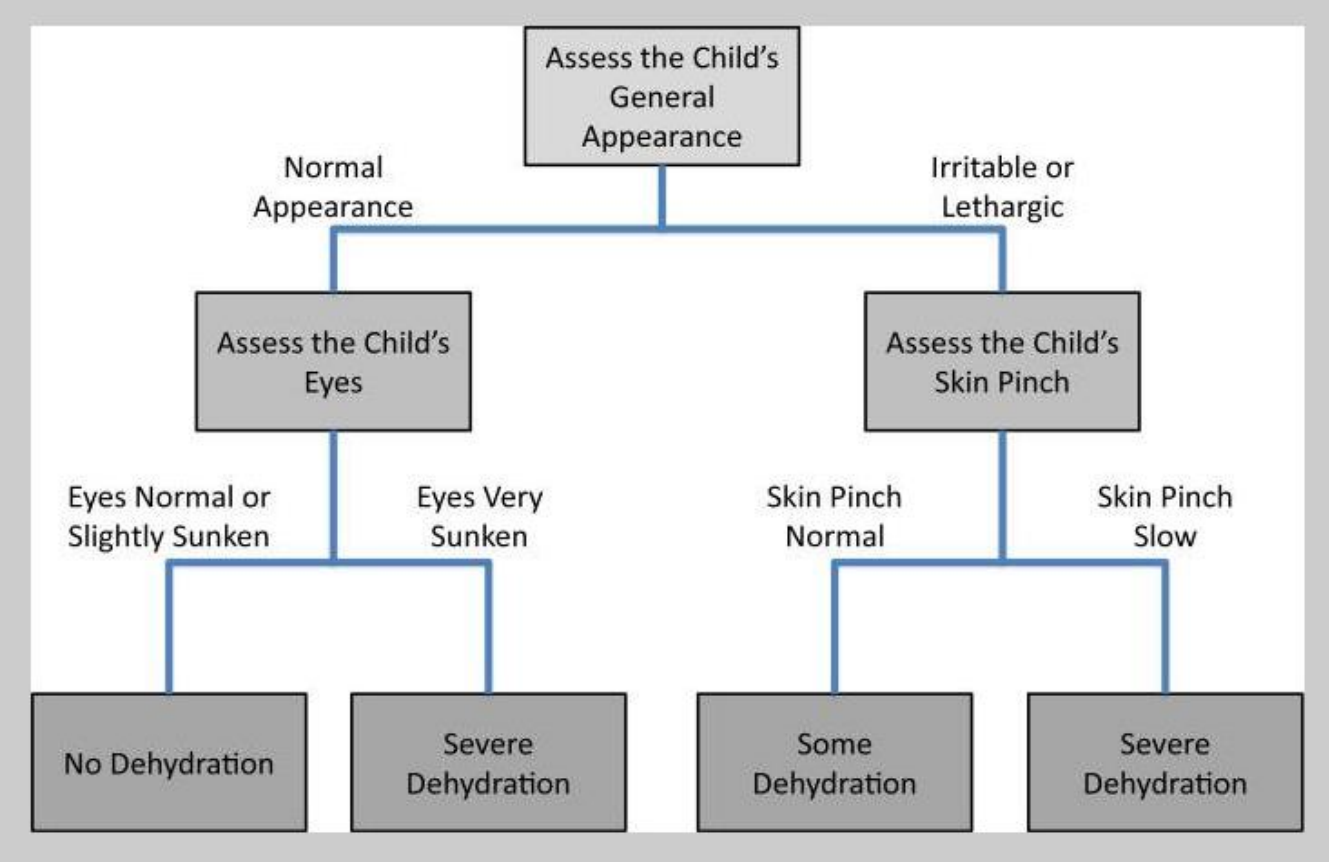

Figure 1.6: DHAKA dehydration tree model[20]

Table 1.3: DHAKA dehydration score[20]

Assessment	Plan A[a]	Plan B[b]	Plan C[c]
General condition	Normal	Irritable/less active (*)	Lethargic / comatose (*)
Eyes	Normal	Sunken	-
Mucosa	Normal	Dry	-
Thirst	Normal	Thirsty	Unable to drink (*)
Radial pulse	Normal	Low Volume (*)	Absent/uncountable (*)
Skin turgor	Normal	Reduced (*)	-
Diagnosis	No dehydration	Some dehydration	Severe dehydration
		At least *two* signs, including at least one key sign (*) are present	Signs of 'some dehydration' plus at least *one* key sign (*) are present
Treatment	Prevent dehydration	Rehydrate with ORS solution unless patient is unable to drink	Rehydrate with IV fluids and ORS
	Reassess periodically	Frequent reassessment	More frequent reassessment

One of three treatment plans may be followed based on the degree of dehydration into which the child is classified, i.e.

[a] *Plan A – no dehydration; outpatient management; continue breastfeeding; normal diet-for-age and encourage intake of fluids.*

[b] *Plan B – some dehydration; observation in a healthcare facility for a minimum of 4 hours, rehydrate using oral rehydration solution (ORS) with the aim of providing 75ml/kg or the facilities rehydration protocol; observe changes in dehydration every 4 hours and repeat ORS administration until patient shows signs of being rehydrated.*

[c] *Plan C – severe dehydration; inpatient status; resuscitation with intravenous fluid.*

There are varied forms of rehydration protocols available, which are often determined by the resources available to the facility and the location thereof. However, these protocols share common characteristics of taking into account the patients age, weight and degree of dehydration. Table 1.4 below is an example of a rehydration protocol using ORS in the hospitalized patient, which is advocated for use by the WHO and WGO.[12]

Table 1.4: Treating dehydration with Oral Rehydration Solution in hospitalized patients[12]

Amount of Oral Rehydration Solution to be offered in the first 4 hours						
Age	≤4 Months	4-11 months	12-23 months	2-4 Years	5-14 Years	≥15 Years
Weight (kg)	<5.0	5.0-7.9	8.0-10.9	11.0-15.9	16.0-29.9	≥30.0
ORS to offer (ml)	200-400	400-600	600-800	800-1200	1200-2200	2200-4000

ORS or oral rehydration therapy (ORT) is described as "the administration of appropriate solutions by mouth to prevent or correct diarrheal dehydration".[4,12] It consists of water and oral rehydration salts, which are supplied in specific amounts to compensate/partially compensate for losses in GE stools. A typical ORS will consist of the following: sodium (75mmol/L), chloride (65mmol/L), anhydrous glucose (75mmol/L), potassium (20mmol/L), and trisodium citrate (10mmol/L) with an overall low osmolarity of 245mmol/L. It can further be used as maintenance fluid therapy to compensate for ongoing losses once rehydration has been achieved.[4,12]

Despite being a cost-effective method of managing AGE and being able to reduce the burden on healthcare systems in both developed and developing countries, UNICEF reports that just over 40 percent of children under-five with GE received the recommended treatment of ORS.[8-10] As is shown in Figure 1.7 below, South Asia and sub-Saharan Africa were found to be the regions with the lowest coverage of the ORS package, and interestingly also the regions with the most GE-related deaths.[8-10]

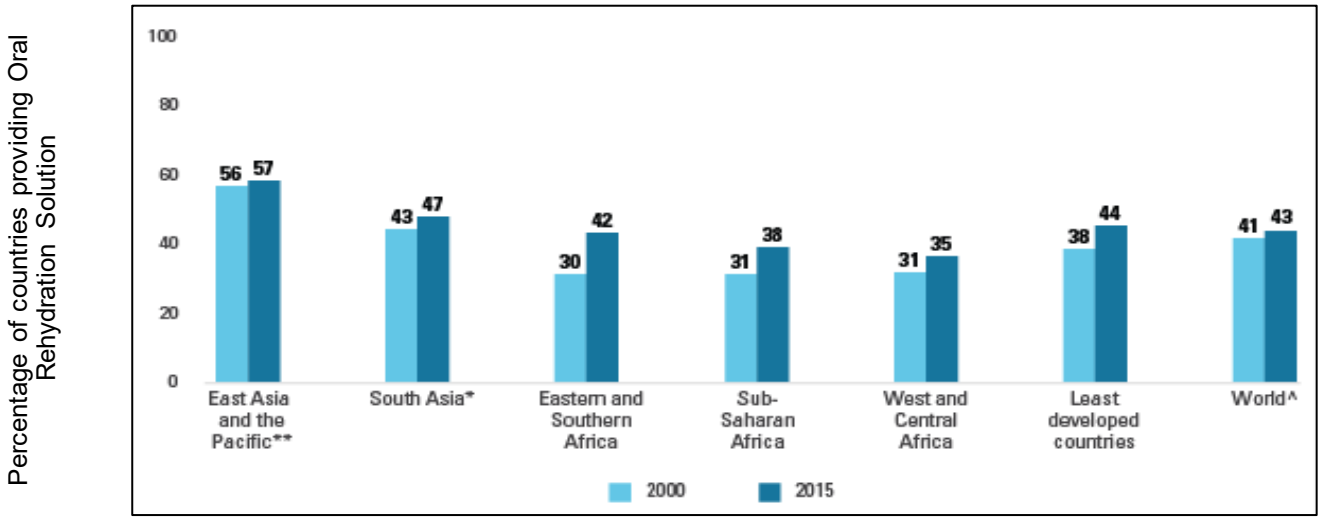

Figure 1.7: Coverage of the recommended Oral Rehydration Solution treatment package is low across regions, particularly in the ones with the highest mortality[8-10]

1.2.4.2 Zinc supplementation

Zinc is a micronutrient that is important for protein synthesis, cell growth and differentiation, immune function and the intestinal transport of water and electrolytes.[9,12,21] Over time, the use of zinc supplementation has become synonymous with the management of AGE as it has been found to reduce the duration and severity of GE episodes and subsequent infections for up to three months.[9,12,21]

In 2004, Fischer Walker & Black conducted a systematic review to estimate (in addition to other infections), the effect of zinc for the treatment of GE on GE mortality.[21] A total of 13 studies were identified for abstraction and a meta-analysis was performed for all outcomes with ≥2 data points. Zinc supplementation was found to decrease a number of parameters, i.e. the proportion of GE episodes which lasted more than 7 days, risk of hospitalization, all-cause mortality and GE mortality. Zinc for the treatment of GE was estimated to decrease GE mortality by 23 percent. The authors concluded that zinc was an effective treatment for GE and will decrease GE morbidity and mortality when used in low-income countries.[21]

Conflicting results were provided by a Cochrane review conducted by Lazzerini & Ronfani.[22] These reviewers conducted a review of 24 randomized controlled trials (RCTs) that compared oral zinc supplementation with placebo in children aged 1 month to 5 years with AGE or PGE, including dysentery. Notably, the majority of the trials were conducted in Asia and in countries known to be at high-risk of a zinc deficiency. Unlike previous reports, these authors reported that there was not enough evidence from well conducted RCTs to confirm that zinc supplementation during AGE reduced death or hospitalization.[22]

In that same year, Lamberti *et al* (2013) conducted a systematic review and meta-analysis on the effect of oral zinc supplementation on AGE in children.[23] Previous reviews confirmed the valuable role zinc supplementation played in treating and managing AGE, but these authors noted that papers used in these reviews were all derived from South Asia. This review included 104 papers, of which 15 sources were non-Chinese and 89 were Chinese. The findings of this review confirmed the benefits of therapeutic zinc supplementation for GE among under-five children, in low- and middle-income countries, i.e. it reduced the duration of GE, stool output, stool frequency and length of hospital stay. These findings were found to be consistent across Chinese and non-Chinese studies as well as for non-specific and Rotavirus GE.[23]

Recommendations from these reviews as well as others have shown that zinc supplementation offered as (10 to 20mg per day) until GE has stopped, significantly reduced the severity and duration of GE in children under-five.[9,12,23] Furthermore, shorter courses of zinc supplementation (10 to 20mg per day for 10 to 14 days) decreased the incidence of GE for 2 to 3 months. Both the WHO and UNICEF recommend zinc treatment for 10 to 14 days as an adjunct therapy that reduces the duration and severity of GE and decreases the likelihood of subsequent infections for up to 3 months following treatment.[4,9,12,23]

1.2.4.3 Ongoing breastfeeding and feeding

The continuation of breastfeeding is of particular importance as both the antimicrobial and antibacterial effects of breastfeeding against GE infections have been well documented.[15-16] Exclusive breastfeeding is known to protect against the common infections of infancy, decreases the frequency and severity of infectious episodes, and promotes the colonization of the intestinal ecosystem with beneficial bacteria (e.g. *Bifidobacteria* and *Lactobacilli*) rather than pathogenic bacteria.[15,16,24-26]

It is therefore clear that the importance of interventions to promote, protect and support improved breastfeeding practices cannot be stressed enough, especially in resource-restricted regions and during the treatment of children suffering the effects of GE.[9,16] Mothers should be educated by trained and skilled healthcare workers on the ongoing benefits of continued breastfeeding, especially during episodes of illness, like during GE.[9,16] The full potential of breastfeeding in reducing mortality associated with GE and therefore overall child mortality can only be realized if all countries accelerate efforts to reach as many infants as possible with effective programs to improve breastfeeding.[9,16] In addition, mothers and caregivers should be educated on the need to offer more frequent breastfeeds (or bottle feeds) to the recovering child. There is no evidence supporting the use of special or diluted formulas.[9,16]

During episodes of GE, it is common “unwarranted” practice to restrict the intake of other foods and fluids for more than 4 hours. Normal feeding should be encouraged in those children who display no signs of dehydration. In children who were initially identified as moderately to severely dehydrated, food should be started about 2 to 4 hours immediately after the dehydration has been corrected with ORS or intravenous rehydration.[9,12,16] In terms of food and meals, infants and young children should be given age-appropriate foods offered as small but frequent meals throughout the day. The energy intake should be advanced as the child is

able to tolerate an increased energy intake. The only caution would be to limit the intake of solutions/fluids that have a high osmolar rate (e.g. canned fruit juices) which can aggravate GE.[9,12,16]

1.3 THE LINK BETWEEN GASTROENTERITIS AND PROBIOTICS

The bacteria that are found in the gastrointestinal tract are a complex ecosystem and able to coexist with the host, as long as a state of balance (equilibrium) is maintained.[6,7,14,15] However, during disruptions in this balanced state, clinical disorders and disease can result. Gastrointestinal disorders, one of which being all forms of GE, can result in an imbalance. One of the goals would then be to restore balance and one of the ways in which this could be done is by restoring the balance in the gastrointestinal bacteria's ecosystem.[6,7,14,15]

Probiotics have been identified as a possible treatment modality to restore beneficial gastrointestinal bacteria to its original balanced state.[14,24-26] The definition of probiotics has evolved, mostly because probiotic bacteria can influence the physiological outcomes, distant from the gut. Probiotics have therefore been defined as any "viable organism that (when ingested) have a beneficial effect in the prevention and treatment of specific pathological conditions".[14,24-26] In order for a probiotic to be viable and biologically active, it must be able to withstand the host's natural defense barriers and arrive at the target site in an active form.[7,24-26] These microorganisms have been shown to act against enteric pathogens by competing for available nutrients and binding sites, increasing the acidity of gut contents, showing tolerance to high concentrations of ethanol and for releasing antimicrobial compounds.[7,24-26]

The ability of an organism to be effective is strain-specific and therefore it is important for these microorganisms to be defined by their genus, species and strain.[7,24-26] Research has shown that the human gastrointestinal tract contains a heterogenous mix of 10^{14} bacteria, of which <0.1 percent is yeast.[7,24-26] The most referenced of all the human gut bacterium are the strains *Lactobacillus acidophilus* and *Lactobacillus rhamnosus-GG*.[7,24-26]

Although yeast might account for only a minority of organisms making up the gut bacteria, each of their cell sizes is 10 times larger than that of bacteria, making yeast a stearic hindrance to pathogens.[7,24] Yeast can also be found in the stomach and colon, signifying their ability to thrive and survive in pH-varied mediums. Owing to its ability to resist stresses like gastrointestinal enzymes, bile salts, varying pH, varying temperatures and organic acids, yeast has demonstrated the ability to play the same role as a probiotic.[7,24]

1.3.1 The yeast probiotic *Saccharomyces boulardii*

One of the most studied yeasts is the genus *Saccharomyces*, with about 20 different species and with applications in commercial settings involving bread making and alcoholic and dairy fermentation.[15,27,28] However, it has also displayed a role in biological control with single-cell protein and vitamin production, synthesis of recombinant proteins and important antagonistic activities towards pathogenic bacteria and fungi.[27,28]

Saccharomyces cerevisiae variety *boulardii*, more commonly referred to as *Saccharomyces boulardii*, is a non-pathogenic yeast that is suitable for human consumption and has also been considered for use in the treatment of inflammatory bowel disorders and several types of GE (e.g. antibiotic-associated diarrhea (AAGE), *Clostridium-difficile*-associated diarrhea, traveler's diarrhea, tube-feeding diarrhea, chronic diarrhea in immuno-compromised individuals and AGE) in adults and children.[15,27,28]

1.3.2 Understanding the action of *Saccharomyces boulardii*

According to Kelesidis & Pothoulakis[6] (2012), there is evidence that resident gastrointestinal bacteria act as a major regulator of the immune system (i.e. the gut and other organs). *Saccharomyces boulardii* has shown clinical and experimental effectiveness in inflammatory gastrointestinal diseases, indicating that these beneficial bacteria might interfere with signaling pathways.[6,27-32] *Saccharomyces boulardii's* mechanism of action has been noted to be three-fold, i.e. luminal, trophic on intestinal mucosa and regulation of the immune response.[6,27-32]

1.3.2.1 Antimicrobial activity

This yeast probiotic displays antimicrobial activity by inhibiting bacterial and parasitic growth, reducing gut translocation of pathogens, neutralizing bacterial virulence factors, and interfering with bacterial colonization by suppressing adherence to the host cell.[6,27-32]

Additionally, *Saccharomyces boulardii* shows antitoxin effects by inhibiting toxin-receptor binding sites, stimulating antibody production against *Clostridium-difficile* toxin A, and allowing for direct proteolysis of pathogenic toxins.[6,27-32] *Saccharomyces boulardii* is also able to exert a symbiotic relationship with resident microorganisms, thereby enhancing their survival and growth. This allows for normal microbiota status to be reestablished more rapidly.[6,27-32]

1.3.2.2 Trophic action

The trophic action of *Saccharomyces boulardii* is numerous, i.e. it reduces the number of infected cells and stimulates the growth and differentiation of intestinal cells in response to trophic factors; it prevents apoptosis and/or synthesis of tumor necrosis factor-α; it reduces mucositis; it restores fluid transport pathways; it stimulates protein and energy production and restores metabolic activities in colonic epithelial cells.[6,27-32]

In addition, this yeast probiotic has also shown the ability to secrete mitogenic factors that enhance cell restitution; it enhances the release of brush-border membrane enzymes; it stimulates the production of glycoproteins in the brush border; it stimulates the production of intestinal polyamines; and it is able to restore normal levels of colonic short chain fatty acids (SCFAs) .[6,27-32]

Saccharomyces boulardii has also displayed an ability to help stabilize gastrointestinal barrier function by strengthening enterocyte tight junctions; by reducing crypt hyperplasia and cell damage in colitis models; and by decreasing intestinal permeability in Crohn's disease patients.[6,27-32]

1.3.2.3 Effect on host's immunity

Innate immunity refers to a nonspecific defense mechanism that comes into play immediately or within a short space of time of an antigen appearing in the body.[6,15,31] It can refer to physical barriers such as the skin, chemicals in the blood, as well as the cells making up the immune system and them being triggered once a foreign cell appears in the body. *Saccharomyces boulardii* has displayed an ability to offer this innate immunity to the host by triggering the activation of complement. This results in the migration of immune system cells like monocytes and granulocytes to the site of activation. In addition, *Saccharomyces boulardii* used in an animal model, was able to enhance the number of Küpffer cells.[6,15,31]

Adaptive immunity refers to a subsystem of the overall immune system that is composed of highly specialized, systemic cells and processes that eliminate or prevent pathogen growth.[6,15,30-32] *Saccharomyces boulardii* was shown to complement the host's adaptive immune system by enhancing the mucosal immune response and secretory immunoglobulin-A intestinal levels; enhancing the systemic immune response and levels of serum immunoglobulin-G to *Clostridium difficile* toxins A and B; helping with early production of interferon gamma, interleukin-12; stimulating regulatory T-cells; inhibiting dendritic cell-induced activation of T cells; and helping to modify the migration of lymphocytes in the chronic inflammatory bowel disease model and lymphocyte adherence to endothelial cells, improving cell rolling and adhesion.[6,15,30-32]

This yeast probiotic was also studied for its ability to reduce pro-inflammatory responses and its ability to promote mucosal anti-inflammatory signaling effects.[6,15,30-32] It was shown to decrease the expression of pro-inflammatory cytokines like interleukin-8, interleukin-6, interleukin-1β, tumor necrosis factor-α and interferon-gamma; it increased the anti-inflammatory cytokines like interleukin-10; and interfered with nuclear factor kappa-mediated signal transduction pathways, in immune and colonic epithelial cells.[6,15,31-32]

In addition, *Saccharomyces boulardii* was able to block the activation of extracellular signal-regulated kinases and mitogen-activated protein kinases; decreased nitric oxide and inhibited the production of inducible nitrous oxide; it modulated T-cell migratory behavior and increased the trapping of T-helper cells into mesenteric lymph nodes; and it also stimulated the production of anti-inflammatory molecules in human colonocytes.[6,15,30-32]

1.3.3 The properties of *Saccharomyces boulardii*

The site of action for *Saccharomyces boulardii* is most commonly the colon and the yeast probiotic has been shown to survive passage to its target organ.[6,15,30-32] Most of the Saccharomyces strains have been shown to work optimally at temperatures between 22^0C to 30^0C – *Saccharomyces boulardii* however, is able to survive temperatures of up to 37^0C, and therefore able to survive human body temperatures. *Saccharomyces boulardii* in a lyophilized form is able to survive gastric acid and bile.[6,15,30-32]

Stool sampling tests done have shown that levels of *Saccharomyces boulardii* can be 100 to 1000 times lower than the oral dose offered, indicating that much of the oral dose is destroyed. Despite this, researchers have

reported that although much of the oral dose consumed is destroyed, surviving doses have been found to be effective.[6,15,30-32]

It is naturally resistant to antibiotics and proteolysis and able to survive in the competitive milieu of the intestinal tract. In human subjects, the concentration in the colon was found to be dose-dependent. When *Saccharomyces boulardii* was given to healthy subjects at doses used therapeutically (1 to 2 x 10^{10}/d), colonic levels were found to be (2 x 10^{8}/gram) stool. Furthermore, when offered orally, *Saccharomyces boulardii* was able to achieve steady-state concentrations within three days and was only cleared within 3 to 5 days after it had been discontinued. It has also demonstrated an ability to coexist and thrive in the presence of other agents e.g. psyllium fiber increased *Saccharomyces boulardii* levels by 22 percent.[6,15,30-32]

1.3.4 Clinical efficacy of *Saccharomyces boulardii* in disease states

Research on the effects of *Saccharomyces boulardii* on chronic conditions such as Crohn's disease, ulcerative colitis, irritable bowel syndrome, parasitic infections, *Amebic colitis, Giardiasis*, *Blastocytosis hominis*, human immunodeficiency virus (HIV)-related diarrhea has been conducted but with no definite guidelines or recommendations.[14,27] It has also featured in the management of more acute conditions like AAGE, *Clostridium difficile* infection, AGE, PGE, enteral nutrition-related GE, traveler's GE and *Helicobacter pylori* infection. For the purpose of this systematic review, the use of *Saccharomyces boulardii* in the management of GE will be investigated.[14,27,28]

AAGE is defined as unexplained GE that occurs in association with the administration of antibiotics.[28-30] A 2010 meta-analysis of ten RCTs involving adults showed that *Saccharomyces boulardii* was significantly protective for AAGE with a pooled relative risk (RR) of 0.47 (95% Confidence Interval (CI): 0.35 - 0.63, $P < 0.001$).[29] An earlier meta-analysis involving five trials and 1076 participants showed similar results, with a significant protective effect being found in patients offered *Saccharomyces boulardii* (pooled RR 0.43; 95% CI: 0.23 - 0.78).[33]

When effects on the pediatric population were investigated, Kotowska *et al* (2005) found a significant increase in the prevention of AAGE in the *Saccharomyces boulardii* group compared to the controls, which ranged between 7.6 and 30.1 percent respectively.[34] *Saccharomyces boulardii* use in pediatric patients diagnosed with *Clostridium difficile* infection is rather limited. One small observational study in children indicated that *Saccharomyces boulardii* may be effective in *Clostridium difficile* infection.[27] But to date, there is not enough evidence to support routine use of such a probiotic in the prevention of *Clostridium difficile* infection.[35]

Other papers have reported of a significant increase in SCFA concentrations in ten enteral-fed patients receiving *Saccharomyces boulardii* compared to 15 healthy controls.[28-30] The relative significant reduction in enteral nutrition-related GE in the *Saccharomyces boulardii* group compared to the control ranged between 5 percent and 8.2 percent therefore warranting more studies to be done.[28-30]

Specific to the management of GE, Szajewska & Mrukowicz (2005) conducted a meta-analysis of five RCTs examining the effectiveness of *Saccharomyces boulardii* in preventing AAGE in a mixed adult and child population consisting of 1079 participants.[32] Despite the cause of the GE differing from that of the current systematic review, these reviewers reported that *Saccharomyces boulardii* offered a significant reduced risk of developing AAGE from 17.2 percent to 6.7 percent when compared to the placebo group, i.e. (RR 0.43; 95% CI: 0.23 to 0.78). Although most of the participants in the included trials were being managed with antibiotics to treat respiratory tract infections, the reviewers concluded that *Saccharomyces boulardii* was moderately effective in preventing AAGE in both the adult and pediatric population.[32]

More specific to the design of the current review is the study conducted by Szajewska, Skorka and Dylag (2007).[33] These reviewers investigated the effectiveness of *Saccharomyces boulardii* in treating AGE in children, although the cause of the AGE was not pre-defined. Five RCTs met the inclusion criteria stipulated by these reviewers. A total of 619 participants were pooled into this systematic review and showed that offering a dose of *Saccharomyces boulardii* between 250 to 750mg per day and over a period of five to six days resulted in statistically significant changes in the *Saccharomyces boulardii* versus the control/placebo group, i.e. the duration of GE in the *Saccharomyces boulardii* group was statistically reduced compared to the control/placebo group (weighted mean difference (WMD) -1,1; 95% CI: -1.3 to -0.83; 4 studies). In addition, Day 2 and Day 4 were significant time points at which the participants in the *Saccharomyces boulardii* group showed possible cure of AGE when compared to their counterparts. Participants receiving *Saccharomyces boulardii* were also less likely to experience GE on Day 3, Day 6 and Day 7 than the control group participants. Overall, the intervention group showed a statistically significant reduction in the risk of GE that lasted more than seven days versus the control/placebo group. These reviewers also reported that other outcomes like vomiting showed no statistical significance between groups, but the duration of hospitalization in the *Saccharomyces boulardii* was statistically lower compared to the control group.[33]

1.3.5 The safety of *Saccharomyces boulardii*

In 2005, a comprehensive review was conducted to establish the relationship between *Saccharomyces boulardii* and the development of a fungal infection called *Saccharomyces fungemia*.[36] These authors reported of ninety two case reports of this Saccharomyces invasive infection, with patients requiring intravascular catheters and antibiotic therapy being the most frequent. *Saccharomyces boulardii* was found to account for 51.3 percent of fungemias, all of which were isolated from blood samples. The affected patients were found to be more frequently immunocompetent and with better prognosis, but each with a good response to intravenous amphotericin B and fluconazole. The authors concluded that special caution should be taken when prescribing this yeast probiotic.[36]

Specific to the pediatric patient, a single-case report highlighted a rare gastrointestinal allergic reaction after *Saccharomyces boulardii* was given to an infant. However, this patient was already diagnosed with a food protein-induced enterocolitis.[14,23]

A 2010 systematic review reported that probiotic products like *Saccharomyces boulardii* have been shown to increase the risk of complications in "vulnerable" patients, like those that are immuno-compromised.[37] Despite these reports, there have been no RCTs confirming any adverse effects observed with the use of *Saccharomyces boulardii*.

Although there is growing research on the subject of probiotics in health, the multiple effects of confounders evident in these studies presents a challenge to researchers, making it difficult for them to make specific recommendations on its use in health and disease prevention. Aside from factors directly related to the type of probiotic used (e.g. strain type, single versus multi-strain, dosage offered, route of administration), the cause of the condition under investigation might also vary. In this case, GE can be as a result of either a bacterial, viral or parasitic cause.

In order to develop clear and concise guidelines on the use of *Saccharomyces boulardii* to treat/manage AGE, research with more rigorous methodology is required. According to research groups and organizations like The Cochrane Collaboration and The Centre for Evidence-based Medicine, the systematic review is considered evidence to be at the top of the research-hierarchy, i.e. level 1A evidence.[1,38] Owing to the use of the gold standard RCT which can be accompanied with the completion of a meta-analysis, the systematic review also promotes the use of an appraisal system which appraises the quality of evidence according to important factors like directness, precision, and consistency.[1,38]

1.4 DESCRIPTION OF A SYSTEMATIC REVIEW

1.4.1 Description of a systematic review

Systematic reviews are becoming increasingly popular as a way of summarizing research evidence.[1,2] These reviews aim to answer a pre-defined research question by reviewing the best available related research after combining the results of several studies. If done properly, systematic reviews are useful in establishing the clinical superiority, ethical appropriateness and cost effectiveness of an intervention. Systematic reviews have the additional benefit of being replicated, especially as it is peer-reviewed.[1,2] The characteristics of a high quality systematic review will identify all relevant published and unpublished evidence; select studies that adequately meet the pre-defined inclusion criteria; assess the quality of each included study; synthesize the findings from each individual study in an unbiased way; interpret the findings and present a balanced and impartial summary of the findings; and acknowledge any weaknesses in the evidence.

When it comes to level of hierarchy of research designs, the systematic review is considered to be at the highest level in evaluating the effectiveness of interventions. When possible, a meta-analysis may be conducted – however, while all meta-analyses are based on the systematic reviews of literature, not all systematic reviews necessarily include a meta-analysis.[1,2]

1.4.2 Steps in developing a systematic review

Firstly, the problem that the reviewer is aiming to address should be specified in the form of a research question (or objective), which should clearly define the population of interest, the intervention to be investigated, the control group to be used and the outcomes to be reviewed. In addition, the reviewer will also need to pre-define eligibility criteria consisting of both inclusion and exclusion criteria to be applied against studies for the review.[1,2]

The next step would be to explicitly describe the exact steps that were followed during every stage of the review, making the likelihood of repeating it very possible.[1,2] Every systematic review should aim to identify all studies that would meet the eligibility criteria. Ideally, both published and unpublished literature should be searched for suitable studies relating to the intervention being investigated. In order to maintain the integrity associated with conducting a systematic review, the literature search needs to be conducted in an unbiased manner so that all possible sources of literature are exhausted i.e. multiple databases are searched using a standardized or customized keyword search string.[1,2]

In any high quality systematic review, the study design that is to be considered as a prerequisite for inclusion makes it a marker of quality.[1,2] This is most applicable when working with randomized studies. Assessing the quality of studies to be included in the systematic review needs to be practiced at every step of the review process. This step is of particular importance as it needs to be conducted in a manner that minimizes any potential for bias, and is therefore carried out by a minimum of two reviewers, conducting assessments independently. The use of checklists for the design-based quality will need to be applied rigorously to all selected studies. Quality assessments will be used for exploring heterogeneity and informing decisions regarding suitability for a meta-analysis. Applying the inclusion and exclusion criteria to selected studies is part of the appraisal of evidence and helps to guide reviewers in their decision to either include of exclude a study from the review.[1,2]

Once this has been achieved, an assessment of the validity of the findings of the included studies should be done.[1,2] This is often achieved by conducting a risk-of-bias assessment for each study and should be conducted independently by each of the reviewers. The Cochrane Collaboration's risk-for-bias tool using generation sequencing, allocation concealment, blinding, incomplete outcome data and loss to follow-up are some of the domains assessed.[1,2]

In addition, the Grades of Recommendation, Assessment, Development and Evaluation (GRADE) tool features in grading the quality of evidence. [1,2,38] The GRADE approach defines the quality of evidence for each individual outcome reported in a systematic review as the extent to which one can be confident that an estimate of effect or association is close to the quantity of specific interest. Quality of a body of evidence involves consideration of within-study risk-of-bias (methodological quality), directness of evidence, heterogeneity, precision of effect estimates and risk of publication bias.[1,2,38]

The extraction of the relevant data from each individual study is important, and should be conducted independently by each of the reviewers.[1,2] Extracted details might include information on authors, publication year, study design, study location, source of funding, duration of study, inclusion criteria, exclusion criteria, causes of the subject under investigation, number of subjects who completed the study, interventions investigated, outcomes, adverse effects and results.[1,2]

The next step would be to synthesize the data that has been extracted – this consists of tabulating study characteristics (e.g. characteristics of included studies), quality and effects (e.g. summary of findings tables).[1,2] Furthermore, identifying the most suitable statistical methods to analyze the differences between studies and combining their effects will need careful thought. Systematic presentation of the findings is crucial, and a meta-analysis must always include a graphic visual display of the results.[1,2]

The interpretation of these findings which will require the reviewer to assess studies for degree of quality, assess the included studies for risk of publication bias and related bias's, explore the heterogeneity amongst studies, and use these findings to offer recommendations. In a systematic review or meta-analysis, assessing the outcomes would refer to interpreting the newfound results from the combined studies. This may then help describe the general trend that was observed, and new hypotheses can be formulated.[1,2]

1.4.3 Strengths and weaknesses associated with a systematic review

Systematic reviews and meta-analyses aim to answer a question by pooling together answers from different sources.[1,2,38] A big challenge for such an attempt is to agree on the comparability of the collected data, in terms of the design, conduct and the presentation of data. Issues such as size of studies, quality of the studies, randomization procedures and time spans should be comparable. Nutritional studies are more difficult, particularly when compared to pharmacological experimental studies, in respect of controlling exposures, and to make sure that all subjects receive exactly the same exposures. In addition, the outcome data used in different studies are not always the same either.[1,2,38]

Another challenge in conducting systematic reviews is to obtain all the relevant literature and to ensure a thorough and complete collection of all studies done on the subject.[1,2,38] If all available literature is not included, the summary estimate may be misleading. When the original studies included in the systematic review are of poor quality, the findings of the systematic review or meta-analysis conducted will also be of poor quality. It is the responsibility of the author of the systematic review to include honest assessments of the study quality, the possible methodological flaws, the risk-of-bias and the comparability of the studies. This will allow for readers to interpret the results with caution, when necessary, and to bear these shortcomings in mind when drawing conclusions. Because of all these challenges, systematic reviews cannot replace clinical reasoning.[1,2,38]

1.4.4 Goal of systematic reviews

In medical practice there is a need for clear and explicit recommendations based on solid facts.[1,2,38] Without conducting a systematic review on a subject, decisions on what should be recommended will be made on personal opinion or hearsay, or on individual trials or single pieces of evidence, which can lead to bias and inaccurate conclusions.[1,2,38]

The Cochrane Collaboration is an international initiative that aims to facilitate an evidence-based approach by bringing together scientific evidence.[1] Its primary aim is to "help people make well-informed decisions about healthcare and health policy by preparing and maintaining high quality systematic reviews." It is a non-profit organization and draws significantly on volunteer effort. The Cochrane Library is published on behalf of The Cochrane Collaboration and includes systematic reviews done on medical topics. Not all systematic reviews done are necessarily included in the Cochrane Collaboration – good systematic reviews can be conducted that are not Cochrane Reviews.[1]

1.5 WHY IS IT IMPORTANT TO DO THIS REVIEW?

There has been growing interest in the use of probiotics in the treatment of infectious conditions like GE owing to the characteristics of these microorganisms, i.e. they display an antagonistic behavior towards all things foreign in the body cavity, compete with such pathogens for binding sites and nutrients, produce and secrete multiple enzymes and chemicals to render the environment unsuitable for foreign bodies to grow and thrive, increase both innate and adaptive immune responses and for the most part, are not harmful.[6,7,24-27] Collectively, there is a huge body of research in this area.

Initially, systematic reviews and meta-analyses were conducted on the clinical applications of mostly probiotics and not so much on yeast-probiotics. Sazawal *et al* (2006)[39] conducted a meta-analysis of thirty four RCTs and reported that probiotics (multiple single strains) reduced the associated risk of AGE in children, but that the effect on AGE was dependent on the age of the host and the genera of the strain used.[39] Johnston *et al* in their systematic review assessed the efficacy of probiotics in treating AAGE in 707 pediatric patients.[25] This was followed five years later by a review of the same subject matter but in a pool of 3432 pediatric patients.[26] The authors concluded that *Lactobacillus rhamnosus* and *Saccharomyces boulardii* at a high dose (5 to 40 billion colony-forming-units (CFUs)/d) may prevent the onset of AAGE, with no serious side effects, but that larger RCTs are required.[25,26]

Specific to the younger population, a 2007 review conducted by Szajewska, Skorka & Dylag investigated the effectiveness of *Saccharomyces boulardii* in treating GE in children.[33] This review consisted of data from five RCTs including children aged between 2 months and 12 years. Off the five studies, only three were placebo-controlled. Participants were offered *Saccharomyces boulardii* at a dose ranging from (250 to 750mg/day) and this was offered over a period of five to six days. The effect of *Saccharomyces boulardii* on duration of GE, stool output and percentage of participants with GE at specific cut-off points were assessed as primary outcomes, whilst secondary outcomes included vomiting, adherence to treatment and adverse effects. The results for

similar outcomes were combined in a meta-analysis and revealed that the *Saccharomyces boulardii* group showed a significant reduction in duration of GE (WMD -1.1, 95% CI: -1.3 to -0.83; 4 RCTs) with a non-significant test for heterogeneity. Participants receiving *Saccharomyces boulardii* were also more likely to have GE resolution at Day 2 and Day 4, much sooner than their counterparts. The *Saccharomyces boulardii* group also showed a statistically significant reduction in the risk of GE lasting more than seven days when compared to the control group. These findings led the authors to conclude that *Saccharomyces boulardii* therapy for GE in healthy infants and children appears to provide a moderate benefit in terms of reducing the duration of GE. However, when the methodology of each of the included studies was scrutinized, it was evident that there were some discrepancies, i.e. three of the five included studies were not considered as having a low risk-of-bias; the bulk of the included studies did not adequately describe how allocation concealment was guaranteed; each of the five studies described withdrawals and drop outs; and only two of the five trials adequately described use of an intention-to-treat (ITT) analysis. Owing to these methodological drawbacks, the authors recommended that the results obtained in this systematic review be considered with caution.[33]

Another systematic review of evidence for the use of probiotics in the management of AGE was conducted in 2010 by Allen *et al*.[15] Following a comprehensive search of the literature on multiple databases, sixty three RCTs met the pre-specified inclusion criteria and this included 8014 participants, both adult and pediatric. Overall the primary outcome of a reduction in the duration of GE was evident, i.e. 39.1 hours in the probiotic group versus 173.5 hours in the control group. Probiotics reduced the mean duration of GE (mean difference (MD) 24.76 hours; 95% CI: 15.9 to 33.6 hours; n=4555; 35 trials); intervention groups were found to have a 59 percent reduced risk of experiencing GE for ≥4 days when compared to control groups (RR 0.41; 95% CI: 0.32 to 0.53; n=2853; 29 trials); and use of probiotics resulted in a decreased stool frequency on Day 2 (MD 0.80; 95% CI: 0.45 to 1.14; n=2751; 20 trials).[15] These reviewers did recommend though that their results should be considered with caution owing to the many different probiotics tested. Off the sixty three included studies, forty six tested a single organism and seventeen tested combinations ranging between two and eight organisms. Although *Saccharomyces boulardii* together with *Lactobacillus casei-GG* and *Enterococcus lactic acid* bacteria were identified as the most common organisms, the true effect of the yeast probiotic cannot be extrapolated from this data. Overall, the pooled results of this 2010 systematic review indicated that probiotics in addition to rehydration therapy resulted in reductions in the duration and severity of GE, and with no adverse effects being reported.[15] The difficulties presented by this review lie in the differences attached to each of the included studies, i.e. the study population consisted of a varied mixture of adult, pediatric and infant patients. Although the primary outcome assessed was duration of GE, a universal definition for GE and resolution thereof was not used, thereby adding to the subjectivity of the results reported in each individual study. There is general consensus that the effects of probiotics are strain-specific and that results obtained with one probiotic cannot be used to explain the effects of other strains. This review by Allen *et al* included a pooled analysis of results obtained from the use of multiple probiotic strains, which were also offered in different settings. Although beneficial outcomes were identified, each of the probiotic strains used are likely to have multiple mechanisms of action.[15] This means that the effect/s it has/had on the host's immunity and gut mucosal barrier integrity will vary. Furthermore, the efficacy of each probiotic might have been impacted on by the environment in which it

was offered. These authors acknowledged the vast heterogeneity that was found between studies and therefore concluded that it would be difficult to use this information to develop evidence-based treatment guidelines.[15]

In that same year, McFarland (2010) conducted a systematic review and meta-analysis of *Saccharomyces boulardii*, but in the adult population.[29] Thirty one RCTs, which were not restricted by language, were used in a meta-analysis which yielded a study population of 5029 participants. *Saccharomyces boulardii* was found to be significantly effective and safe in 84 percent of the treatment arms. When the types of GE were more closely examined, the author found that *Saccharomyces boulardii* was particularly effective in preventing AAGE, i.e. adult patients with a background of receiving antibiotic therapy and offered *Saccharomyces boulardii* prophylactically, were 53 percent less likely to experience AAGE as compared to those patients not receiving *Saccharomyces boulardii* (RR 0.47; 95% CI: 0.35 to 0.63; $P < 0.001$). Unlike the previous systematic review, this reviewer reported that *Saccharomyces boulardii* was both safe and beneficial for use in the adult population. Furthermore, recommendations for its use for specific infectious conditions was suggested, with a typical daily dose of $>10^9$ CFUs/day. Based on the disease state, the yeast probiotic could be offered for a minimum of seven days, up to six months, and either as a single treatment modality or as adjunct treatment. However, for more chronic disease states (e.g. Crohns disease, irritable bowel syndrome), the reviewer recommended that more RCTs are needed.[29]

The systematic review conducted by Pan *et al* (2012) was perhaps the only piece of literature that best mimicked the study design of the current systematic review.[40] These authors also assessed the effect of either placebo/no additional intervention versus *Saccharomyces boulardii* on children with AGE, with primary outcomes being duration of GE, stool frequency and adverse effects. A total of eight RCTs met the inclusion criteria warranting advancement to analysis and pooling of data. The eight included studies resulted in a pooled sample population of 978 children ranging between the ages of 1 month and 12 years. The intervention group consisted of 487 participants receiving the yeast probiotic (250 to 500mg per day), versus the control group consisting of 491 participants. The results of the meta-analysis showed that *Saccharomyces boulardii* was more effective in decreasing the duration of GE compared to the control group (MD -0.92; 95% CI: -1.32 to -0.52). This was especially applicable for stool frequency on Day 3 (MD -1.92, 95% CI: -1.63 to -0.95), Day 4 (MD -0.51; 95% CI: -0.89 to -0.33), and Day 7 (MD -0.44; 95% CI: -0.72 to -0.16). In addition, none of the included studies reported on any adverse events occurring. These authors therefore concluded that *Saccharomyces boulardii* in children with AGE has displayed therapeutic benefits, but more RCTs involving bigger sample sizes and with improved methodology is needed.[40]

Based on these reporting's, it is evident that therapeutic benefit from the use of *Saccharomyces boulardii* has the potential to be the sole or adjunct treatment in treating AGE. Despite the results of the systematic reviews and studies discussed above being derived from mostly RCTs, the number of potential areas where confounding and bias is possible is far too many. As a result, the aim of this research project is to provide a systematic up-to-date review of published studies, specifically assessing the efficacy and safety of *Saccharomyces boulardii* on the treatment of AGE in the pediatric population.

CHAPTER 2
METHODOLOGY

2.1 INTRODUCTION

Guidelines on the management of GE in the pediatric hospitalized patient are currently available.[8-10] However, UNICEF still reports that GE remains a leading cause of death in children, i.e. GE was responsible for 9 percent of all deaths among children under-five in 2015.[8-10] Although simple effective treatments are available, 1400 children are still reported to be dying each day from this symptom. The use of the yeast probiotic *Saccharomyces boulardii* has been researched as a possible treatment option for GE and for restoring gut microflora.[25-33] The data generated by this research was systematically collated in this review. This chapter serves to describe the following components required for the planning, and conducting of this systemic review: the purpose and objectives of this study, study design, study population, the method of data collection and analysis, and the piloting methods used.

2.1.1 Purpose of the study

The purpose of this systematic review was to assess the efficacy and safety of the yeast probiotic *Saccharomyces boulardii* in the treatment of AGE in the pediatric hospitalized population.

2.1.2 Research Objectives

2.1.2.1 Primary outcomes

The primary objective of this systematic review was two-fold, i.e. to assess the overall efficacy of *Saccharomyces boulardii* on the duration of diarrhea in the pediatric patient admitted to a hospital setting with AGE; and to establish the safety of this yeast probiotic for use in the pediatric hospitalized patient.

2.1.2.2 Secondary outcomes

The secondary objectives of this systematic review were to assess how use of *Saccharomyces boulardii* as part of a treatment regimen impacted on the pediatric patients' length of stay in hospital and therefore associated costs.

2.1.2.3 Other outcomes

Additional findings on optimal dosing, mode of delivery, frequency of treatment, duration of treatment and timing of delivery of this intervention were also investigated.

2.2 CRITERIA FOR SELECTING STUDIES FOR THIS REVIEW

2.2.1 Types of studies

Only RCTs, involving human participants, investigating the efficacy and safety of *Saccharomyces boulardii* were considered for inclusion. Trials were included regardless of the lack of blinding or placebo treatment. All other

study designs (e.g. cross-over trials, quasi-controlled trials, case studies, observational studies, retrospective studies, non-randomized, non-controlled, expert opinion, and traditional reviews) were excluded.

2.2.2 Types of participants

Infants and pediatric patients, aged between 0 and 16 years, admitted to a hospital setting with a diagnosis of AGE (≥ 3 unformed stools in the last 24 hours and of ≤ 48 hour duration). Studies including patients with the following characteristics were excluded from this review: chronic illnesses, under-nutrition, severe dehydration, known allergies, recent history of use of one or a combination of probiotics, antibiotics and anti-diarrhea medication.

2.2.3 Types of interventions

Only studies using *Saccharomyces boulardii* as the intervention were included. Any study, in which the *Saccharomyces boulardii* intervention was confounded by another intervention and without a proper control, was excluded. Use of other strains of Saccharomyces (as the intervention) was not included.

2.2.4 Types of outcome measures

Outcome measures which required daily record keeping included the following:

- Duration of diarrhea in days
- Mean number of stools passed per day
- Mean number of episodes of diarrhea at follow up
- Frequency of diarrhea at start, mid-point and end of intervention
- Stool frequency
- Changes in stool consistency post intervention
- Duration of hospital stay in days

Modifiers and confounders included:

- Active ingredients offered concurrently with the intervention (e.g. antibiotics)
- The intervention being offered as part of a cocktail treatment
- Differences in dosages offered and method of administration

2.3 SEARCH METHODS FOR IDENTIFICATION OF STUDIES

A comprehensive literature search of computerized databases was conducted with the guidance of a qualified Medical Librarian (Mrs. Wilhelmine Pool). Databases searched included: Medline (accessed via PubMed); EBSCO host (Elton B Stephen's Company), including Academic Search Premier, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Africa Wide and CAB Abstracts (produced by CABI Publishing, which covers the significant research and development literature in agriculture, forestry, human nutrition, veterinary medicine and the environment); Cochrane Library which includes the Cochrane Databases of Systematic Reviews (CDSR, Cochrane Reviews), Cochrane Central Register of Controlled Trials (CENTRAL; Clinical

Trials), Databases of Abstracts of Reviews of Effects (DARE; Other Reviews); ISI Web of Knowledge – Web of Science; Scopus (abstract and citation database of peer-reviewed literature); ProQuest Medical Library; Science Direct; and SABINET (South African Bibliographic Information Network).

Additional literature was obtained through hand searching and reviewing of reference lists of articles and systematic reviews which appeared in the primary search. Studies were selected regardless of language, publication date or status, with electronic searches commencing 10 April 2014 and ending 27 January 2015.

The final search string used was: (probiotic OR *Saccharomyces boulardii*) AND (diarrh* OR gastroent*) AND (clinical trial* OR randomized control trial* OR random allocation OR placebo* OR random research OR comparative OR evaluation stud* OR follow up OR prospective* OR control* OR volunteer* OR single mask* OR double mask* OR treble mask* OR tripl* mask* OR double mask* OR treble mask* OR tripl* mask* OR single-blind OR double-blind OR treble blind OR tripl* blind). The only limits applied whilst using this search string was human and child (birth to 16 years), and therefore foreign language articles were included. This search string was adapted where relevant and applied across all databases that were mentioned above.

2.4 DATA COLLECTION AND ANALYSIS

Preliminary screening was conducted by one reviewer (MP). Articles that were clearly non-relevant to the current systematic review were filtered out of the search pool (e.g. multi-species trials, not related to AGE but rather inflammatory bowel disease, updates and commentaries). Following this process, the screening steps that followed were completed independently and in duplicate by each of the two identified reviewers (MP and EV).

2.4.1 Selection of studies

Following removal of clearly non-relevant articles, two reviewers (MP and EV) independently screened the titles and abstracts of the articles identified by the search and applied the pre-defined inclusion criteria in order to identify eligible studies. The form used to standardize this process was one that was adapted from the Cochrane Handbook, the “Study Eligibility Form” (see Appendix 6.1). In the event of there being disagreement with the eligibility of a specific abstract for inclusion in this research review, the reviewers documented this and proceeded to obtain the full text article for further clarification. Where the two reviewers were not able to achieve consensus on such a matter, a third opinion was sought from the primary research team. Studies that initially appeared to be relevant but subsequently excluded are discussed in the section “Excluded studies”, together with the reasons for exclusion.

2.4.2 Data extraction and management

A Data Extraction Form (see Appendix 6.2) that was developed using the Cochrane Library resources was piloted using three of the full text articles that were identified as not applicable for placement in the list of included studies. Following piloting, this standardized Data Extraction Form was then used by each of the two reviewers (MP and EV) to independently extract data from the full text articles used in this research review.

For each study, a review title/ID was assigned and the following information was recorded:

- General information (i.e. surname of first author and year published; authors contact details; publication type; name of the reviewer completing the form; date on which the form was completed);
- Methods (i.e. aim of the study; study design type; method of recruitment; inclusion and exclusion criteria; informed consent obtained; ethical approval needed/obtained; funding being clear to indicate both the source and amount; statistical methods used);
- Participants (i.e. population description; setting; total number randomized; age; gender; ethnicity; baseline imbalances; withdrawals/exclusions; severity of illness; co-morbidities; other socio-demographics; subgroups measured; subgroups reported);
- Intervention group/s (i.e. group name; description; duration of treatment period; timing; delivery of intervention; providers; co-interventions; economic information; resource requirements);
- Outcomes (i.e. outcome type; outcome name; time points measured; time points reported; outcome definition; person measuring/reporting; unit of measurement; imputation of missing data; assumed risk estimate; power);
- Risk-of-bias assessment (i.e. random sequence generation; allocation concealment; blinding of participants and personnel; blinding of outcome assessment; incomplete outcome data; selective outcome reporting; other bias);
- Data analysis (i.e. comparison; outcome; subgroup/s; time point; post-intervention or change from baseline; other results; number of missing participants; reason for missing; number of participants that moved groups and reason for the move; unit of analysis); and
- Other information (i.e. key conclusions of study authors; relevant references).

2.5 ASSESSMENT OF METHODOLOGICAL QUALITY OF INCLUDED STUDIES

Each of the two reviewers independently assessed the components of each of the included studies for risk-of-bias. This was done by using a risk-of-bias tool (see Appendix 6.3), as described by the Cochrane Handbook for Systematic Review of Interventions (see Table 2.1).[1] This tool helped to evaluate the potential sources of bias in the methodology of the included studies. The methodological domains of the studies were evaluated and classified as adequate, inadequate or unclear, as shown in Table 2.1 below. More detail regarding how this evaluation was conducted will be discussed later.

The domains of the methodology that were assessed are sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, and other potential sources to affect validity.[1,2,38] Assessment was done by answering a pre-specified question about the adequacy of the study in relation to the entry, in such a way that the judgment of 'yes' can be indicative of low risk-of-bias, 'no' can be indicative of high risk of bias, and 'unclear' can be indicative of uncertain risk of bias.[1,2,38] Disagreements between each of the reviewers' judgments were resolved by discussion, and consensus was reached in all cases.

"Summary of findings" tables were used to display the risk-of-bias for important outcomes within and across studies. RCTs were considered high quality evidence, but were further extrapolated into limitations such as: risk-of-bias, consistency, directness, imprecision, and reporting bias.

The GRADE system for rating overall quality of evidence for the most relevant outcomes was applied. The quality of evidence was further categorized as either high (confident that the true effect lies close to that of the estimate of effect), moderate (moderately confident in the effect estimate), low (confidence in the effect estimate is limited) and very low (very little confidence in the effect estimate).[38]

Table 2.1: The Cochrane Collaboration's tool for assessing risk-of-bias[1]

Domain	Description	Review Author's Judgement
Sequence generation	Describe the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.	Was the allocation sequence adequately generated?
Allocation concealment	Describe the method used to conceal the allocation sequence in sufficient detail to determine whether the intervention allocations could have been foreseen in advance of, or during enrolment.	Was allocation adequately concealed?
Blinding of participants, personnel and outcome assessors	Describe all measures used to blind study personnel and participants from knowledge of which intervention relating to whether the intended blinding was effective.	Was knowledge of the allocated intervention adequately prevented during the study?
Incomplete outcome data	Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared to total randomized participants), reasons were reported, and any re-inclusions in analyses performed by the review authors.	Were incomplete outcome data adequately addressed, regarding the amount, nature and handling of incomplete data?
Selective outcome reporting	State how the possibility of selective outcome reporting was examined by the review authors, and what was found.	Are reports or the study free of suggestion of selective outcome reporting?
Other sources of bias	State any important concerns about bias bot being addressed in the other domains of the tool.	Was the study free of other problems, not covered elsewhere in the table, making it high risk?

2.6 MEASUREMENTS OF TREATMENT EFFECT

2.6.1 Dichotomous data

All dichotomous data resulted in the following information being extracted from each treatment group: the number of participants with the event and the total number of participants. Risk ratios (RRs) were calculated for all dichotomous data.

2.6.2 Continuous data

All continuous data resulted in the following information being extracted from each treatment group: the arithmetic mean, Standard Deviation (SD) and the number of participants. The SD was calculated using the 95% CI and MDs was calculated for continuous data where applicable.

2.6.3 Incidence data

As an outcome, the included studies did not report on the incidence of GE. Therefore, incidence rate ratios are not applicable.

2.6.4 Dealing with duplicate publications

Owing to a comprehensive search across multiple electronic databases, duplications of the same references, but from different sources were encountered. These references were double-checked to confirm duplication by reviewing the date of publication, and once this was confirmed, only one of these (duplicated) references was included.

2.6.5 Assessment of heterogeneity

Assessment of heterogeneity was achieved through the visual inspection of the forest plots.[1,2] CIs were assessed and considered to have statistical heterogeneity if there was poor overlap of the results of individual studies. A Chi^2 test for heterogeneity (significance level $P < 0.1$) was conducted and the I^2 statistic calculated. The following guidelines were used for the interpretation of the I^2 values[1,2]:

- 0% to 40%: might not be important
- 30% to 60%: may represent moderate heterogeneity
- 50% to 90%: may represent substantial heterogeneity
- 75% to 100%: considerable heterogeneity

The value of I^2 depended on the magnitude and direction of the effects, as well as on the strength of evidence for heterogeneity (e.g. $P < 0.1$ from the Chi^2 test, or a CI for I^2).[1,2]

2.6.6 Assessment of reporting bias

Funnel plots are usually used to explore the possibility of small study bias.[1,2] Different explanations are used to explain funnel plot asymmetry such as publication bias, the effect of different study sizes and poor study design. Tests for funnel plot asymmetry should only be used when there are a least 10 studies included in a meta-analysis, as fewer studies would result in the power of the tests being too low to identify chance versus real asymmetry.[1,2] Since we did not have a meta-analysis of ten or more studies, we did not construct a funnel plot to assess publication bias.

2.6.7 Data synthesis and analyses

Data were analyzed using Review Manage 5 (RevMan 2008) software. A random effects model of meta-analysis was used due to the presence of significant heterogeneity of treatment effects between studies. When homogeneity was displayed, a fixed effects model was applied. The Mantel-Haenszel method of meta-analysis

was applied for dichotomous outcomes and the Inverse-Variance method was applied for continuous outcomes. In the event of participants in included studies not receiving the assigned intervention as stipulated in the protocol, or being lost to follow-up, an ITT analysis was applied. In the event of missing data, the primary authors for the relevant studies were contacted for additional information. This was in done in the form of an email, providing the relevant study information and the data missing.

See Appendix 6.4 for an example of the correspondence forwarded to primary authors of studies used. As per the protocol, all uncertain findings that were not appropriate for inclusion in the pooled analyses were reported in the review.

2.6.8 Unit of analysis

During data analyses, the reviewers took into account at what level the randomization of study subjects took place. The reviewers also assessed the included studies to make sure that the number of observations made in the analysis matched the number of "units" that were randomized in the study.

2.6.9 Sub group analysis and investigations of heterogeneity

If statistical heterogeneity was present ($P < 0.1$), potentially influential study characteristics were further investigated by conducting subgroup analyses.

2.6.10 Sensitivity analysis

The researchers planned to perform sensitivity analyses in the event of there being other influencers on study results.

2.7 ETHICAL & LEGAL ASPECTS

No ethical approval is required for a systematic review, as it is not a formal study requiring human participation. The Health Research Ethics Committee of Stellenbosch University was notified of the proposed systematic review and for record purposes, the project was registered (Ethics Number X14/07/012, see Ethics letter, Appendix 6.5).

The protocol was also registered at the Prospective Register of Ongoing Systematic Reviews (PROSPERO), which is an international database of prospectively registered systematic reviews on health and social care. The registration number for this systematic review is CRD42014009913 (see Appendix 6.6).

CHAPTER 3
RESULTS

3.1 DESCRIPTION OF STUDIES

3.1.1 Results of the search

The revised search string across multiple computerized databases yielded a large number of hits (>2200) resulting in the need for a preliminary screening. The result of this step yielded 190 papers, with no additional references being added. During the identification process, 10 papers were removed owing to duplication. A further 68 papers were removed during the screening process for the following reasons: 13 were the wrong design or wrong setting; 4 carried out in the wrong population (adult/animal); 8 involved the concurrent use of other active treatments; 27 addressed non-acute GE; and 16 addressed causes other than Rotavirus. The removal of a further 71 papers was necessary owing to the design being that of a systematic review.

At this point, two independent reviewers (MP and EV) considered the remaining 41 papers for inclusion in this systematic review. Of this pool, 7 were foreign language papers, of which 3 were written by the same author. Attempts at obtaining the English translations of these papers were made by contacting the relevant authors via email addresses found in each of the papers (see Appendix 6.4). Only one of these authors responded stating that the papers were only available in French. In addition, attempts at accessing translated versions of the respective articles via the Stellenbosch University library resources were also futile. As a result, the research team took the decision to remove these references, citing this as one of the shortcomings of this systematic review.

Full text articles for 25 of the 34 remaining eligible papers were obtainable. The remaining 9 papers were removed from the steps that follow owing to the following reasons: 1 paper used the wrong study population; 1 paper was a repetition of the foreign language paper that was not available in English; 7 papers were inaccessible. Despite attempts by the Medical Librarian to access these remaining 7 papers, they were still found to be inaccessible owing to online versions starting after the date of publication (see Appendix 6.4 for communication with research authors and the Medical Librarian). The removal of these final 7 papers owing to inaccessibility would therefore be cited as a possible limitation of this systematic review, resulting in only 25 papers advancing through to the study eligibility phase (see Figure 3.1 below).

Whilst conducting independent assessments of these 25 studies, the two reviewers (MP and EV) agreed on a further 12 studies being removed: 1 study involved the concurrent use of other active ingredients, 2 studies were conducted in an outpatient setting, 2 were a wrong study type (e.g. *in vivo*), 2 were in the wrong study population (i.e. 18 to 60 years), and 5 addressed AGE caused by antibiotic therapy and not Rotavirus. The remaining thirteen studies[41-53] then advanced to the data extraction phase. During this process, the 2 reviewers agreed on data extraction and inclusion of ten[41,42,43,44,45,47,48,49,50,53] of the studies. However, the remaining three studies were found to be inappropriate for inclusion: 2 studies[46,52] included participants that were severely dehydrated, one study[51] addressed GE caused by agents other than Rotavirus (see Figure 3.1 below for a summary of the

study selection process). Therefore, only ten[41,42,43,44,45,47,48,49,50,53] studies advanced to the next step of this systematic review, i.e. data analysis.

3.1.2 Excluded studies

Some of the main reasons for study exclusion were as follows: 71 took the form of a commentary, updates, guidelines, reviews and/or meta-analysis; 27 addressed GE that presented in forms other than acute; 16 included a diagnosis other than Rotavirus-causing GE; 13 took place in a non-hospital setting; 10 were repetitions; 9 were not accessible either online or were not published; 8 involved the concurrent use of other active ingredients; 7 were references in a foreign language; and 4 involved inappropriate study participants. The Table of Excluded Studies (Appendix 6.7) provides more information on the aforementioned references.

3.1.3 Included studies

Ten studies[41,42,43,44,45,47,48,49,50,53] met the inclusion criteria and were included in this systematic review. The ten included studies were published between the years 2006 and 2013. Important information about these studies can be found in the "Characteristics of Included Studies" table (see Appendix 6.8). A total of 1401 participants were included from the combined ten studies, with the smallest study[47] involving 27 participants and the largest study[48] involving 480 participants. All ten included studies were conducted in a hospital setting, but in multiple locations across the world i.e. 1 was conducted in Pakistan[43], 2 conducted in India[44,49], 1 conducted in Brazil[45], 1 conducted in Myanmar[55], and 5 in different hospitals within Turkey[41,42,47,48,50].

All ten included studies adopted a study design that included both an intervention and control/placebo group, being monitored in parallel. The intervention arm consisted of one or more interventions, but with *Saccharomyces boulardii* always being used as an independent intervention. Across all ten studies, *Saccharomyces boulardii* was used at a dosage ranging from 200mg[45] to 250mg[41,43,44,47,48,49,50,53] with only one study[42] offering the yeast probiotic at a slightly higher dosage of 282.5mg. In terms of frequency of treatment, 50 percent of the studies offered the intervention dose once[42,44,45,48,50] per day and 50 percent offered the intervention dose twice per day[41,43,47,49,53].

Most studies[41,44,45,48,49,50,53] considered the first five days as the "active" treatment days, with one study[43] using six days as the active treatment days. Only one study[47] required the intervention to be implemented over a seven day period. One study[42] did not specify the minimum "active" treatment phase, but provided information on the mean duration-time of GE in all study groups of (5.9 ± 2.0) days. Of all the included studies, only one[43] followed participants for two months post discharge to assess incidence of GE episodes post intervention.

Not all included studies indicated or implemented the use of a placebo in their study designs, i.e. six studies[41,42,43,44,48,53] did not describe or make use of a placebo, whilst the remaining four studies[45,47,49,50] mentioned/described the placebo treatment used.

The four studies that described use of a placebo did so in different ways, i.e. one study[50] offered both the intervention and an identical-looking placebo diluted in water or juice (as advised by the manufacturer); one study[47] offered both the intervention and placebo dissolved in water; one study[49] offered both the intervention and placebo in identical packets mixed with puffed rice powder; and one study[45] offered both the intervention and placebo in capsule form, which were prepared by a faculty pharmacy.

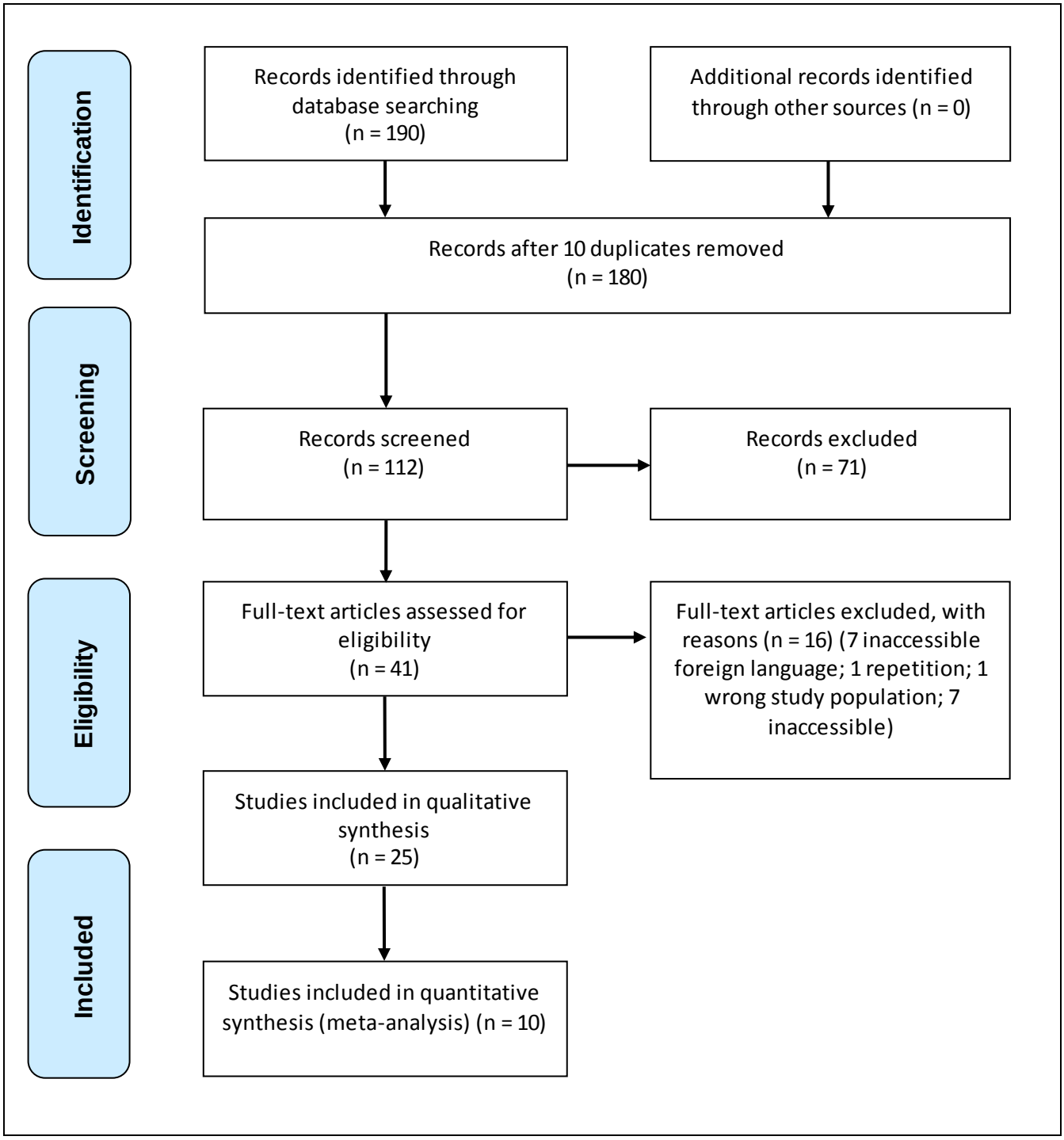

Figure 3.1 PRISMA study flow diagram

3.2 RISK-OF-BIAS IN INCLUDED STUDIES

By using the "Risk-of-Bias Tool" (see Appendix 6.3), the two reviewers (MP and EV) independently assessed each of the ten included studies for any risk-of-bias. Six methodological quality domains were addressed, i.e. adequate sequence generation, allocation concealment, blinding, incomplete outcome data, selective reporting and other potential sources of bias.

Appendix 6.9 summarizes the consensus-judgments reached for each of these ten studies, together with supporting comments.

3.2.1 Adequate sequence generation

Random sequence generation was adequate in four[41,44,48,53] of the 10 studies (see Figure 3.2 and 3.3). The remaining 6 studies[42,43,45,47,49,50] did not clearly indicate how adequate sequence generation was achieved. None of the studies led to the conclusion of being at high risk-of-bias in this domain.

3.2.2 Allocation concealment

Two[45,49] of the ten studies clearly described how adequate allocation concealment was achieved (see Figure 3.2 and 3.3). Two[41,43] studies posed a high risk-of-bias. The remaining six studies[42,44,47,48,50,53] did not clearly describe how this domain was achieved.

3.2.3 Blinding

The blinding of participants and personnel was found to be adequate in four[45,47,49,50] of the ten studies. Three[41,42,53] studies posed a high risk to blinding practices and the remaining three studies[43,44,48] did not provide enough details to be totally clear about bias infringements in this domain (see Figure 3.2 and 3.3).

3.2.4 Blinding of outcome assessment

Fifty percent of the studies[42,43,47,48,53] did not clearly indicate how blinding of outcome assessment was guaranteed. The remaining studies consisted of only one study[50] that did not provide for adequate blinding of this domain and four studies[41,44,45,49] achieving adequate blinding (see Figure 3.2 and 3.3).

3.2.5 Incomplete outcome data

Six studies[41,43,44,45,48,49] provided enough information to be considered adequate prevention of attrition bias. The remaining four studies[42,47,50,53] were assessed as unclear owing to insufficient details on how this domain was achieved (see Figure 3.2 and 3.3).

3.2.6 Selective outcome reporting

Eight studies[38,40,41,42,44,45,46,50] all clearly reported on all outcomes initially mentioned. Only one study[39] did not adequately accommodate for reporting bias. One study[47] did not provide sufficient information on all outcomes reported (see Figure 3.2 and 3.3).

3.2.7 Other potential sources of bias

Sources of funding could possibly play a role as a potential source of bias. Two of the included studies[43,47] were funded and supported by pharmaceutical companies, with one study[47] declaring no conflict of interest in relation to the study. One study[44] acknowledged receiving financial support from a university affiliated with the hospital where the study was conducted. Another study[45] reported support from a government council involved with scientific and technological development. Fifty percent of the included studies[41,42,48-50] did not disclose any information about source of funding or financial support received. However, one of these studies[49] made a simple declaration that no conflict of interest and no funding were received for the study. The one remaining study[53] was the only study where authors commented that it was completed with a very limited budget owing to there being no involvement of the company commercializing the yeast probiotic that was used in the interventional arm.

Other areas of bias did not appear to be a concern in eight of the included studies[41,42,43,45,47,49,50,53] and was considered adequate. Only two studies[44,48] provided insufficient information making it challenging to remove other sources of bias from their study designs (see Figure 3.2 and 3.3).

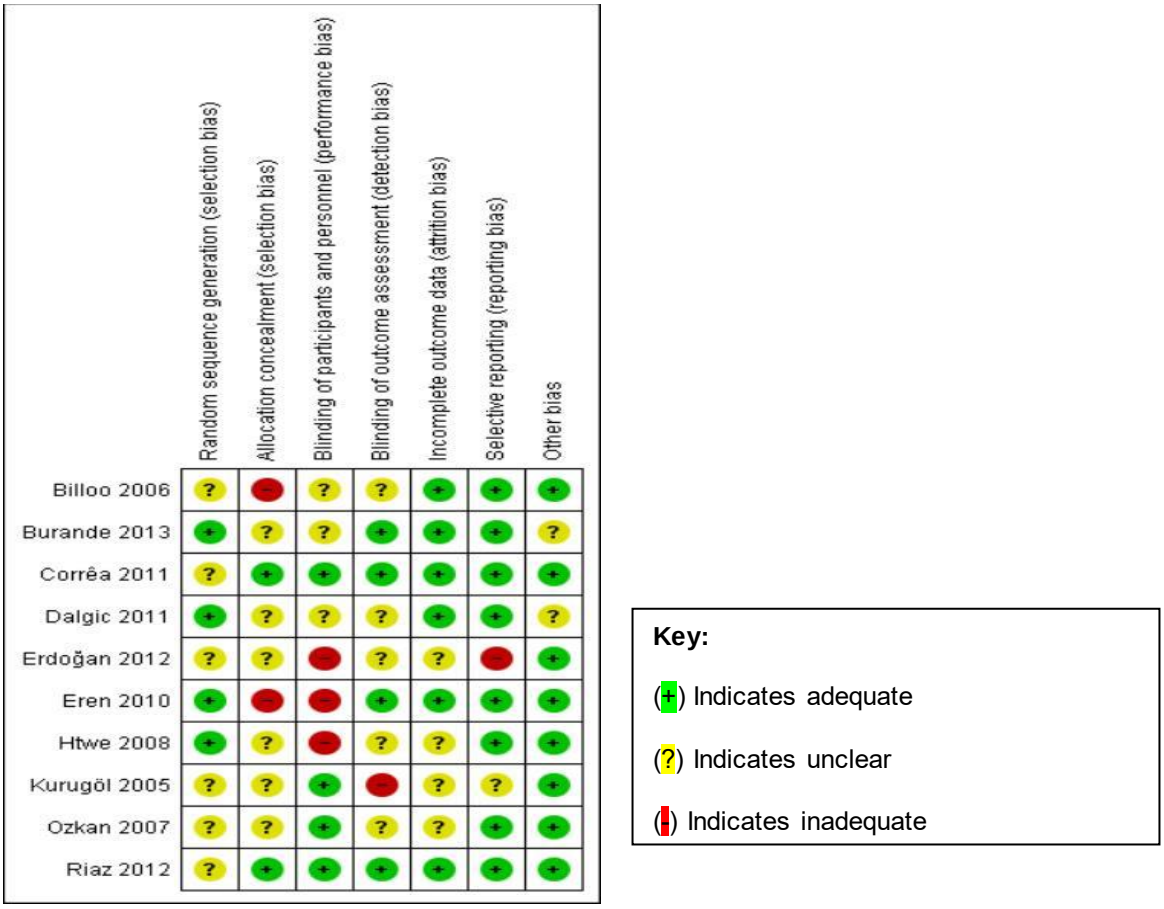

Figure 3.2: Methodological quality summary: judgments about each methodological quality item for each included study (10)

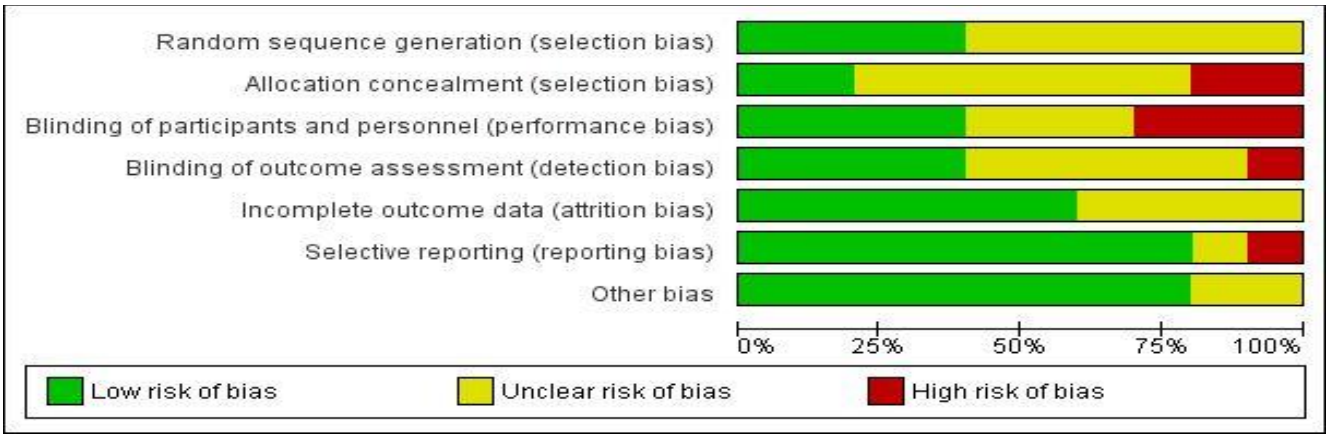

Figure 3.3: Methodological quality graph: judgments about each methodological quality item presented as percentages for all included studies (n=10)

3.3 METHODOLOGICAL QUALITY OF GROUPS OF STUDIES

The methodological quality of the included studies was then assessed using the GRADE tool. Using GRADE-pro software, the following, most relevant outcomes were assessed for overall methodological quality: duration of diarrhea, mean number of stools per day, frequency of diarrhea, number having less than three stools per day and duration of hospital stay. A summary of findings table was generated (see Table 3.1 below).

The first outcome for which methodological quality was assessed was that of "duration of diarrhea (in days). Five studies[42,44,48,49,50] were pooled in a random effects meta-analysis, resulting in a total sample population of 548 participants. The quality of evidence was downgraded (-1) owing to 4 studies[42,44,48,50] showing increased risk-of-bias owing to a combination of selection bias[42,44,48,50], performance bias[42,44,48], detection bias[42,48], attrition bias[42],reporting bias[50] and other bias[48]. However, there was no downgrading for inconsistency as 4 of the 5 included studies displayed no heterogeneity (I^2 = 0%) and CIs overlapped, indicating that any variation in the size of effect was more likely as a result of chance. Overall, this outcome produced a quality assessment that was rated moderate.

The second outcome assessed for standard of quality was "mean number of stools per day". This outcome consisted of only 1 study[47] and the overall quality of this study was found to be low owing to the following reasons: (-1) for risk-of-bias as both selection bias (no detail of how randomization and allocation concealment were achieved) and detection bias (no detail on the outcome assessment technique used) were present. In addition, downgrading (-1) for inconsistency was applied as only Day 0 out of the five days intercepted the line of no effect meaning that any variation in the size effect is not due to chance (I^2 = 95.3%, P = < 0.00001).

The study[45] investigating frequency of diarrhea being less than three times per day was found to display an overall quality rating of high. The reasons for this are as follows: this was a well-controlled study which displayed zero risk of bias, downgrading (-1) was applied owing to inconsistency as heterogeneity was present (I^2 = 96%, P < 0.00001), and only one day (Day 1) of the study overlapped the line of no effect. This quality of

this study's[45] evidence was upgraded as it produced a RR of 0.66, indicating that the *Saccharomyces boulardii* were more likely to experience fewer stools per day versus the control group.

The quality assessment for a study[53] consisting of an overall sample pool of 700 participants was found to be moderate for assessing the number of participants passing less than three stools per day during the first seven days after starting intervention. Overall, (-1) was applied for risk-of-bias as both performance and detection bias were noted. In addition, (-1) was applied for inconsistency as the forest plot completed indicated non-overlapping CIs, high I^2 (95%) and an accompanying low *P* value (< 0.00001). However, upgrading of the quality of this evidence was applied as a RR (1.13) was indicative of the *Saccharomyces boulardii* group being 1.13 times more likely to experience less than three stools per day quicker than the control group.

The final test for methodological quality involved two studies[48,50] which investigated the effect of use of this yeast probiotic on length of stay on hospital. Despite the two studies producing a pooled sample study population of 320 participants, the quality of the evidence was found to be low owing to the following: (-1) for risk-of-bias in areas of selection bias[48], reporting bias[48]. In addition, this particular study[48] was also concerning as of the 480 participants originally recruited, these authors reported that all 480 of them completed the study, with no withdrawals, exclusions of loss-to-follow-up. In addition, (-1) for inconsistency was applied (variation in size of effect likely no due to chance, high heterogeneity with I^2 = 95%, and very low *P* value of < 0.0001).

Table 3.1: Summary of findings table using GRADE - *Saccharomyces boulardii* compared to Control or Placebo for AGE

Patient or population: patients with AGE
Settings: in pediatric, hospitalized patients
Intervention: *Saccharomyces boulardii*
Comparison: Control or Placebo

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk **Control or Placebo**	Corresponding risk ***Saccharomyces boulardii***				
Duration of diarrhea measured in days Follow-up: mean 5-7 days		The mean duration of diarrhea in the intervention groups was **0.57 lower** (0.83 to 0.3 lower)		548 (5 studies)	⊕⊕⊕⊖ **moderate**[1,2]	
Mean number of stools per day number of stools per day Follow-up: mean 7 days		The mean number of stools per day in the intervention groups was **0.97 lower** (1.56 to 0.39 lower)		133 (1 study)	⊕⊕⊖⊖ **low**[3]	
Frequency of diarrhea Evacuation frequency was <3 times per day Follow-up: mean 5 days	**Study population**		**RR 0.66** (0.35 to 1.23)	528 (1 study)	⊕⊕⊕⊕ **high**[4,5,6]	
	775 per 1000	**512 per 1000** (271 to 953)				
	Moderate					
	802 per 1000	**529 per 1000** (281 to 986)				
Number having <3 stools per day stools passed Follow-up: mean 7 days	**Study population**		**RR 1.13** (0.97 to 1.31)	700 (1 study)	⊕⊕⊕⊖ **moderate**[7,8]	
	657 per 1000	**743 per 1000** (637 to 861)				
	Moderate					
	780 per 1000	**881 per 1000** (757 to 1000)				
Duration of hospital stay (days) in days		The mean duration of hospital stay (days) in the intervention groups was **0.12 lower** (1.9 lower to 1.65 higher)		320 (2 studies)	⊕⊕⊖⊖ **low**[9,10,11]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** Risk ratio

GRADE Working Group grades of evidence:
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] SELECTION BIAS (Kurugol 2005, Erdogan 2012, Burande 2013, Dalgic 2011); REPORTING BIAS (Kurugol 2005, Erdogan 2012); BLINDING (Erdogan 2012, Burande 2013, Dalgic 2011); OTHER BIAS (Dalgic 2011, Riaz 2013).
[2] No dow ngrading for inconsistency as: 4 of 5 studies have CIs that overlap meaning that any variation in the size of effect is more likely a result of chance; I^2 value of 0% indicating no heterogeneity; non-significant *P*-value.
[3] Dow ngrading for inconsistency as only Day 0 out of 5 days intercepted the line of no effect meaning that any variation in the size of effect is not due to chance; I^2 value is very high 95.3% indicating heterogeneity; very low *P* value (<0.00001).
[4] Dow ngrading for inconsistency as the forest plot for this outcome show s that of each of the 3 days of assessment, only results for Day 1 overlap w ith the line of no effect; the overall test for heterogeneity show ed a high I^2 of 96% and a very low *P* value (<0.00001).
[5] No dow ngrading as this outcome show s a w ide CI w ith the effect on the side favoring benefit, a large number of events (148+200) and a large sample size (270+258).
[6] Corrêa 2011: A RR of 0.66 indicating that the *Saccharomyces boulardii* group w as 34% more likely to experience fewer stools per day versus the control group.
[7] Htw e 2008: not all CIs overlap the line of no effect; I^2 value quite high at 95% and accompanied by a very low *P* value (<0.00001).
[8] Htw e 2008: Overall, this analysis showed a RR (1.13) indicating that the *Saccharomyces boulardii* group w as 1.13 times more likely to experience <3 stools per day quicker than the control group.
[9] Only Dalgic 2011 and Kurugol 2005 assessed impact of *Saccharomyces boulardii* on length of hospital stay.
[10] Dalgic 2011: SELECTION BIAS w as unclear as no information w as given on how allocation concealment w as achieved. REPORTING BIAS as no mention is made regarding the training of parents for reporting of symptoms like "appearance of stools", "w atery GE", "GE". OTHER BIAS: 480 participants w ere recruited and all 480 w ere reported to have completed the study, w ith no w ithdraw als, exclusions or loss to follow -up?
[11] Dow ngrading for inconsistency as neither study truly overlaps w ith the line of no effect meaning that any variation in the size of effect is not due to chance; I^2 value is very high 95% indicating heterogeneity; very low *P* value (<0.0001).

3.4 EFFECTS OF INTERVENTION

All ten of the included studies investigated the effects of *Saccharomyces boulardii* on AGE, but in two different comparisons, i.e. nine of the studies[42,43,44,45,47,48,49,50,53] compared *Saccharomyces boulardii* against a control or placebo, whilst one study[41] compared *Saccharomyces boulardii* against a yoghurt fluid containing *Lactobacillus bulgaricus* and *Streptococcus thermophiles*.

One study[48] evaluated *Saccharomyces boulardii* versus the following multiple comparisons: zinc; lactose-free formula; (*Saccharomyces boulardii* + zinc); (*Saccharomyces boulardii* + lactose-free formula); (zinc + lactose-free formula); (*Saccharomyces boulardii* + zinc + lactose-free formula); (ORS alone). In order to avoid the unit of analysis error due to multiple comparisons, a one pair-wise comparison was used for analysis, i.e. group 7 (*Saccharomyces boulardii* + zinc + lactose-free formula) versus group 6 (zinc + lactose-free formula). This was included in first comparison because it was assessing *Saccharomyces boulardii* versus control.

3.4.1 Comparison group: *Saccharomyces boulardii* versus control or placebo

Nine studies evaluated *Saccharomyces boulardii* versus control[42,43,44,48,53] or placebo[45,47,49,50] group. These were analyzed together since there were no active ingredients in either the control or the placebo.

3.4.1.1 Primary outcomes

All of the included studies investigated the efficacy of *Saccharomyces boulardii* on GE caused by Rotavirus, but reported their findings in somewhat different ways. Seven studies[42,43,44,48,49,50,53] reported duration of diarrhea (in days). One study[44] reported the outcome as recovery from loose motions. Five studies[42,44,48,49,50] were pooled in a random effects meta-analysis which showed that *Saccharomyces boulardii* significantly shortened the duration of diarrhea (in days), compared to the control or placebo group (MD -0.57; 95% CI: -0.83 to -0.30; n=548 children; 5 studies). Furthermore, there was no significant heterogeneity detected between the trials (Tau^2=0.00; Chi^2=1.57; df=4; *P*=0.81; I^2=0%) (see Figure 3.4 below).

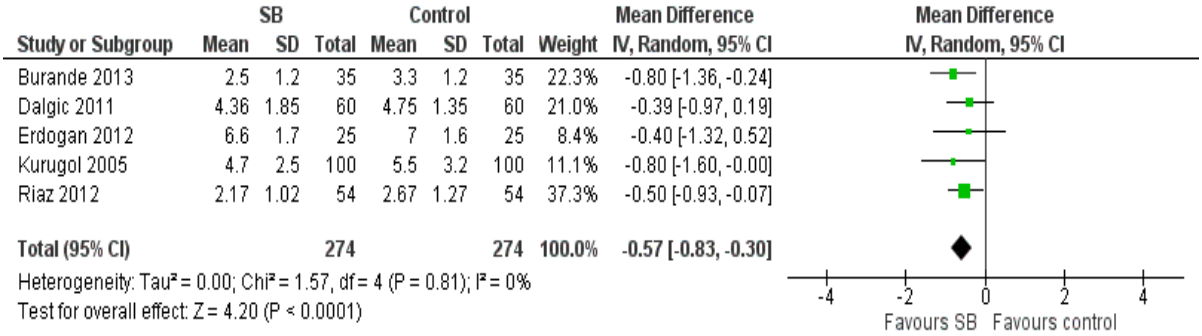

Figure 3.4: Forest plot: difference in duration of diarrhea (in days; *Saccharomyces boulardii* versus control)

Although two studies[43,53] reported the mean duration of diarrhea without the corresponding SDs, and therefore could not be included in the above meta-analysis, the study authors reported that *Saccharomyces boulardii* significantly shortened the duration of diarrhea (in days), compared to the control group in both studies, i.e. one study[43] (MD -1.2 (3.6 versus 4.8); n=100 children; 1 study; *P* = 0.001); and one study[53] (MD -1.6 (3.08 versus 4.68); n=100 children; 1 study; *P* < 0.05).

Another three studies[43,47,49] reported on the mean number of stools per day, with one study[49] also reporting the mean number of stools per day for 0 to 24 hours, 25 to 48 hours, and 49 to 72 hours. However, the results of the latter study were skewed (mean <2 SDs) and therefore no meta-analysis could be done. Although the other study[43] reported the mean number of stools per day, no corresponding SDs were reported, rendering it unsuitable for inclusion in the meta-analysis. These authors[43] however, did report that use of *Saccharomyces boulardii* offered statistically significant effects on the number of stools per day compared to the control group for Day 3 (MD -1.6 (2.8 versus 4.4); *P* = 0.01) and Day 6 (MD -1.7 (1.6 versus 3.3); *P* = 0.001), but not for Day 0 (MD (9.5 versus 8.8); *P* = 0.37). Results from the remaining study[47] showed a significant difference in the mean number of stool per day between the *Saccharomyces boulardii* group and the control group for Day 1 (MD -0.86; 95% CI: -1.15 to -0.57), Day 2 (MD -1.21; 95% CI: -1.49 to -0.93), Day 3 (MD -1.68; 95% CI: -1.93 to -1.43) and Day 4 (MD -1.38; 95% CI: -1.65 to -1.11), but there was no difference on Day 0 (MD 0.31, 95% CI: -0.06 to 0.68). Overall, the pooled effect size for the duration of treatment of AGE in this study favored the *Saccharomyces boulardii* group (*P* = 0.001) (see Appendix 6.10).

Only one study[43] reported on the mean number of episodes of diarrhea after Month 1 and Month 2 but there were no SDs reported. Even though the CIs for the MD between the *Saccharomyces boulardii* and control groups could not be calculated, the authors[43] reported that *Saccharomyces boulardii* significantly shortened the mean number of episodes of diarrhea compared to the control group for both Month 1 (MD -0.44 (0.2 versus 0.64); n=100 children; *P* = 0.001) and Month 2 (MD -0.24 (0.32 versus 0.56); n=100 children; *P* = 0.04).

One study[45] reported the number of children having diarrhea at each day after starting the intervention and the results show that *Saccharomyces boulardii* significantly reduced the risk of diarrhea compared to the control group for Day 2 (RR 0.54; 95% CI: 0.42 to 0.70; n=176 children) and Day 3 (RR 0.54; 95% CI: 0.38 to 0.77; n=176 children) but not on Day 1 (RR 0.96; 95% CI: 0.87 to 1.05; n=176 children). Overall, the effect of *Saccharomyces boulardii* for the first three days of treatment did not demonstrate superiority over the control, producing a non-significant result (*P* = 0.19) (see Appendix 6.11).

One study[53] reported on the number of children having less than 3 stools per day after starting the intervention and the results show that significantly more children were having less than three stools per day in the *Saccharomyces boulardii* group (n=50) compared to the control group (n=50) on Day 2 (RR 1.80; 95% CI: 1.10 to 2.95), Day 3 (RR 1.39; 95% CI: 1.05 to 1.85), and Day 4 (RR 1.23; 95% CI: 1.05 to 1.44). On Day 1, none of the children had less than three stools per day in both groups. On Day 6 and Day 7, all the children had less than three stools per day. On Day 5, there was no difference in the number of children having less than three

stools per day in the two groups (RR 1.04; 95% CI: 0.97 to 1.11). Although this analysis appeared to moderately favor the *Saccharomyces boulardii* group, it was not statistically significant (*P* = 0.11) (see Appendix 6.12).

One study[53] reported on the number of children having solid stools per day, after starting intervention and the results show that significantly more children were having solid stools in the *Saccharomyces boulardii* group (n=50) compared to the control group (n=50) on Day 2 (RR 3.00; 95% CI: 0.32 to 27.87), Day 3 (RR 3.17; 95% CI: 1.89 to 5.31), Day 4 (RR 1.63; 95% CI: 1.30 to 2.06) and Day 5 (RR 1.25; 95% CI: 1.08 to 1.44). On Day 1, none of the children had solid stools in both groups. On Day 7, all the children had solid stools. On Day 6, there was no difference in the number of children having solid stools between the two groups (RR 1.04; 95% CI: 0.97 to 1.11). Although the results appeared to favor the *Saccharomyces boulardii* group, it was not statistically significant (*P* = 0.06) (see Appendix 6.13).

In addition to the above, one other primary outcome of the current systematic review was to investigate the safety of use of this yeast probiotic in the pediatric hospitalized patient. None of the included studies reported on any significant side effects associated with *Saccharomyces boulardii* use.

3.4.1.2 Secondary and other outcomes

Two studies[48,50] reported on the duration of hospital stay (in days) and their results were combined in a meta-analysis that resulted in significant statistical heterogeneity (Tau^2=1.55; Chi^2=18.94; df=1; *P* < 0.0001; I^2=95%). Therefore, their results are reported separately: the first study[48] found a longer duration of hospital stay in the *Saccharomyces boulardii* group (MD 0.81; 95% CI: 0.09 to 1.53; n=120 children) compared to the control group. However, the second study[50] showed that *Saccharomyces boulardii* significantly shortened the duration of hospital stay, in days, compared to the placebo group (MD -1.00; 95% CI: -1.38 to -0.62; n=200 children) (see Figure 3.5 below).

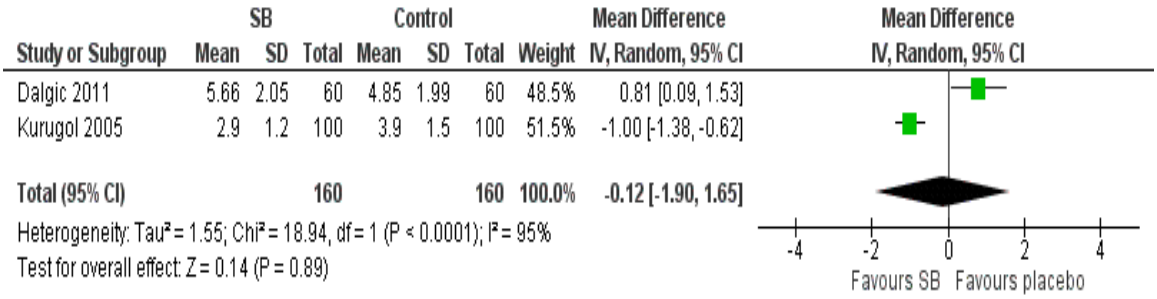

Figure 3.5: Forest plot: duration of hospital stay (in days, *Saccharomyces boulardii* versus control)

None of the ten studies evaluated other outcomes (e.g. cost-effectiveness, optimal dosing and delivery method, frequency/duration of treatment, timing of delivery of *Saccharomyces boulardii*).

3.4.2 Comparison group: *Saccharomyces boulardii* versus yoghurt fluid containing *Lactobacillus bulgaricus* and *Streptococcus thermophiles*

Results for the above comparison[41] were found to be non-significant for both the primary outcomes (i.e. duration of diarrhea in days was not different between the two groups: MD -0.93, 95% CI: -2.26 to 0.40; resolution of diarrhea at Day 3 and Day 5 showed that diarrhea had resolved significantly more in children in the *Saccharomyces boulardii* group on Day 3: MD 2.06, 95% CI: 1.02 to 4.15, but with no difference on Day 5 and an overall non-significant result of $P = 0.26$; and daily stool frequency reduction between the two groups yielded no significant difference: MD 0.96, 95% CI: -0.72 to 2.64) and secondary outcomes and therefore not discussed any further.[41]

CHAPTER 4

DISCUSSION

4.1 OVERALL COMPLETENESS AND APPLICABILITY OF EVIDENCE

Any factor that disrupts the bowels multifaceted ecosystem can result in the development of gastrointestinal disease, with GE being one of the most documented symptoms. The difficulty this presents is that GE can be categorized in various ways, i.e. according to cause (e.g. bacterial, viral, parasitic)[4,7-10], or by severity (e.g. mild, moderate and severe).[4,7-10] This systematic review was very specific as it aimed to include only those studies addressing mild-moderate GE caused by the Rotavirus. In addition, subjects needed to be between 0 and 16 years, be in a generally healthy condition with no other comorbidities, and qualify for hospitalization. The addition of studies investigating the effects of *Saccharomyces boulardii* only made this a very challenging search for supporting studies. Owing to these specifications, the initial search strategy was amended so as to be as inclusive as possible. This resulted in reviewers identifying ten RCTs involving a combined 1401 participants between the ages of 0 and 16 years for inclusion in this systematic review.

The study settings within which each of the included studies took place were in many different countries across the globe (i.e. Pakistan, India, Brazil, Myanmar, and Turkey). Aside from varied geographical settings, the included studies also included participants that were from varied backgrounds, socio-economic status's, with different research resources, different research teams and therefore varied methodological quality standards.

One of the secondary outcomes of the current systematic review was to investigate the effect of *Saccharomyces boulardii* on the days of hospitalization. Of the ten included studies, only three studies[41,48,50] reported on this outcome and each with a different result.

4.2 SUMMARY OF MAIN RESULTS

The objective of this systematic review was to assess the effectiveness and safety of *Saccharomyces boulardii* in the management of AGE in the pediatric hospitalized population. A comprehensive electronic search of potential studies, without language restrictions was carried out and resulted in ten studies (involving 1401 participants) being included in this systematic review. The included studies all considered *Saccharomyces boulardii* administration to pediatric patients being diagnosed with GE that was defined by WHO[4] as ≥ three loose/watery stools in a 24 hour period, but without display of severe dehydration. The quality of the individual included studies ranged between low and moderate, with unclear risk-of-bias displayed for especially the first four domains i.e. random sequence generation, allocation concealment, blinding of participants/personnel and blinding of outcome assessment (see Figure 3.2 and 3.3). Some of the results for some outcomes showed clear differences between groups within single studies. However, the manner in which outcomes were reported (i.e. number of stools per day, days to < three stools per day, mean number of stools) resulted in only one meta-analysis being done.

4.2.1 Primary outcomes

Five studies[42,43,44,48,53] compared *Saccharomyces boulardii* with a control, while four studies[45,47,49,50] compared *Saccharomyces boulardii* with a placebo. No active ingredients were present in either the control or placebo, thereby presenting no risk of any confounding effects. However, only five studies[42,44,48,49,50] reported the primary outcome of duration of GE in days and were therefore included in the first meta-analysis (Figure 3.4). Of these five studies[42,44,48,49,50], a total sample size of 274 participants was achieved and of the five studies, three[44,49,50] showed individual statistical significance not intercepting the line of no effect and therefore favoring the use of *Saccharomyces boulardii*. Overall, the participants in the *Saccharomyces boulardii* group produced a statistically significant result ($P < 0.0001$) indicating that participants exposed to the *Saccharomyces boulardii* intervention experienced a shorter duration of GE (MD -0.57; 95% CI: -0.83 to -0.30) than the control/placebo groups. These findings would have been more robust had a larger number of studies of similar design and investigating similar outcomes were available.

Analyzing data from the remaining four studies[43,45,47,49] was not straight-forward, as one study[45] reported findings as frequency of GE after three days of starting the *Saccharomyces boulardii* intervention, whilst the remaining three studies[43,47,49] reported on the "mean number of stools per day". Data provided by two of the studies[43,49] could not be combined in a meta-analysis with data from the remaining study[47], i.e. one study[49] produced skewed results (mean <2 SDs) resulting in it not being possible to calculate treatment effects; and the other study[43] reported the mean number of stools per day without corresponding SDs. Analysis of data produced by the remaining study[47] revealed changes in GE episodes from inclusion day (Day 0) up to and including Day 4. No difference in treatment effects were noted on Day 0, but this was not the case for other specified days. Results for this outcome showed that there was a significant difference in the mean number of stools per day between the *Saccharomyces boulardii* and the control group for Day 1 (MD -0.86; 95% CI: -1.15 to -0.57), Day 2 (MD -1.21; 95% CI: -1.49 to -0.93), Day 3 (MD -1.68; 95% CI: -1.93 to -1.43) and Day 4 (MD -1.38; 95% CI: -1.65 to -1.11).

Although the study by Corrëa *et al*[45] was aiming to assess the frequency of GE during the first three days after starting the *Saccharomyces boulardii* intervention, it is interesting that these researchers also found no notable difference between each of the groups on Day 0, but there were statistically significant reductions in the frequency of a loose stool being experienced on intervention Day 2 (RR 0.54; 95% CI: 0.42 to 0.70; $P < 0.00001$) and Day 3 (RR 0.54; 95% CI: 0.38 to 0.77).

The study by Riaz *et al*[49] provided further support to these findings with the post-intervention GE between the *Saccharomyces boulardii* group (52.85 ± 24.6 hours) being significantly less than the placebo group (64.61 ± 30.9 hours). In addition, significance was found with the time of appearance of the first semi-formed stool in the *Saccharomyces boulardii* group (39.48 ± 23.09 hours) versus the placebo group (54.13 ± 28.21 hours). However, the results from each of these studies[45,49] did differ in terms of the test for overall effect as Corrëa *et al*[45] found no statistical significance supporting the use of *Saccharomyces boulardii* in the management of AGE

whereas Riaz *et al*[49] found favor in the *Saccharomyces boulardii* group (MD -0.97; 95% CI: -1.56 to -0.39; *P* = 0.001).

The objective of this systematic review was to assess the efficacy of *Saccharomyces boulardii* on AGE in pediatric patients that were admitted for management to a hospital setting. As a result, post discharge assessments were not supposed to be tracked. However, one study[43] did assess episodes of diarrhea for participants post discharge, at both Month 1 and Month 2. It was interesting to find that these authors[43] found a residual treatment effect of *Saccharomyces boulardii* in the interventional arm, with a significantly short mean number of episodes of GE compared to the control arm, at both Month 1 (*P* = 0.001) and Month 2 (*P* = 0.04) follow-ups. This study[43] is the first one to report on the reduction in number of GE episodes during the post-treatment follow-up period. This finding is in support of reports by earlier papers[13-15] that this yeast probiotic has a mechanism of action which stimulates the host's immunity and enhances the trophic activity of mucosa by releasing polyamines, which contributes to its long-term activity of reestablishing normal microbiota status.

The study carried out by Htwe *et al*[53] chose to assess the number of participants passing fewer than three stools per day following commencement on the seven-day intervention study. Similar to results reported by other studies[43,45], no difference was recorded between the two groups in the first 24 hours. However, like results reported by three other studies[41,45,47], significant differences in stool frequency was recorded for Day 2, Day 3 and Day 4 of the study, i.e. the *Saccharomyces boulardii* group was 1.80 times on Day 2 (95% CI: 1.10 to 2.95, *P* = 0.02), 1.39 times on Day 3 (95% CI: 1.05 to 1.85, *P* = 0.02) and 1.23 times on Day 4 (95% CI: 1.05 to 1.44, *P* = 0.01) more likely to experience less than three stools per day than the control group. Little/no difference in stool frequency was noted between the two groups for Day 5, Day 6 and Day 7. Although not found to be statistically significant (*P* = 0.11), the overall effect of this study was more in favor of the *Saccharomyces boulardii* group.

The study by Htwe *et al*[53] also aimed to report on the effects of *Saccharomyces boulardii* on the consistency of stool being passed over the study period. The RR for Day 3, Day 4, and Day 5 were in favor of the *Saccharomyces boulardii* intervention group as these participants were more likely to pass solid stools as compared to the control group, i.e. Day 3 (RR 3.17; 95% CI: 1.89 to 5.31; *P* < 0.0001), Day 4 (RR 1.63; 95% CI: 1.30 to 2.06; *P* < 0.0001), and Day 5 (RR 1.25; 95% CI: 1.08 to 1.44; *P* < 0.002). Although not a strongly statistically significant result (*P* = 0.06), the overall effect of offering *Saccharomyces boulardii* to pediatric subjects with AGE was found to offer an advantage as these subjects were 1.41 times more likely to pass a solid stool sooner than the control group subjects. It must be noted though that the manner in which the "stool consistency" was assessed is questionable, i.e. according to these authors, information about the changes in stool consistency was assessed/recorded by the subjects mother or attendant. Although these authors[53] provided specific definitions for the outcomes in their study, they failed to report on any parent/personnel training or on the use of standardized stool-assessment tool/s that were used to train the mothers and/or attendants.

Safety of use of the yeast probiotic was the other primary outcome under investigation, and of the ten included studies, only one study[50] reported of a single participant complaining of "meteorism" which is defined as excess gas accumulating in the gastrointestinal system and causing abdominal distension.[3] No additional information was provided by the authors and neither was there mention of the participant needing to be removed from the trial. Other than this reporting, no serious adverse reaction in the *Saccharomyces boulardii* group were registered during any of the included studies.

4.2.2 Secondary outcomes

Duration of hospital stay will have both clinical and economic implications. In this systematic review, three studies[41,48,50] reported on the duration of hospital stay (in days). Treatment groups for two studies[48,50] were comparable and their results were combined. However, owing to significant heterogeneity (I^2 = 95%), each of their results were reported separately. The one study[48] reported that the *Saccharomyces boulardii* group was found to have a longer stay in hospital as compared to the control group (MD 0.81, 95% CI: 0.09 to 1.53). In direct contrast, the second study[50] reported that the use of *Saccharomyces boulardii* resulted in a statistically significant (P < 0.001) impact on duration of stay in hospital (MD -1.00, 95% CI: -1.38 to -0.62), i.e. *Saccharomyces boulardii* group (2.9 days) versus the placebo group (3.9 days). The remaining third study[41] found no significant difference in number of days spent in hospital between the *Saccharomyces boulardii* group and the yoghurt fluid group (MD 0.45; 95% CI: -0.64 to 1.54; P = 0.42).

4.3 QUALITY OF EVIDENCE

The ten studies included in this review lacked meticulousness when it came to methodological quality. All ten trials met the prerequisite of being RCTs. When judgment about each methodological quality item for each included study was performed, shortcomings across some of the domains for some of the studies were noted. Selection bias was clearly prevented in four studies[41,44,48,53] as methods at simple randomization were described i.e. computer-generated random numbers[44,48], according to identification numbers[41] and simple alternated allocation to treatment and control groups.[53] The remaining six studies[42,43,45,47,49,50] reported carrying out randomization but details on how this was achieved were unclear.

The manner in which allocation concealment was achieved was unclear in six studies[42,44,47,48,50,53], while two studies[45,49] adequately reported on this domain. The remaining two studies[41,43] did not report/describe the use of a placebo.

Four of the studies[45,47,49,50] adequately reported on controlling for performance bias by providing detailed information on the identical appearance of placebos offered. Three of the studies[43,44,48] did not describe how personnel and participants were blinded from treatment options and therefore designated an unclear risk status. The remaining three studies failed at preventing performance bias as one study[41] was an open clinical trial and therefore both participants and personnel were not oblivious to allocated treatment options. Both Htwe *et al*[53] and Erdogan *et al*[42] made no mention of any placebo being offered to control group participants.

Four studies[41,44,45,49] were found to have low risk for detection bias, while five other studies[42,43,47,48,53] provided insufficient information and were therefore categorized as unclear. The study by Kurugol *et al*[50] was found to be of high detection bias as a subjective method of reporting symptoms was described, i.e. parents of participants were contacted by the research team to gather data on stool numbers and consistency. These authors[50] did not describe any training offered to the parents, nor was a standard assessment tool used to categorize the quality and quantity of stools being passed. In addition, no standard descriptions of different GE types were stipulated in this study's[50] methodology e.g. watery GE versus GE.

The bulk of the studies[41,43,44,45,48,49] were found to achieve low attrition bias status. The remaining four studies[42,47,50,53] did not provide sufficient detail on how this domain was addressed and therefore marked as unclear risk.

All the studies[41,43,44,45,47,48,49,53] except for two[42,50] fully reported on all outcomes described at the onset. However, the reporting of results in the study by Kurugol *et al*[50] was unclear as, although data was provided in a table format, the authors failed to describe in detail their observations of duration of watery GE, vomiting, fever and length of hospital stay. The remaining study by Erdogan *et al*[42] was identified as high risk for reporting bias as all outcomes except for stool consistency were reported on. It must also be borne in mind that even though declared, one study[43] did receive support and funding from a pharmaceutical company i.e. this company supplied the yeast probiotic preparation which was used in the *Saccharomyces boulardii* group.

Overall, except for two studies[44,48] with unclear risk of other biases, the studies included in this systematic review did not display other forms of bias.

In order to assess the methodological quality of the included studies, a GRADE assessment was conducted (see Table 3.1). Of all the outcomes analyzed, five of the most relevant were investigated further, i.e. duration of diarrhea, mean number of stools per day, frequency of diarrhea, number having less than 3 stools per day and duration of hospital stay. Despite the absence of high quality evidence and uncertain values/preferences being presented, the overall quality of evidence across all critical outcomes was found to be moderate.

4.4 POTENTIAL BIASES IN OVERVIEW PROCESS

One of the biggest "threats" to systematic reviews is publication bias, defined as "the publication or non-publication of research findings, depending on the nature and direction of results".[1,2] As a result, this would impact on the "true" nature of the research topic under investigation. This systematic review is no exception as there is always the possibility that applicable research papers could have been missed or overlooked during various stages of the search and selection process. The use of two reviewers (MP and EV), independently assessing studies for inclusion in this systematic review would have helped to address this form of bias, but is not 100 percent full-proof.

In addition, the final search strategy did not apply any language restrictions and yielded nine foreign language abstracts, of which one appeared to be a study that required full-text reviewing. However, owing to the inaccessibility of the translated version of this article, the research team took to the decision to remove this study from the "included studies" category.

Even though two reviewers (MP and EV) independently carried out study selection, data extraction/analysis and quality assessments, these all remain subjective judgments.

4.5 AGREEMENTS AND DISAGREEMENTS WITH OTHER REVIEWS

Like the current systematic review, other researchers like Szajewska *et al* (2007)[33], McFarland (2010)[29], Allen *et al* (2010)[15] and Pan *et al* (2012)[40] have attempted to review and possibly put forward treatment guidelines for the use of *Saccharomyces boulardii* in the management of GE, but often in a mixed population of both pediatric and adult participants.

Except for the non-specification that participants needed to be admitted to hospital for the duration of the intervention, the systematic review conducted by Szajewska *et al* (2007)[33] is the only closest match to the inclusion criteria of the current systematic review. These authors[33] conducted a systematic review of only RCTs to test the effectiveness of *Saccharomyces boulardii* in treating AGE in children. Five RCTs involving 619 participants were included. The combined data showed that *Saccharomyces boulardii* significantly reduced the duration of diarrhea when compared to the control arm. Using a fixed model and random effects model, this yeast probiotic still produced a WMD of -1.1 days (95% CI: -1.2 to -0.8). Although a smaller study sample (n=548) and a smaller WMD of -0.57 days (95% CI: -0.83 to -0.30), the current systematic review also produced results in favor of use of *Saccharomyces boulardii* to treat AGE, but specifically in the pediatric patient. Again, like results from McFarland (2010)[29] and the current systematic review, significant changes in GE experienced by the *Saccharomyces boulardii* versus the control group were noted on Day 3, in addition to Day 6 and Day 7. Furthermore, these authors[33] reported than in one RCT study (n=88), the risk of diarrhea lasting in excess of 7 days was significantly reduced in the *Saccharomyces boulardii* versus the control group (RR 0.25; 95% CI: 0.08 to 0.83; number-needed-to-treat=5, 95% CI: 3 to 20). As a result, these authors[33] concluded that *Saccharomyces boulardii* displayed moderate clinical benefit in otherwise healthy infants and children with AGE.

The systematic review conducted by McFarland (2010)[29] used only RCTs and pre-clinical studies to assess the efficacy and safety of *Saccharomyces boulardii* on various forms of GE, one of which was AGE. The number of studies reporting on the use of *Saccharomyces boulardii* to treat AGE was limited, with these authors only referring to two studies in which *Saccharomyces boulardii* or a placebo was offered to a small sample (n=92) of the adult population. In the first study, participants receiving *Saccharomyces boulardii* at a dose of (8×10^9 /day) for eight consecutive days showed a significant improvement in GE severity score by Day 3 of treatment (5.5 ± 6.8; $P = 0.04$) compared to the placebo group (6.7 ± 8.7). The time point of Day 3 is significant as similar findings were found in the current systematic review. During the treatment period for four of the included

studies[41,45,47,53], Day 3 of treatment was a turning point where participants showed a significant reduction in stool frequency, some even with resolution of GE.

The second study reported by McFarland (2010)[29] involved fewer patients (n=57) whom were offered *Saccharomyces boulardii* as (1.5 x 10^{10} /day) and for a longer period of ten days. It was noted that after four weeks of treatment, all participants receiving *Saccharomyces boulardii* were cured compared with only 19 percent of those not given the yeast probiotic. Overall, the results of the meta-analysis conducted by McFarland (2010)[29] show that in 27 out of 31 studies (including 5029 study participants), *Saccharomyces boulardii* was found to be most efficacious and safe in 84 percent of the treatment arms. This reporting on efficacy complements findings in the current systematic review, i.e. participants offered *Saccharomyces boulardii* were likely to experience duration of GE at least half a day less than participants not offered *Saccharomyces boulardii*. Although this figure might not seem high, it was found to be statically significant ($P < 0.0001$).

However, it must be noted that the overall results from the meta-analysis conducted by McFarland (2010)[29] differed in many areas with the meta-analysis conducted in the present systematic review. Aside from McFarland (2010)[29] using a study population (i.e. adult) different from the current systematic review (i.e. pediatrics between 0 to 16 years), McFarland (2010)[29] also included use of *Saccharomyces boulardii* in treating GE that resulted from a wide variety of causes. By way of example, one of the studies mentioned above treated participants with acute *Entamoeba histolytica* dysentery. The current systematic review was specific to only include studies addressing GE caused by Rotavirus. Furthermore, in- and out-patient status, duration of treatment period and dosages offered differed drastically between the two systematic reviews.

The Cochrane Review carried out by Allen *et al* (2010)[15] was another systematic review aimed at assessing the effect and, like the current systematic review, safety of probiotics, including *Saccharomyces boulardii*, in treating GE. This review was much larger than the systematic reviews mentioned earlier as it included 63 studies with a combined 8014 participants. Within this large pool of studies, 56 included infants and young children. The included studies took the form of either RCTs or quasi-RCTS that compared the effect of a specified probiotic with either a placebo/no probiotic in people with AGE. The overall result was indicative of probiotics (including *Saccharomyces boulardii*), having the ability to reduce the duration of GE. But like the assessment made by McFarland (2010)[29], these authors[15] also acknowledged challenges faced with conducting their systematic review. Included studies in their systematic review varied in their definitions of both AGE and AGE-resolution, the studies were all undertaken in a wide range of different settings and there was variation in terms of the organism tested, dosage offered and participant characteristics. The authors[15] concluded that if used alongside ORS, probiotics (including *Saccharomyces boulardii*), appeared to be safe and has the *potential* to reduce AGE duration and reduce stool frequency.

The systematic review conducted by Pan *et al* (2012)[40] was similar to the current systematic review in that three of the studies[43,50,53] included in the current systematic review also featured in Pan *et al*'s (2012)[40] list of included studies. Similar to challenges experienced with the current systematic review, these authors also had difficulty retrieving suitable RCTs for inclusion, i.e. only eight included studies from a total pool of 678. These eight studies included participants that ranged between the ages of 1 month up to 12 years, were all described as being randomized into either the *Saccharomyces boulardii* or the control (commercialized ORS) group, received about the same dosage of *Saccharomyces boulardii* (500mg/d) but with only 2 studies indicating smaller doses of *Saccharomyces boulardii* (250mg/d) for participants <12 months. All participants received the intervention for a period of five to seven days, with only one study continuing to follow the participants up until Day 14. Although only 25 percent of the included studies reported on the cause of the GE, and not all studies were carried out in a hospital setting, the authors reported that the results of their meta-analysis showed that the *Saccharomyces boulardii* group was more effective than the control group with decreasing the following: duration of diarrhea (MD -0.92, 95% CI: -1.32 to -0.52), stool frequency on Day 3 (MD -1.92, 95% CI: -1.63 to -0.95), Day 4 (MD -0.51, 95% CI: -0.89 to -0.33), and Day 7 (MD -0.44, 95% CI: -0.72 to -0.16), respectively. Despite only 25 percent of included studies indicating the cause of the diarrhea in each of their studies, the authors concluded that *Saccharomyces boulardii* displayed therapeutic effects in treating children with AGE.

Based on the above few reviews, it would seem justified to conclude that *Saccharomyces boulardii* has displayed no harmful effects and has shown *consistent potential* to significantly decrease the duration of AGE in the (-adult and-) pediatric population.

4.6 STRENGTHS AND LIMITATIONS OF THIS SYSTEMATIC REVIEW

There have been multiple studies, including RCTs done on probiotics (including *Saccharomyces boulardii*) and its effects on human health. It was challenging to access studies that met all the inclusion criteria stipulated in this systematic review, i.e. *Saccharomyces boulardii* + pediatric population + no active ingredients aside from *Saccharomyces boulardii* + diagnosed with Rotavirus-causing AGE + admitted to a hospital setting for the duration of the study. From the initial search, only ten studies were able to successfully meet these inclusion requirements. That being said, these specifications did lead to a more appropriate comparison, and therefore pooling of results between the intervention and control groups of each individual study.

During the literature search phase and owing to the use of an all-inclusive search string, >2200 hits were obtained, some being clearly non-relevant. A single reviewer (MP) conducted the initial screening phase so as to sharpen the research team's focus on studies that appeared to be most relevant to the current systematic review. This might be viewed as an area of bias in this systematic review. However, based on the final ten studies included in this review, and the inclusion of the same/similar studies in other systematic reviews, it is more than likely that this screening process was not highly compromised.

The area that might be considered an absolute weakness in this systematic review is the use of English-language studies only. As mentioned earlier, an all-inclusive search string was used initially. Once foreign language studies were identified, attempts were made to access English versions of these references, i.e. the primary authors were emailed directly, or the Medical Librarian tried sourcing the respective articles via the University's library. In both instances, the primary reviewer (MP) failed to access these foreign language studies, i.e. study authors either did not respond to the enquiry or replied in the negative, or the University library was not able to access the journal or study based on its publication date and access restrictions. As a result, the research team was forced to collectively agree to remove these studies from the list of potential studies for inclusion, and acknowledge this to be a limitation in this systematic review.

Another concern would be the various geographical settings in which the ten included studies were conducted. Five different countries were identified, namely Pakistan, Myanmar, Turkey, India and Brazil. The nutritional status of the participants would vary drastically e.g. the study by Ozkan *et al*[47] was conducted in Turkey where mild/moderate malnutrition was seen in only 12.5 percent of the *Saccharomyces boulardii* group and 9.1 percent in the control group, with mortality statistics considered low in this pediatric population.[8-10] Myanmar, which is where one study[53] was conducted, would vary drastically from the aforementioned study because it is a country considered as having a high childhood mortality risk.[8-10] That being said, one of the criteria stipulated in this research review was to exclude studies including under-nourished participants, thereby removing any associated confounding effect.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSIONS

The purpose of this systematic review was to assess the efficacy and safety of the yeast probiotic *Saccharomyces boulardii* in the treatment of AGE in the pediatric hospitalized population. The primary outcomes under investigation were to assess the overall efficacy of *Saccharomyces boulardii* on the duration of GE in the pediatric patient, and to establish the safety of this yeast probiotic for use in the pediatric hospitalized patient. Additional secondary outcomes included the impact of this yeast probiotic on length of hospital stay and associated costs.

Overall, the results indicate that *Saccharomyces boulardii* shortened the duration of AGE caused by Rotavirus (in days) when compared to the control/placebo group, with the included studies displaying little/no heterogeneity. In addition, no adverse effects were associated with the use of this yeast probiotic in treating AGE in otherwise healthy children. Therefore, the results of the current systematic review indicate that there is a *potential benefit* associated with the use of *Saccharomyces boulardii* to treat AGE in the pediatric patient.[15,17,18,24-27,32,44-45,47-49,53]

Offering this unique yeast probiotic at a dose of 250mg once to twice per day for up to five to seven days has shown some statistically significant benefit with decreasing the duration of AGE. Although no statistical difference was noted between the groups with the number of days in hospital, the days to appearance of the first semi-formed stool were found to be less in the *Saccharomyces boulardii* group as compared to the control group.[15,17,18,24-27,32,44-45,47-49,53]

However, owing to factors such as small sample sizes, unclear and inconsistent quality of methodology, reporting bias owing to source of funding and support, a definitive conclusion and recommendation for the use of a specific probiotic like *Saccharomyces boulardii* to be used as treatment or treatment adjunct for AGE in the pediatric hospitalized patient cannot yet be made.

5.2 RECOMMENDATIONS FOR FUTURE RESEARCH

Future research initiatives investigating the subject of the benefits/harm associated with the use of *Saccharomyces boulardii* must therefore endeavor to consist of larger RCTs which:

- Minimize heterogeneity associated with study participants enrolled,
- Clearly pre-define aetiologies' e.g. GE, AGE etcetera
- Minimize methodological variability (e.g. blinding),
- Standardize the presentation in which the intervention is offered, and
- Single-strain probiotic investigations.

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APPENDICES

APPENDIX 6.1: Study Eligibility Form

Eligibility Form: A systematic review of the efficacy and safety of *Saccharomyces boulardii* in the treatment of acute gastroenteritis in the pediatric population.

Study Eligibility Form
***Saccharomyces boulardii* in the treatment of AGE in the pediatric population**

Reviewer ID
Study ID

Type of study			
Is the study a randomised controlled trial?	YES ↓ Go to next question	UNCLEAR ↓	NO ↓ Exclude:
Types of participants			
Are the participants between 0-16 years?	YES ↓ Go to next question	UNCLEAR ↓	NO ↓ Exclude:
Trial Intervention			
Did the participants in the study have acute gastroenteritis as defined by WHO?	YES ↓ Go to next question	UNCLEAR ↓	NO ↓ Exclude:
Was the intervention a *Saccharomyces boulardii* supplement?	YES ↓ Go to next question	UNCLEAR ↓	NO ↓ Exclude:
Was the intervention administered in a hospital setting?	YES ↓ Go to next question	UNCLEAR ↓	NO ↓ Exclude:
Types of comparison			
Was a proper control used [placebo, no intervention, other supplement(s)]?	YES ↓ Go to next question	UNCLEAR ↓	NO ↓ Exclude:
Outcomes			
Was at least one of the pre-specified outcomes in the protocol addressed?	YES ↓ Go to next question	UNCLEAR ↓	NO ↓ Exclude:
Other			
Any other reasons for excluding study? Please specify.	NO ↓ Include, subject to clarification of 'unclear' points		YES ↓ Exclude because of:
Final Decision:	Include		Exclude

Reviewer ID
Study ID

Type of study			
Is the study a randomised controlled trial?	YES ↓ Go to next question	UNCLEAR ↓	NO ↓ Exclude:
Types of participants			
Are the participants between 0-16 years?	YES ↓ Go to next question	UNCLEAR ↓	NO ↓ Exclude:
Trial Intervention			
Did the participants in the study have acute gastroenteritis as defined by WHO?	YES ↓	UNCLEAR ↓	NO ↓ Exclude:
Was the intervention a *Saccharomyces boulardii* supplement?	YES ↓	UNCLEAR ↓	NO ↓ Exclude:
Was the intervention administered in a hospital setting?	YES ↓ Go to next question	UNCLEAR ↓	NO ↓ Exclude:
Types of comparisons			
Was a proper control used [placebo, no intervention, other supplement(s)]?	YES ↓ Go to next question	UNCLEAR ↓	NO ↓ Exclude:
Outcomes			
Was at least one of the pre-specified outcomes in the protocol addressed?	YES ↓ Go to next question	UNCLEAR ↓	NO ↓ Exclude:
Other			
Any other reasons for excluding study? Please specify.	NO ↓ Include, subject to clarification of 'unclear' points		YES ↓ Exclude because of:
Final Decision:	Include		Exclude

APPENDIX 6.2: Data Extraction Form

General Information

Review title / ID	
Study ID ***(surname of first author and year)***	
Authors contact details &/or reference citation	
Publication type	
Name of review author completing this form	
Date form completed	
Notes	

Methods

	Descriptions as stated in report/paper			Location in text or source
Aim of study *(e.g. efficacy)*				
Design ***(e.g. parallel, RCT)***				
Method of recruitment				
Inclusion criteria				
Exclusion criteria				
Informed consent	Y	N	Unclear	
Ethical approval needed / obtained	Y	N	Unclear	
Funding (source/amount)				
Statistical methods used and their appropriateness				
Notes:				

Participants

	Description			Location in text or source
Population description				
Setting				
Total no. randomised				
Age *(range, mean, SD)*				
Gender	Male	Female		
Ethnicity				
Baseline imbalances				
Withdrawals / exclusions				
Severity of illness				
Co-morbidities				
Other socio-demographics				
Subgroups measured				
Subgroups reported				
Notes:				

Intervention groups

	Description as stated in report/paper	Location in text or source
Group name		
No. randomised to group		
Description		
Duration of treatment period		
Timing *(e.g. frequency, duration of each episode)*		

Delivery of intervention *(e.g. in stages, timing, frequency, duration, how?)*		
Providers (e.g. who delivers the intervention, the no. of providers; training of providers in delivery of intervention)		
Co-interventions		
Economic information		
Resource requirements		
Notes:		

Outcomes

	Description as stated in report/paper	**Location in text or source**
Outcome type		
Outcome name		
Time points measured *(specify whether from start or end of intervention)*		
Time points reported		
Outcome definition *(with diagnostic criteria if relevant)*		
Person measuring/ reporting		
Unit of measurement		
Imputation of missing data *(e.g. assumptions made for ITT analysis)*		
Assumed risk estimate *(e.g. baseline or population risk noted in Background)*		
Power *(e.g. power & sample size calculation, level of power achieved)*		
Notes:		

Risk of bias assessment

Domain	**Risk of bias rating**			**Support for judgment**	**Location in text**
	Low	High	Unclear		
Random sequence generation *(selection bias)*	Low	High	Unclear		
Allocation concealment *(selection bias)*	Low	High	Unclear		
Blinding of participants and personnel *(performance bias)*	Low	High	Unclear		
Blinding of outcome assessment *(detection bias)*	Low	High	Unclear		
Incomplete outcome data *(attrition bias)*	Low	High	Unclear		
Selective outcome reporting? *(reporting bias)*	Low	High	Unclear		
Other bias	Low	High	Unclear		
Notes:					

Data and analysis

Continuous outcome	**Description as stated in report/paper**	**Location in text or source**
Comparison		
Outcome		
Subgroup		
Time point		
Post-intervention or change from baseline?		

Outcome	**Timing of outcome** (days/months)	**Intervention S boulardii & ORS**	**Control ORS alone**	**Notes**
Mean duration of diarrhea				

Any other results reported			
No. missing participants			
Reasons missing			
No. participants moved from other group			
Reasons moved			
Unit of analysis			
Notes:			

Other information / miscellaneous

	Description as stated in report/paper	**Location in text or source**
Key conclusions of study authors		
References to other relevant studies		
Correspondence required for further study information *(from whom, what and when)*		
Notes:		

Adapted from **http://cccrg.cochrane.org/author-resources**

APPENDIX 6.3: Risk-of-bias tool

Sequence generation Was the allocation sequence adequately generated? (Adequate sequence generation?)	
Criteria for a judgment of "YES" (i.e. low risk of bias)	The investigation describes a random component in the sequence generation process such as: • Referring to a random number table; • Using a computer random number generator; • Coin tossing; • Shuffling cards or envelopes; • Throwing dice; • Drawing of lots; • Minimization
Criteria for the judgment of "NO" (i.e. high risk of bias)	The investigators describe a non-random component in the sequence generation process. Usually, the description would involve some systematic, non-random approach, for example: • Sequence generated by odd or even date of birth; • Sequence generated by some rule based on date (or day) of admission; • Sequence generated by some rule based on hospital or clinic record number; Other non-random approaches happen much less frequently than the systematic approaches mentioned above and tend to be obvious. They usually involve judgment or some method of non-random categorization of participants, for example: • Allocation by judgment of the clinician; • Allocation by preference of the participant; • Allocation based on the results of a laboratory test or a series of tests.
Criteria for the judgment of "UNCLEAR" (uncertain risk of bias)	Insufficient information about the sequence generation process to permit judgment of "Yes" or "No".
Allocation generation Was allocation adequately concealed? (Adequate allocation concealment?)	
Criteria for the judgment of "Yes" (i.e. loss risk of bias)	Participants and investigators enrolling participants could not foresee assignment because of one of the following, or an equivalent method, was used to conceal allocation: • Central allocation (including telephone, web-based and pharmacy-controlled randomization); • Sequentially numbered drug containers of identical appearance.
Criteria for the judgment of "NO" (i.e. high risk of bias)	Participants or investigators enrolling participants could possibly foresee assignments and thus introduce selection bias, such as allocation based on: • Using an open random allocation schedule (e.g. a list of random numbers); • Assignment envelopes were used without appropriate safeguards (e.g. if envelopes were unsealed or non-opaque or not sequentially numbered); • Alternation or rotation; • Date of birth; • Case record number
Criteria for the judgment of "UNCLEAR" (uncertain risk of bias)	Insufficient information to permit judgment of "Yes" or "No". This is usually the case if the method of concealment is not described or not described in sufficient detail to allow a definite judgment – for example, if the use of assignment envelopes is described, but it remains unclear whether envelopes were sequentially numbered, opaque and sealed.
Blinding of participants, personnel and outcome assessors Was knowledge of the allocated interventions adequately prevented during the study? (Blinding?)	
Criteria for the judgment of "Yes" (i.e. loss risk of bias)	Any one of the following: • No blinding, but the review authors judge that the outcome/outcome measurement are not likely to be influenced by lack of blinding; • Blinding of participants and key study personnel ensured; blinding unlikely to have been broken.
Criteria for the judgment of "NO" (i.e. high risk of bias)	Any one of the following: • No blinding or incomplete blinding, and the outcome or outcome measurement is likely to be influenced by lack of blinding; • Blinding of key study participants and personnel attempted, but likely that the blinding could have been broken.
Criteria for the judgment of "UNCLEAR" (uncertain risk of bias)	Any one of the following: • Insufficient information to permit judgment of "Yes" or "No"; • The study did not address this outcome.

Incomplete outcome data Were incomplete outcome data adequately addressed?	
Criteria for the judgment of "Yes" (i.e. loss risk of bias)	Any one of the following: • No missing outcome data; • Reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias); • Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; • For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; • For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes not enough to have a clinically relevant impact on observed effect size.
Criteria for the judgment of "NO" (i.e. high risk of bias)	Any one of the following: • Reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups; • For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; • For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect size; • "As-treated" analysis done with substantial departure of the intervention received from that assigned at randomization.
Criteria for the judgment of "UNCLEAR" (uncertain risk of bias)	Any one of the following: • Insufficient reporting of attrition/exclusions to permit judgment of "Yes" or "No" (e.g. number randomized not stated, no reasons for missing data provided); • The study did not address this outcome.
Selective outcome reporting Are reports of the study free of suggestion of selective outcome reporting? (Free of selective reporting?)	
Criteria for the judgment of "Yes" (i.e. loss risk of bias)	The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way.
Criteria for the judgment of "NO" (i.e. high risk of bias)	Any of the following: • Not all of the study's pre-specified primary outcomes have been reported; • One or more primary outcomes is reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified; • One or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect); • One or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis.
Criteria for the judgment of "UNCLEAR" (uncertain risk of bias)	Insufficient information to permit judgment of "Yes" or "No". It is likely that the majority of studies will fall into this category.
Other potential threats to validity Was the study apparently free of other problems that could put it at a risk of bias? (Free of other bias?)	
Criteria for the judgment of "Yes" (i.e. loss risk of bias)	The study appears to be free of other potential sources of bias.
Criteria for the judgment of "NO" (i.e. high risk of bias)	There is at least one important risk of bias. For example, the study: • Had a potential source of bias related to the specific study design used; or • Stopped early due to some data-dependent process (including a formal-stopping rule); or • Had extreme baseline imbalance; or • Has been claimed to have been fraudulent.
Criteria for the judgment of "UNCLEAR" (uncertain risk of bias)	There may be risk of bias, nut there is either: • Insufficient information to assess whether an important risk of bias exists; or • Insufficient rationale or evidence that an identified problem will introduce bias.

APPENDIX 6.4: Letters to Research Authors

Article enquiry: SB in AGE

Tuesday, July 7, 2015 1:52 PM

From: "Nisha Padayachee" <nisha_padayachee@yahoo.com>

To: seema_alam@hotmail.com

Cc: estelleviljoen00@gmail.com

Full Headers Printable View

Dear Author,

I am currently involved with completing a Masters in Human Nutrition Degree with Stellenbosch University (Cape Town, South Africa). The area of interest that I have based my research on is the "Safety and efficacy of Saccharomyces Boulardii in the management of Acute Gastroenteritis in the pediatric population".

I have reviewed the references and am particularly interested in the article "Efficacy and Safety of Saccharomyces boulardii in Acute Childhood Diarrhea: A Double Blind Randomised Controlled Trial". There seems to be some discrepancy in the reporting i.e. the total number of participants randomized is 108, with 54 in each the intervention and control groups. However, when totalling the numbers in Table 1, the intervention group only adds up to 47 and the control group 61.

I will sincerely appreciate it if you can clarify these discrepancies.

Kind regards,

Miss Nisha (M) Padayachee
(Registered Dietitian, Certified Nutrition Support Clinician)

Sourcing full text versions

Tuesday, April 7, 2015 5:09 PM

From: "Nisha Padayachee" <nisha_padayachee@yahoo.com>

To: wp@sun.ac.za&amp WP@sun.ac.za

Cc: estelleviljoen00@gmail.com "jconrad@sun za" <jconrad@sun.ac.za>

Full Headers Printable View

1 Files | 10KB | Download All

XLSX 10KB

Articles for Wilhelmin e to

Save

Hello Wilhelmine,

Hope you are returning from a lovely Easter break.

I have been busy working through the searches you have so thoroughly completed for me - thank you.
There are a few of them for which I am having difficulty sourcing, i.e. they are either housed in unfamiliar journals or are not in English.
Please can you help with sourcing them or steering me in the right direction.

Best wishes and have a pleasant work week,

Nisha

English version of published article

Tuesday, April 7, 2015 4:33 PM

From: "Nisha Padayachee" <nisha_padayachee@yahoo.com>

To: bleluyer@ch-havre.fr

Full Headers Printable View

Dear Author,

I am currently involved with completing a Masters in Human Nutrition Degree with Stellenbosch University (Cape Town, South Africa). The area of interest that I have based my research on is the "Safety and efficacy of Saccharomyces Boulardii in the management of Acute Gastroenteritis in the pediatric population". With the help of a medical librarian, a comprehensive literature search was conducted and a large number of hits (>2200) was obtained, of which (35) were foreign language studies.

I would be most grateful if you could indicate the availability of a translated version of the following article:
B. Le Luyer, G. Makhoul, J.-F. Duhamel. Étude multicentrique, contrôlée en double insu d'une formule adaptée enrichie en Saccharomyces boulardii dans le traitement des diarrhées aiguës du nourrisson Original Research Article Archives de Pédiatrie, Volume 17, Issue 5, May 2010, Pages 459-465.

Kind regards,

Miss Nisha Padayachee
(Registered Dietitian, Certified Nutrition Support Clinician)

English version of published articles

Friday, April 3, 2015 6:44 PM

From: "Nisha Padayachee" <nisha_padayachee@yahoo.com>

To: cdupont@uwaterloo.ca

Full Headers Printable View

Dear Author,

I am currently involved with completing a Masters in Human Nutrition Degree with Stellenbosch University (Cape Town, South Africa). The area of interest that I have based my research on is the "Safety and efficacy of Saccharomyces Boulardii in the management of Acute Gastroenteritis in the pediatric population". With the help of a medical librarian, a comprehensive literature search was conducted and a large number of hits (>2200) was obtained, of which (35) were foreign language studies.

I would be most grateful if you could indicate the availability of a translated version of the following 3 articles:
1. "Flore du nourrisson et immunité intestinale: implications et perspectives en alimentation infantile pour les prébiotiques Original Research Article Archives de Pédiatrie, Volume 7, Supplement 2, May 2000, Pages 252s-255s"
2. "Diarrhées aiguës de l'enfant Original Research Article Journal de Pédiatrie et de Puériculture, Volume 23, Issue 2, May 2010, Pages 84-95"
3. "Diarreas agudas del niño Original Research Article EMC - Pediatría, Volume 44, Issue 4, 2009, Pages 1-9"

Kind regards,

Miss Nisha Padayachee
(Registered Dietitian, Certified Nutrition Support Clinician)

English version of published article

Friday, April 3, 2015 4:22 PM

From: "Nisha Padayachee" <nisha_padayachee@yahoo.com>

To: bleluyer@ch-havre.fr

Full Headers Printable View

Dear Author,

I am currently involved with completing a Masters in Human Nutrition Degree with Stellenbosch University (Cape Town, South Africa). The area of interest that I have based my research on is the "Safety and efficacy of Saccharomyces Boulardii in the management of Acute Gastroenteritis in the pediatric population". With the help of a medical librarian, a comprehensive literature search was conducted and a large number of hits (>2200) was obtained, of which (35) were foreign language studies.

I would be most grateful if you could indicate the availability of a translated version of the following article:
A multicentric study of a lactose free formula supplemented with Saccharomyces boulardii in children with acute diarrhea. Archives de Pédiatrie; 17(5), Issy-les-Moulineaux:Elsevier Masson SAS,2010,459-465 (Journal Article)

Kind regards,

Miss Nisha Padayachee
(Registered Dietitian, Certified Nutrition Support Clinician)

A multicentric study of a lactose free formula supplemented with Saccharomyces boulardii in children with acute diarrhea.

RE:Clinics and Research in Hepatology and Gastroenterology Enquiry: English version of 2 published articles

Friday, April 3, 2015 6:24 PM

From: "GOULET Olivier" <olivier.goulet@nck.aphp.fr>

To: "Nisha Padayachee" <nisha_padayachee@yahoo.com>

Full Headers Printable View

These papers are in french
Sincerely
OG

Olivier Goulet MD, PhD
Professor of Pediatrics
Head of the Division of Pediatric Gastroenterology-Hepatology-Nutrition
National Reference Center for Rare Digestive Disease
Reference Center for Home Parenteral Nutrition
Hôpital Necker Enfants Malades-University Paris Descartes
149 rue de Sèvres 75015 Paris
tel: 00 33 1 44 49 25 60
fax: 00 33 1 44 49 25 01

De : Elsevier [stjournalsjhtp@elsevier.com] de la part de Nisha Padayachee [nisha_padayachee@yahoo.com]
Envoyé : vendredi 3 avril 2015 17:06
À : GOULET Olivier
Objet : Clinics and Research in Hepatology and Gastroenterology Enquiry: English version of 2 published articles

The following enquiry was sent via the Elsevier website:

-- Sender --
First Name: Nisha
Last Name: Padayachee
Email: nisha_padayachee@yahoo.com

-- Message --
I am currently involved with completing a Masters in Human Nutrition Degree with Stellenbosch University (Cape Town, South Africa). The area of interest that I have based my research on is the "Safety and efficacy of Saccharomyces Boulardii in the management of Acute Gastroenteritis in the pediatric population". With the help of a medical librarian, a comprehensive literature search was conducted and a large number of hits (>2200) were obtained, of which (35) were foreign language studies.

I would be most grateful if you could indicate the availability of a translated version of the following 2 articles:
1. "La flore intestinale : un monde vivant à préserver Original Research Article Journal de Pédiatrie et de Puériculture, Volume 22, Issue 3, May 2009, Pages 102-106".
2. " Effets de Saccharomyces boulardii dans le traitement et la prévention des diarrhées de l'enfant Original Research Article, Journal de Pédiatrie et de Puériculture, Volume 22, Issues 7–8, November–December 2009, Pages 337 -340

Kind regards,

Miss Nisha Padayachee
(Registered Dietitian, Certified Nutrition Support Clinician)

APPENDIX 6.5: Ethics letter

UNIVERSITEIT·STELLENBOSCH·UNIVERSITY
jou kennisvennoot • your knowledge partner

Ethics Letter

15-Jul-2014

Ethics Reference #: X14/07/012
Title: A systematic review of the efficacy and safety of Saccharomyces boulardii in the treatment of acute gastroenteritis in the pediatric population.

Dear Ms Morgambal Padayachee,

Thank you for your application. The application is for a systematic review using only data that is available in the public domain therefore the cluster head for Research Ethics has considered this proposal to be exempt from ethical review.

This letter confirms that this project is now registered and you can proceed with the work.

If you have any queries or need further help, please contact the REC Office 0219389207.

Sincerely,

REC Coordinator
Mertrude Davids
Health Research Ethics Committee 2

APPENDIX 6.6: PROSPERO Registration

PROSPERO Registration message [9913]

Tuesday, May 27, 2014 2:15 PM

From: "crd-register@york.ac.uk" <crd-register@york.ac.uk>

To: nisha_padayachee@yahoo.com

Full Headers Printable View

Dear Miss Padayachee
Thank you for submitting details of your systematic review *A systematic review of the efficacy and safety of Saccharomyces boulardii in the treatment of acute gastroenteritis in the pediatric population* to the PROSPERO register. We are pleased to confirm that the record has been published on the database.
Your registration number is: CRD42014009913
You are free to update the record at any time, all submitted changes will be displayed as the latest version with previous versions available to public view. Please also give brief details of the key changes in the Revision notes facility. You can log in to PROSPERO and access your records at http://www.crd.york.ac.uk/PROSPERO
An email reminder will be sent to you on the anticipated completion date, prompting you to update the record.
Comments and feedback on your experience of registering with PROSPERO are welcome at crd-register@york.ac.uk
Best wishes for the successful completion of your review.

Yours sincerely
Jimmy Christie
PROSPERO Administrator
Centre for Reviews and Dissemination
University of York
York YO10 5DD
t: +44 (0) 1904 321040
f: +44 (0) 1904 321041
e: CRD-register@york.ac.uk
www.york.ac.uk/inst/crd
CRD is part of the National Institute for Health Research and is a department of the University of York.
Email disclaimer: http://www.york.ac.uk/docs/disclaimer/email.htm

APPENDIX 6.7: Table of Excluded Studies

Reason for exclusion: inappropriate study setting or study design (n=12)
Assathiany R, Guedj R, Bocquet A, Thiebault G, Salinier C, Girardet JP. Pratiques de prise en charge des gastro-entérites aiguës: enquête auprès de 641 pédiatres libéraux [Treatment of acute gastroenteritis in private practice: a survey of 641 pediatricians]. *Archives de Pédiatrie.* 2013; 20(10): 1113-1119.
Bhutta ZA, Das JK, Walker N, Rizvi A, Campbell H, Rudan I. Interventions to address deaths from childhood pneumonia and diarrhoea equitably: what works and at what cost? *The Lancet.* 2013; 381(9875): 1417-1429.
Buccigrossi V, Laudiero G, Russo C, Miele E, Sofia M, Monini M, Ruggeri FM, Guarino A. Chloride Secretion Induced by Rotavirus Is Oxidative Stress-Dependent and Inhibited by *Saccharomyces boulardii* in Human Enterocytes. *PLoS ONE.* 2014; 9(6): e99830.
Canani RB, Cirillo P, Terrin G, Cesarano L, Spagnuolo MI, De Vincenzo A, Albano F, Passariello A, De Marco G, Manguso F, Guarino A. Probiotics for treatment of acute diarrhea in children: randomized clinical trial of five different preparations. *British Medical Journal Online First.* 2007;1-6.
Guzganu IL. Severe diarrhea in a 4-month-old baby girl with acute gastroenteritis: a case report and review of the literature. *Case Reports in Gastrointestinal Medicine*. 2012; 2012: 920375.
Hudson LE, Fasken MB, McDermott CD, McBride SM, Kuiper EG, Guiliano DB, Corbett AH, Lamb TJ. Functional heterologous protein expression by genetically engineered probiotic yeast *Saccharomyces boulardii*. *PLoS ONE.* 2014; 9(11): art. no. e112660.
Kullen MJ, Bettler J. The Delivery of Probiotics and Prebiotics to Infants. *Curr Pharm Des.* 2005; 11(1): 55-74.
Phavichitr N, Puwdee P, Tantibhaedhyangkul R. Cost-benefit analysis of the probiotic treatment of children hospitalized for acute diarrhea in Bangkok, Thailand. *Southeast Asian J Trop Med Public Health*. 2013; 44(6): 1065-71.
Sur D, Manna B, Niyogi SK, Ramamurthy T, Palit A, Nomoto K, Takahashi T, Shima T, Tsuji H, Kurakawa T, Takeda Y, Nair GB, Bhattacharya SK. Role of probiotic in preventing acute diarrhea in children: a community-based, randomized, double-blind placebo-controlled field trial in an urban slum. *Epidemiology and Infection*. 2011; 139(6): 919-26.
Thomas SB, Przesdzing IB, Metzke DB, Schmitz JC, Radbruch AC, Baumgart DCAB. *Saccharomyces boulardii* inhibits lipopolysaccharide-induced activation of human dendritic cells and T cell proliferation. *Clinical and Experimental Immunology.* 2009; 156(1): 78-87.
Thomas MB, Vaidyanathan M, Radhakrishnan K, Raichur AM. Enhanced viability of probiotic *Saccharomyces boulardii* encapsulated by layer-by-layer approach in pH responsive chitosan–dextran sulfate polyelectrolytes. *Journal of Food Engineering*. 2014; 136:1-8.
Villarruel G, Rubio DM, Lopez F, Cintioni J, Gurevech R, Romero G, Vandenplas Y. *Saccharomyces boulardii* in acute childhood diarrhea: a randomized, placebo-controlled study. *Acta Pædiatrica.* 2007; 1-4.
Reason for exclusion: inappropriate study participants (n=4)
El Samad Y, Havet HE, Bentayeb B, Olory B, Canarelli JF, Lardanchet Y, Douadi F, Rousseau FX, Lescure P, Mertl FE, Schmit JL. Traitement des infections ostéoarticulaires par clindamycine chez l'adulte [Treatment of osteoarticular infections with clindamycin in adults]. *Médecine et Maladies Infectieuses*. 2008; 38(9): 465-470.
Justino PFC, Melo LFM, Nogueira AF, Costa, JVG, Silva LMN, Santos CM, Mendes WO, Costa MR, Franco AX, Lima AA, Ribeiro RA, Souza MHLP, Soares PMG. Treatment with *Saccharomyces boulardii* reduces the inflammation and dysfunction of the gastrointestinal tract in 5-fluorouracil-induced intestinal mucositis in mice. *British Journal of Nutrition*. 2014; 111(9): 1611-1621.
Maioli TU, De Melo Silva B, Dias MN, Paiva NC, Cardoso VN, Fernandes SO, Carneiro CM, Dos Santos Martins F, De Vasconcelos Generoso S. Pretreatment with *Saccharomyces boulardii* does not prevent the experimental mucositis in Swiss mice. *Journal of Negative Results in BioMedicine*. 2014; 13(1).
Van Gossum A. Prise en charge à long terme du grêle court (adulte). *Nutrition Clinique et Métabolisme*. 2000; 14(4): 310-319.

Reason for exclusion: commentary, updates, practice guidelines, reviews, meta-analysis (n=71)

Allen SJ, Martinez EG, Germana V, Dans LF. Probiotics for treating acute infectious diarrhea. *Cochrane Database of Systematic Reviews*. 2010; 11(CD003048).

Amparo A, Mach N. Efecto de los probióticos en el control de la obesidad en humanos: hipótesis no demostradas. *Revista Española de Nutrición Humana y Dietética.* 2012; 16(3): 100-107.

Anon (Review Group). World Gastroenterology Organization practice guideline: Probiotics and prebiotics. *Arab Journal of Gastroenterology.* 2009; 10(1): 33-42.

Anon (Conference proceedings). European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Annual Meeting. *Journal of Pediatric Gastroenterology and Nutrition.* 2010; 50(suppl 2): E1-E217.

Applegate JA, Fischer-Walker CL, Ambikapathi R, Black RE. Systematic review of probiotics for the treatment of community-acquired acute diarrhea in children. *BMC Public Health.* 2013; 13(SUPPL.3; art. no. S16).

Butel MJ. Les probiotiques et leur place en médecine humaine. *Journal des Anti-infectieux*, In Press, Corrected Proof, Available online 25 February 2014.

Buts JP, Bernasconi P. *Saccharomyces boulardii*: Basic science and clinical applications in gastroenterology. *Gastroenterology Clinics of North America.* 2005; 34(3).

Canani B, Cucchiara S, Cuomo R, Pace F, Papale F. *Saccharomyces boulardii*: A summary of the evidence for gastroenterology clinical practice in adults and children. *European Review for Medical and Pharmacological Sciences* 2011; 15(7): 809-822.

Cézard JP, Bellaiche M, Viala J, Hugot JP. Traitement médicamenteux des diarrhées aiguëis du nourrisson et de l'enfant. *Archives de Pédiatrie.* 2007; 14(suppl 3): S169-S175.

Cherbut C. Gastro-intestinal effects of probiotics and prebiotics: Where do w e stand? *Agro-food-industry hi-tech (Milano - Teknoscience).* 2006; 17(2): 18-20.

Close AL. The effect of probiotics in reducing the duration of acute infectious diarrhea in children: a literature review. *International Journal of Probiotics and Prebiotics.* 2010; 5(4): 183-192.

Czerucka D, Piche T, Rampal P. Review article: Yeast as probiotics - *Saccharomyces boulardii*. *Alimentary Pharmacology and Therapeutics.* 2007; 26(6): 767-778.

Czerucka D, Rampal P. Experimental effects of *Saccharomyces boulardii* on diarrheal pathogens. *Microbes and Infection.* 2002; 4(7): 733-739.

Czerwionka-Szaflarska M, Adamska I, Szaflarska-Popław ska A, Romańczuk B. *Saccharomyces boulardii* w profilaktyce biegunki zw iązanej z antybiotykoterapią – dośw iadczenia własne [*Saccharomyces boulardii* in the prevention of diarrhea associated w ith antibiotic therapy – ow n experience]. *Pediatria Polska.* 2012; 87(1): 14-18.

Czerwionka-Szaflarska M, Gaw ryjołek J, Jakubow ska-Zając B, Kuczyńska R. Możliw ości leczenia ostrej biegunki u dzieci w w arunkach podstaw ow ej opieki zdrow otnej (Review Article). *Pediatria Polska.* 2013; 88(3): 263-266.

Didari T, Solki S, Mozaffari S, Nikfar S, Abdollahi M. A systematic review of the safety of probiotics. *Expert Opinion on Drug Safety.* 2014; 13(2): 227-239.

Dinleyici EC, Eren M, Ozen M, Yargic ZA, Vandenplas Y. Effectiveness and safety of *Saccharomyces boulardii* for acute infectious diarrhea. *Expert Opin Biol Ther.* 2012; 12(4): 395-410.

Feizizadeh S, Salehi-Abargouei A, Akbari V. Efficacy and safety of *Saccharomyces boulardii* for acute diarrhea. *Pediatrics.* 2014; 134(1): e176-e191.

Gąsiorowska J, Czerw ionka-Szaflarska M. Bezpieczeństw o stosow ania probiotyków u now orodków , niemowląt, kobiet ciężarnych i karmiących piersią. *Pediatria Polska.* 2010; 85(6): 624-628.

Gąsiorowska J, Czerw ionka-Szaflarska M. Znaczenie ekosystemu mikrobiontów przew odu pokarmow ego u dzieci – w ybrane zagadnienia. *Pediatria Polska.* 2011; 86(6): 639-645.

Grammatikos AP, Mantadakis E, Falagas ME. Meta-analyses on Pediatric Infections and Vaccines. *Infectious Disease Clinics of North America.* 2009; 23(2): 431-457.

Guandalini S. Probiotics for children w ith diarrhea - An update. *Journal of Clinical Gastroenterology.* 2008; 42(Suppl. 2): S53-7.

Guandalini S. Probiotics for Prevention and Treatment of Diarrhea. *J Clin Gastroenterol.* 2011; 45(Supp. 3): S149–S153.

Guarino A, Albano F, Ashkenazi S, Gendrel D, Hoekstra JH, Shamir R, Szajewska H. European society for paediatric gastroenterology, hepatology, and nutrition/European society for paediatric infectious diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe. *Journal of Pediatric Gastroenterology and Nutrition.* 2008; 46(5): 619-21.

Gutiérrez PC, Allué IP, Salazar LE. Manejo de la gastroenteritis aguda en menores de 5 años: un enfoque basado en la evidencia: Guía de práctica clínica Ibero-Latinoamericana [An evidence based Iberic-Lation American guideline for acute gastroenteritis management in infants and prescholars]. *Anales de Pediatría*. 2010; 72(3): e1-220.

Hatoum R, Labrie, S, Fliss I. Antimicrobial and probiotic properties of yeasts: From fundamental to novel applications. *Frontiers in Microbiology.* 2012; 3(Article 421).

Hempel S, Newberry S, Ruelaz A, Wang Z, Miles JN, Suttorp MJ, Johnsen B, Shanman R, Slusser W, Fu N, Smith A, Roth B, Polak J, Motala A, Perry T, Shekelle PG. Safety of probiotics used to reduce risk and prevent or treat disease. *Evid Rep Technol Assess.* 2011; 200: 1-645.

Huang JS, Bousvaros A, Lee JW, Diaz A, Davidson EJ. Efficacy of probiotic use in acute diarrhea in children: a meta-analysis. *Digestive Diseases and Sciences.* 2002; 47(11): 2625-2634.

Huebner ES, Surawicz CM. Probiotics in the Prevention and Treatment of Gastrointestinal Infections. *Gastroenterology Clinics of North America.* 2006; 35(2): 355-365.

Ignyś I, Piątkowska P, Cichy W. Probiotyki i prebiotyki w żywieniu i leczeniu dzieci. *Pediatria Polska.* 2008; 83(1): 68-75.

Jach ME, Sajnaga E, Kozak E, Malm A. Use of yeasts for prevention and therapy. *Current Issues in Pharmacy and Medical Sciences.* 2013; 26(2): 198-202.

Jiang Y, Liu J, Ren HY. Probiotics and gastrointestinal diseases. *World Chinese Journal of Digestology*. 2011; 19(17): 1813-1818.

Johnston BC, Goldenberg JZ, Vandvik PO, Sun X, Guyatt GH. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database of Systematic Reviews.* 2011, Issue 11.

Johnston BC, Supina AL, Vohra S. Probiotics for pediatric antibiotic-associated diarrhea: a meta-analysis of randomized placebo-controlled trials. *Canadian Medical Association Journal*. 2006; 175(4): 377-383.

Kelesidis T, Pothoulakis C. Efficacy and safety of the probiotic *Saccharomyces boulardii* for the prevention and therapy of gastrointestinal disorders. *Therapeutic Advances in Gastroenterology.* 2012; 5(2): 111–125.

Loś-Rycharska E, Czerwionka-Szaflarska M. Zastosowanie *Lactobacillus rhamnosus GG* w profilaktyce i leczeniu wybranych chorób przewodu pokarmowego - Review article. *Pediatria Polska.* 2008; 83(4): 402-08.

Madsen KL. The use of probiotics in gastrointestinal disease. *Can J Gastroenterology.* 2001; 15(12): 817-22.

McFarland LV. Probiotics and diarrhea. *Annals of Nutrition and Metabolism.* 2010; 57(suppl 1): 10-11.

Mearin F, Perelló A, Balboa A. Trastornos funcionales y motores digestivos. *Gastroenterologia y Hepatologia.* 2008; 31(suppl 4): 3-17.

Melchior JC, Hanachi M, Crenn P. Quand et comment conduire une nutrition entérale au cours de l'anorexie mentale? *Nutrition Clinique et Métabolisme.* 2007; 21(4): 201-208.

Moslehi-Jenabian S, Pedersen LL, Jespersen L. Beneficial effects of probiotic and food borne yeasts on human health. *Nutrients.* 2010; 2(2): 449-473.

Mugambi MN, Musekiwa Alfred, Lombard M, Young T, Blaauw R. Synbiotics, probiotics or prebiotics in infant formula for full term infants: a systematic review. *Nutrition Journal.* 2012; 11: 81.

Noone M. Management of acute gastroenteritis in children: review article. *Paediatrics and Child Health.* 2012; 22(10): 426-431.

Pan J, Hu J, Qi X, Tu Z. A meta-analysis of the efficacy of *Saccharomyces boulardii* in children with acute diarrhea. *African Journal of Pharmacy and Pharmacology.* 2012; 6(21): 1508-1514.

Pieścik-Lech M, Shamir R, Guarino A, Szajewska H. Review article: The management of acute gastroenteritis in children. *Alimentary Pharmacology and Therapeutics.* 2013; 37(3): 289-303.

Pothoulakis C. Review article: Anti-inflammatory mechanisms of action of *Saccharomyces boulardii*. *Alimentary Pharmacology and Therapeutics.* 2009; 30(8): 826-833.

Pothoulakis C. Recent advances in *Saccharomyces boulardii* research. *Gastroenterol Clin Biol.* 2010; 34(Suppl 1): S62-70.

Pooneh S, Shekoufeh N, Mohammad A. A Meta-Analysis and Systematic Review on the Effect of Probiotics in Acute Diarrhea. *Inflammation & Allergy - Drug Targets.* 2012; 11(1): 3-14.

Saavedra J. Probiotics and infectious diarrhea. *The American Journal of Gastroenterology.* 2000; 95(1): S16-S18.

Saavedra JM, Tschernia A. Human studies with probiotics and prebiotics: clinical implications. *BJN.* 2002; 87(Suppl 2); S241-6.

Salazar-Lindo E, Allué IP, Gutiérrez-Castrellón P. Guía de práctica clínica ibero-latinoamericana sobre el manejo de la gastroenteritis aguda en menores de 5 años: tratamiento farmacológico. *Anales de Pediatría.* 2014; 80(Suppl 1): 15–22.

Salvatore S, Hauser B, Devreker T, Vieira MC, Luini C, Arrigo S, Nespoli L, Vandenplas Y. Probiotics and zinc in acute infectious gastroenteritis in children: are they effective? *Nutrition*. 2007; 23(6): 498-506.

Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Efficacy of probiotics in prevention of acute diarrhea: a meta-analysis of masked, randomised, placebo-controlled trials. *The Lancet Infectious Diseases.* 2006; 6(6): 374-382.

Schneider SM, Giudicelli S. Probiotiques Et Autres Pathologies Digestives: Perspectives. *Cahiers de Nutrition et de Diététique.* 2007; 42(Suppl 2): 60-66.

Sudha M, Bhonagiri R, Kumar S, Asin M. Oral consumption of potential probiotic *Saccharomyces boulardii* strain Unique 28 in patients with acute diarrhea: a clinical report. *Benef Microbes.* 2012; 3(2): 145-50.

Sullivan A, Nord CE. The place of probiotics in human intestinal infections. *International Journal of Antimicrobial Agents*. 2002; 20(5): 313-319.

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APPENDIX 6.8: Characteristics of included studies

Billoo AG, Memon MA, Khaskheli SA, Murtaza G, Iqbal K, Shekhani MS, Siddiqi AQ. Role of a probiotic (*Saccharomyces boulardii*) in management and prevention of diarrhea. World Journal of Gastroenterology 2006 July 28;12(28):4557-4560.		
Methods	Study design: RCT Study duration: 5 day active treatment phase, followed for 2 months afterwards. Study location: Low income community, Kharadar General Hospital, Karachi	
Participants	Number of participants: 100 children ranging 2months to 12 years Intervention group: 50; Control group: 50	
Interventions	Intervention: Sb, 250mg twice per day x 5 days, WHO-CDD protocol. Control: WHO-CDD protocol only.	
Outcomes	Primary outcomes: Duration of diarrhea, mean number of stools per day, number of episodes of diarrhea, percentage weight gain.	
Notes	No placebo mentioned/described. Funding: Acknowledged support of Laboratoires Biocodex and Hilton Pharma for this study. Hilton Pharma supplied Sb (Enflor) and logistic support for the follow up of patients during the course of the study	
Bias	**Authors judgment**	**Support for judgment**
Random sequence generation	Unclear risk	Quote “100 children were randomized into two groups”.
Allocation concealment	High risk	No description/mention of any placebo used in the control group.
Blinding of participants/personnel	Unclear risk	All personnel and participants were blinded during the 5-day treatment period.
Blinding of outcome assessment	Unclear risk	There is the possibility that follow up could have been blinded, but parents knew if their child received the dissolved treatment or not.
Incomplete outcome data	Low risk	
Selective reporting	Low risk	
Other bias	Low risk	

Burande MA. Comparison of efficacy of Saccharomyces boulardii strain in the treatment of acute diarrhea in children: A prospective, single-blind, randomized controlled clinical trial. Journal of Pharmacology and Pharmacotherapeutics July-September 2013;4(3):205-208.		
Methods	Study design: Prospective, parallel, single-blinded RCT Study duration: July 2009 to July 2011 Study location: tertiary care hospital attached with Medical College, India	
Participants	Number of participants: 72 Intervention group: 35; Control group: 35	
Interventions	Intervention: Sb, 250mg x 2 daily for 5 days, ORS and zinc Control: ORS and zinc supplement only	
Outcomes	1. Days to recovery from loose motions 2. Days to recovery from vomiting	
Notes		
Bias	**Authors judgment**	**Support for judgment**
Random sequence generation	Low risk	Patients were assigned a study number corresponding to their entry in the trial. They were randomized by simple randomization with the help of computer-generated random numbers.
Allocation concealment	Unclear risk	**Quote:** "As per the allocation, drugs were prescribed to the patients by the pediatrician". **Comment:** It is not clear if the parents knew of the different treatment groups.
Blinding of participants/personnel	Unclear risk	Single blind study with parents being blind to allocation used. However, no placebo was given, so parents could compare treatments and differences.
Blinding of outcome assessment	Low risk	The passage of two consecutive formed stools as per the Kings scoring system or having no stool till the 12 hour mark.
Incomplete outcome data	Low risk	No obvious missing data or missing outcomes.
Selective reporting	Low risk	No obvious missing data or missing outcomes.
Other bias	Unclear risk	**Quotes:** "After approval from institutional ethical committee … attached to a Medical College." **Comment:** No details about funding etc.

Corrêa NBO, Penna FJ, Lima FLMS, Nicoli JR, Filho LAP. Treatment of Acute Diarrhea with *Saccharomyces boulardii* in Infants. Journal of Pediatric Gastroenterology and Nutrition. June 20 2011;53:497-501.		
Methods	Study design: Double blinded, randomized, placebo-controlled, parallel-group study Study duration: 5 days Study location: Two hospitals in Goiaˆnia, Goia´s, Brazil.	
Participants	Number of participants: 186 mixed gender children, 6-48 months with no other diarrhea episode or antibiotic use 2 weeks before trial, and AGE within 72 hrs before hospitalization. Intervention: 90; Control: 86	
Interventions	Intervention: Sb, 200mg capsules, offered every 12 hours for 5 days. Control: Placebo offered every 12 hours for 5 days.	
Outcomes	1. Clinical cure of diarrhoea. 2. Frequency of diarrhoea during the first 3 days after start of intervention. 3. Frequency of diarrhoea 3 days after start of intervention for patients presenting or not presenting with rotavirus.	
Notes	Funding not clear: “The study was supported by Conselho Nacional de Desenvolvimento Cientı´fico e Tecnolo´gico (CNPq) and Coordenac¸a˜o de Aperfeic¸oamentodo Pessoal de Ensino Superior (CAPES).”	
Bias	**Authors judgment**	**Support for judgment**
Random sequence generation	Low risk	Patients assigned a study no corresponding to their entry into trial; randomized by simple randomization with computer-generated random nos.
Allocation concealment	Low risk	Capsules were randomly coded by computer-generated numbers and distributed to the attending staff, which was composed of 2 physicians, 2 nurses and 2 nutritionists.
Blinding of participants/personnel	Low risk	Both placebo and lyophilized Sb were packaged in identical capsules. Powders on both types of capsules were similar in texture and color, and the attending staff was unaware which product was being administered.
Blinding of outcome assessment	Low risk	Clearly defined as – when evacuation frequency was <3 times per day or the stool consistency improved for at least 24hrs. If no improvement was noted in 4 days, therapy was stopped and child was remanded for further treatment of diarrhea.
Incomplete outcome data	Low risk	ITT and PP analyses performed.
Selective reporting	Low risk	Clearly stated outcomes were used.
Other bias	Unclear risk	None.

Dalgic N, Sancar M, Bayraktar B, Pullu M, Hasim O. Probiotic, zinc and lactose-free formula in children with rotavirus diarrhea: Are they effective? Pediatrics International January 11 2011;53:677-682.		
Methods	Study design: Prospective, randomized, single-blind, controlled trial. Study duration: September 2008 and June 2010 Study location: Sisli Etfal Training and Research Hospital, Turkey	
Participants	Number of participants: 480 children, ages 1 to 28 months Participants: 60 in each of the 8 groups.	
Interventions	Group 1 (Sb, 250mg/d x 5 days) Group 2 (Zinc acetate x 20mg/d x 5 days) Group 3 (Lactose-free formula offered as required) Group 4 (Sb, 250mg/d + Zinc acetate x 20mg/d) x 5 days Group 5 (Sb, 250mg/d + Lactose-free formula as required) x 5 days Group 6 (Zinc acetate 20mg/d + Lactose-free formula) x 5 days Group 7 (Sbx250mg/d+Lact-free form+Zinc acetate x20mg/d) x 5 days Group 8 (only oral and/or parenteral rehydration solutions)	
Outcomes	1. Duration of diarrhoea. 2. Duration of hospitalization. 3. Time to resolution of vomiting. 4. Time to resolution of fever.	
Notes	For rehydration, patients were offered ORS with a composition as recommended by the ESPGHAN. If necessary, because of excessive vomiting and clinical signs of dehydration, parenteral rehydration was established. The study preparation was given right after randomization, rehydration was not awaited.	
Bias	**Authors judgment**	**Support for judgment**
Random sequence generation	Low risk	The patients were randomly assigned from a computerized admissions list to 1 of 8 different treatment groups described.
Allocation concealment	Unclear risk	How participants/caregivers and researchers were kept in the dark is not clearly stated.
Blinding of participants/personnel	Unclear risk	This is a single blind study. However, it is not clear how the different treatments were made to look alike to the patients.
Blinding of outcome assessment	Unclear risk	The authors do not provide definitions for all outcomes; who conducted these assessments is not clear.
Incomplete outcome data	Low risk	Reported that all 480 participants completed the study.
Selective reporting	Low risk	All stated outcomes were reported clearly.
Other bias	Unclear risk	**Comments:** Question the likelihood that all 480 participants completed the study. Intervention "lactose-free formula" not well described in terms of amounts and duration of use.

Erdoğan Ö, Tanyeri B, Torun E, Gönüllü E, Arslan, Erenberk U, Öktem F. The Comparition of the Efficacy of Two Different Probiotics in Rotavirus Gastroenteritis in Children. Journal of Tropical Medicine 2012;Article ID 787240:1-5.		
Methods	Study design: Prospective, randomized trial Study duration: October 2009 and May 2010 Study location: Bezmialem Hospital, Turkey	
Participants	Number of participants: 75 children aged 5 months and 5 years. Intervention 1= 25; Intervention 2 = 25; Control = 25	
Interventions	Group 1 (ORS, rapid refeeding with a normal diet, 282.5mg/d *Saccharomyces boulardii*) Group 2 (ORS, rapid refeeding with a normal diet, 30mg/d *Bifidionbacterium lactis*) Group 3 (ORS, rapid refeeding with a normal diet)	
Outcomes	1. Duration time of diarrhoea; 2. Vomiting episodes at follow up.	
Notes		
Bias	**Authors judgment**	**Support for judgment**
Random sequence generation	Unclear risk	Simple comment that patients were divided into 3 groups of 25; no detail on how this was done randomly.
Allocation concealment	Unclear risk	No details given on attempts to conceal allocation
Blinding of participants/personnel	High risk	Control groups no placebo, not blinded.
Blinding of outcome assessment	Unclear risk	**Quote:** "post discharged follow up were done by telephone to elicit … stool characteristics and consistency, and episodes of vomiting per day". **Comment:** No training was provided to parents regarding reporting of these outcomes. No details regarding who conducted the telephonic interviews.
Incomplete outcome data	Unclear risk	No value vomiting episodes/d for group 3 on day 5.
Selective reporting	High risk	Regarding diarrhea: subjects and methods (page 2) states they wanted to measure the frequency of diarrhea, plus the stool consistency. But they only reported the duration time of the diarrhea.
Other bias	Low risk	

Eren M, Dinleyici EC, Vandenplas Y. Clinical Efficacy Comparison of *Saccharomyces boulardii* and Yogurt Fluid in Acute Non-Bloody Diarrhea in Children: A Randomized Controlled, Open Label Study. American Journal of Tropical Medicine and Hygiene 2010;82(3):488–491.		
Methods	Study design: randomized, prospective open-label study Study duration: April 2007 to January 2009 Study location: Eskisehir Osmangazi University Hospital, Turkey	
Participants	Number of participants: 55 children aged 5 months to 16 years. Group 1 = 28; Group 2 = 27	
Interventions	Group 1 (*Saccharomyces boulardii* 250mg x 2 daily if >/= 2 years or 125mg x 2 daily if <2 years) Group 2 (Yoghurt fluid containing *Lactobacillus bulgaricus* and *S.* thermophiles, 10^7 microorganisms/100ml; 30ml x 2 daily if >/= 2 years or 15ml x 2 daily if <2 years)	
Outcomes	1. Duration of diarrhoea. 2. Resolution of diarrhoea at days 3 and 5. 3. Days of hospitalization. 4. Duration of vomiting. 5. Cost-effectiveness of both interventions.	
Notes	All patients that were mild or moderately dehydrated were treated according to WHO recommendations with ORS and zinc supplements (10mg/d in infants ≤ 6 months and 20mg/d in patients ≥ 6 months).	
Bias	**Authors judgment**	**Support for judgment**
Random sequence generation	Low risk	Patients were randomized according to their patient ID number and enrolled in 2 groups. Patients with odd ID numbers made up group A and patients with even ID numbers made up group B.
Allocation concealment	High risk	Randomized, prospective but OPEN clinical trial – the two interventions differed visibly.
Blinding of participants/personnel	High risk	Randomized, prospective but OPEN clinical trial – the two interventions differed visibly.
Blinding of outcome assessment	Low risk	All patients were examined by 1 pediatric gastroenterologist on admission and re-evaluated every morning by the same doctor until resolution of diarrhea and discharged.
Incomplete outcome data	Low risk	ITT & PP analyses were completed for two of the outcomes.
Selective reporting	Low risk	Reported on all 5 outcomes initially mentioned.
Other bias	Low risk	

Htwe K, Yee KS, Tin M, Vandenplas Y. Effect of *Saccharomyces boulardii* in the Treatment of Acute Watery Diarrhea in Myanmar Children: A Randomized Controlled Study. American Journal of Tropical Medicine and Hygiene 2008;78(2):214–216.		
Methods	Study design: prospective, randomized controlled trial Study duration: not mentioned Study location: North Okkalapa General Hospital, Myanmar	
Participants	Number of participants: 100 children aged 3 months to 10 years. Intervention group = 50; Control group = 50.	
Interventions	Group 1 (Standard ORS to manage watery AGE, as per WHO guidelines x 5 days) Group 2 (*Saccharomyces boulardii* 250mg x 2 daily, standard ORS to manage watery AGE, as per WHO guidelines x 5 days)	
Outcomes	1. Duration of diarrhea. 2. Stool frequency per day.	
Notes		
Bias		
Random sequence generation	Low risk	Patients were alternately assigned to receive the active product (*Saccharomyces boulardii*) in addition to ORS or ORS alone.
Allocation concealment	Unclear risk	No details given regarding allocation concealment.
Blinding of participants/personnel	High risk	Treatment group received ORS plus treatment and control group received only ORS; no placebo for blinding effect of participants.
Blinding of outcome assessment	Unclear risk	Outcomes were recorded according to the information provided by the mother or attendant. Specific definitions provided for each outcome, but not clear if mother and attendants were trained on this.
Incomplete outcome data	Unclear risk	Table information indicates all subjects completed the study, but authors don't discuss this in detail.
Selective reporting	Low risk	All outcomes reported on.
Other bias	Low risk	

Kurugöl Z, Koturoğlu G. Effects of *Saccharomyces boulardii* in children with acute diarrhoea. Acta Pædiatrica 2005;94:44–47.		
Methods	Study design: Randomized placebo controlled study Study duration: not mentioned Study location: Pediatric Department, Ege University in Izmir, Turkey	
Participants	Number of participants: 200 children aged 3 months to 7 years. Intervention group = 100; Control group = 100.	
Interventions	Group 1 (*Saccharomyces boulardii*, 250mg per day x 5 days) Group 2 (identical looking placebo diluted in water or juice x 5 days)	
Outcomes	1. Duration of diarrhea. 2. Duration of watery diarrhea. 3. Duration of fever. 4. Duration of vomiting. 5. Length of hospital stay.	
Notes	ORT and normal food for their ages; parenteral rehydration if needed. No serious adverse reactions in the *Saccharomyces boulardii* group were registered during the clinical study. One child had a complaint of meteorism.	
Bias		
Random sequence generation	Unclear risk	**Quote:** "The patients were randomly allocated" **Comment:** No further info given about how randomization was achieved.
Allocation concealment	Unclear risk	No information given about allocation concealment.
Blinding of participants/personnel	Low risk	Group 1 (*Saccharomyces boulardii*) received 250mg/d; Group 2 received an identical placebo.
Blinding of outcome assessment	High risk	Parents were contacted telephonically to obtain data regarding stools and temperature. Many flaws with this method as there is no mention of training being given to parents and there are no definitions for "watery diarrhea" versus "diarrhea".
Incomplete outcome data	Unclear risk	Data of 32 children who were excluded does not appear in any analyses (ITT); no reasons given.
Selective reporting	Low risk	Stated to have observed adverse effects but very little info is given on this in the results section. Not clearly stating to have observed duration of watery diarrhea, vomiting and fever, length of hospital stay, but this is very well reported in Table II.
Other bias	Unclear risk	

Ozkan TB, Sahin E, Erdemir G, Budak F. Effect of *Saccharomyces boulardii* in Children with Acute Gastroenteritis and Its Relationship to the Immune Response. The Journal of International Medical Research 2007;35:201–212.		
Methods	Study design: Randomized, double-blinded, placebo-controlled trial Study duration: October 2004 to March 2005 Study location: Uludag University, Bursa, Turkey	
Participants	Number of participants: 27 children aged 6 months and 10 years. Intervention group = 16; Control group = 11.	
Interventions	Group 1 ((*Saccharomyces boulardii* 250mg x 2 daily x 7 days) Group 2 (Identical placebo x 2 daily x 7 days)	
Outcomes	Daily stool frequency	
Notes	All patients were given ORS and a lactose-free diet.	
Bias		
Random sequence generation	Unclear risk	**Quote:** "Patients were randomly allocated to one of two treatment groups". **Comment:** A general statement was made that patients were randomly allocated to one of two treatment groups, but no further details on how this was done is described.
Allocation concealment	Unclear risk	No details on how allocation concealment was achieved are provided.
Blinding of participants/personnel	Low risk	**Quote:** "Control group (group 2) was given a placebo treatment that had identical characteristics and appearance".
Blinding of outcome assessment	Unclear risk	No details on the outcome assessment technique used have been reported.
Incomplete outcome data	Unclear risk	No information on missing data.
Selective reporting	Low risk	All outcomes were reported on.
Other bias	Low risk	

Riaz M, Alam S, Malik A, Ali SM. Efficacy and Safety of *Saccharomyces boulardii* in Acute Childhood Diarrhea: A Double Blind Randomised Controlled Trial. Indian Journal of Pediatrics April 2012;79(4):478–482.		
Methods	Study design: double blind RCT. Study duration: May 2008 to September 2009 Study location: Diarrhea Training and Treatment Unit, India	
Participants	Number of participants: 108 children aged 3 months and 59 months. Group 1: 54; Group 2 = 54	
Interventions	Group 1 ((*Saccharomyces boulardii* mixed with puffed rice powder, 250mg x 2 daily x 5 days) Group 2 (Placebo mixed with puffed rice powder, 2 daily x 5 days)	
Outcomes	1. Duration of post intervention diarrhea (time form enrolment to recovery). 2. Frequency of stools. 3. Time of first semi-formed stool.	
Notes		
Bias		
Random sequence generation	Unclear risk	**Quote:** "After informed consent the children were randomly given either a placebo …".
Allocation concealment	Low risk	**Quote:** "A non-departmental colleague not involved in study randomized (block randomization) these identical packets of placebo or Sb".
Blinding of participants/personnel	Low risk	**Quote:** "… placebo or *Saccharomyces boulardii* (SB) in identical packets mixed with puffed rice powder".
Blinding of outcome assessment	Low risk	Used clear discharge and recovery criteria, observed by the mother (who was blinded) and then personnel.
Incomplete outcome data	Low risk	ITT and PP analysis were done.
Selective reporting	Low risk	All stated outcomes were reported on.
Other bias	Low risk	

APPENDIX 6.9: Risk-of-bias judgments for included studies

BILLOO 2006

ITEM	Authors judgments	Description
Adequate sequence generation?	Unclear	Only a comment made that "100 children were randomized into two groups".
Adequate allocation concealment?	No	*Saccharomyces boulardii* was dissolved in water or semi-solid food, but the control group received nothing; but should have ideally received a placebo powder.
Adequate blinding of participants and personnel?	Unclear	All personnel and participants were unblended during the 5-day treatment period.
Adequate blinding of outcome assessment?	Unclear	There is the possibility that follow-up personnel could have been blinded, but parents knew if their child received the dissolved treatment or not.
Incomplete outcome data addressed?	Yes	
Free of selective reporting?	Yes	
Free of other bias?	Yes	

BURANDE 2013

ITEM	Authors judgments	Description
Adequate sequence generation?	Yes	Patients were assigned a study number corresponding to their entry in the trial. They were randomized by simple randomization with the help of computer-generated random nos.
Adequate allocation concealment?	Unclear	As per the allocation, drugs were prescribed to the patients by the pediatrician. It is not clear if parents were aware of the different treatment groups.
Adequate blinding of participants and personnel?	Unclear	Single blind study with parents being blind to allocation used. However, no placebo was given, so parents could compare treatments and differences.
Adequate blinding of outcome assessment?	Yes	The passage of two consecutive formed stools as per the Kings scoring system or having no stool till the 12 hour mark.
Incomplete outcome data addressed?	Yes	No obvious missing data or missing outcomes.
Free of selective reporting?	Yes	No obvious missing data or missing outcomes.
Free of other bias?	No	The study was funded by DY Patil University and Management. Dr Pravin Chavan helped with data collection. No specific details provided regarding how much funding and where it was used.

CORRÊA 2011

ITEM	Authors judgments	Description
Adequate sequence generation?	Unclear	No information on the randomization of participants into 2 groups.
Adequate allocation concealment?	Yes	The capsules were randomly coded by computer-generated numbers and distributed to the attending staff,
Adequate blinding of participants and personnel?	Yes	Both the placebo and lyophilized *Saccharomyces boulardii* were packaged in identical capsules. Powders in both types of capsules were similar in texture and color; attending staff were unaware which product was being administered.
Adequate blinding of outcome assessment?	Yes	Clearly defined as when evacuation frequency was <3 times per day or the stool consistency improved for at least 24 ours. During the trial period, if no improvement was observed in 4 days of intervention, then the therapy was stopped and the child was sent for further treatment of diarrhea.
Incomplete outcome data addressed?	Yes	PP and ITT were completed.
Free of selective reporting?	Yes	Clearly stated outcomes were used.
Free of other bias?	Yes	None

DALGIC 2011

ITEM	Authors judgments	Description
Adequate sequence generation?	Yes	The patients were randomly assigned from a computerized admissions list to one of the eight different treatment groups described.
Adequate allocation concealment?	Unclear	No information about allocation concealment from participants, caregivers and researchers was achieved.
Adequate blinding of participants and personnel?	Unclear	This is a single blind study but its not clear how the different treatments were made to "look alike" to the participants.
Adequate blinding of outcome assessment?	Unclear	It is not clear who performed the outcome assessments, but clear definitions are given.
Incomplete outcome data addressed?	Yes	Authors report that all 480 participants completed the study.
Free of selective reporting?	Yes	All outcomes mentioned in methods section are reported on in the results section.
Free of other bias?	Unclear	The authors reported that among the 480 participants, no-one was lost to withdrawal or exclusion or loss to follow-up. Not enough information is given regarding the intervention "lactose-free formula" is given (e.g. amount offered and duration of use).

ERDOGAN 2012

ITEM	Authors judgments	Description
Adequate sequence generation?	Unclear	A comment with no supporting details was made that participants were divided into 3 groups of 25.
Adequate allocation concealment?	Unclear	No details given on attempts made to conceal allocation. Control group was not offered a placebo and therefore no blinding.
Adequate blinding of participants and personnel?	No	Control group received no placebo (only the ORT and diet, like the other two groups) and were therefore not blinded.
Adequate blinding of outcome assessment?	Unclear	No training was provided to parents regarding reporting of these outcomes. No details regarding who conducted the telephonic interviews.
Incomplete outcome data addressed?	Unclear	No value supplied for the rate of vomiting episodes per day for group 3 on day 5.
Free of selective reporting?	No	Stool characteristics and consistency were not reported on in the results section.
Free of other bias?	Yes	

EREN 2010

ITEM	Authors judgments	Description
Adequate sequence generation?	Yes	Patients were randomized according to their patient ID number and enrolled in 2 groups. Patients with an odd ID number composed group A and those with an even ID number composed group B.
Adequate allocation concealment?	No	The two interventions differed visibly.
Adequate blinding of participants and personnel?	No	This study design was described as a randomized prospective but open clinical trial.
Adequate blinding of outcome assessment?	Yes	All patients were examined by 1 pediatric gastroenterologist on admission and re-evaluated by the same doctor until resolution of diarrhea and discharge. A standard evaluation tool (Bristol criteria) was used to evaluate participants at each visit.
Incomplete outcome data addressed?	Yes	ITT and PP analyses were completed for two of the outcomes.
Free of selective reporting?	Yes	Authors reported on all 5 outcomes initially mentioned.
Free of other bias?	Yes	

HTWE 2008

ITEM	Authors judgments	Description
Adequate sequence generation?	Yes	Patients were alternately assigned to treatment groups.
Adequate allocation concealment?	Unclear	No details given regarding allocation concealment.
Adequate blinding of participants and personnel?	No	The treatment group received ORS plus treatment and the control group received only ORS – no placebo was mentioned.
Adequate blinding of outcome assessment?	Unclear	Outcomes were recorded according to the information provided by the mother or attendant. Specific definitions provided for outcomes, but it was not made clear if mothers or attendants were trained for this.
Incomplete outcome data addressed?	Unclear	Table information indicates all subjects completed the study, but authors don't discuss this in any detail.
Free of selective reporting?	Yes	All outcomes were reported on.
Free of other bias?	Yes	

KURUGOL 2005

ITEM	Authors judgments	Description
Adequate sequence generation?	Unclear	A general statement that patients were randomly allocated to treatment groups was made but no further information on how this was done is provided.
Adequate allocation concealment?	Unclear	No information given about allocation concealment.
Adequate blinding of participants and personnel?	Yes	The patients in group 1 (*Saccharomyces boulardii*) received 250gm/d diluted with water or juice in accordance with the manufacturer's instructions, whilst those in group 2 (placebo) received an identical placebo.
Adequate blinding of outcome assessment?	No	Parents were contacted telephonically to obtain data reading stools and temperature. This practice would present multiple opportunities for subjective reporting. No mention of training being done for parents regarding how to assess changes in the participant's diarrheal status (e.g. watery diarrhea versus diarrhea).
Incomplete outcome data addressed?	Unclear	The data of the 32 participants who were excluded does not appear in any analysis (e.g. ITT) and reasons for this exclusion are not mentioned.
Free of selective reporting?	Unclear	Researchers report that adverse effects were observed but little/no information was given regarding this in the results section.
Free of other bias?	Yes	

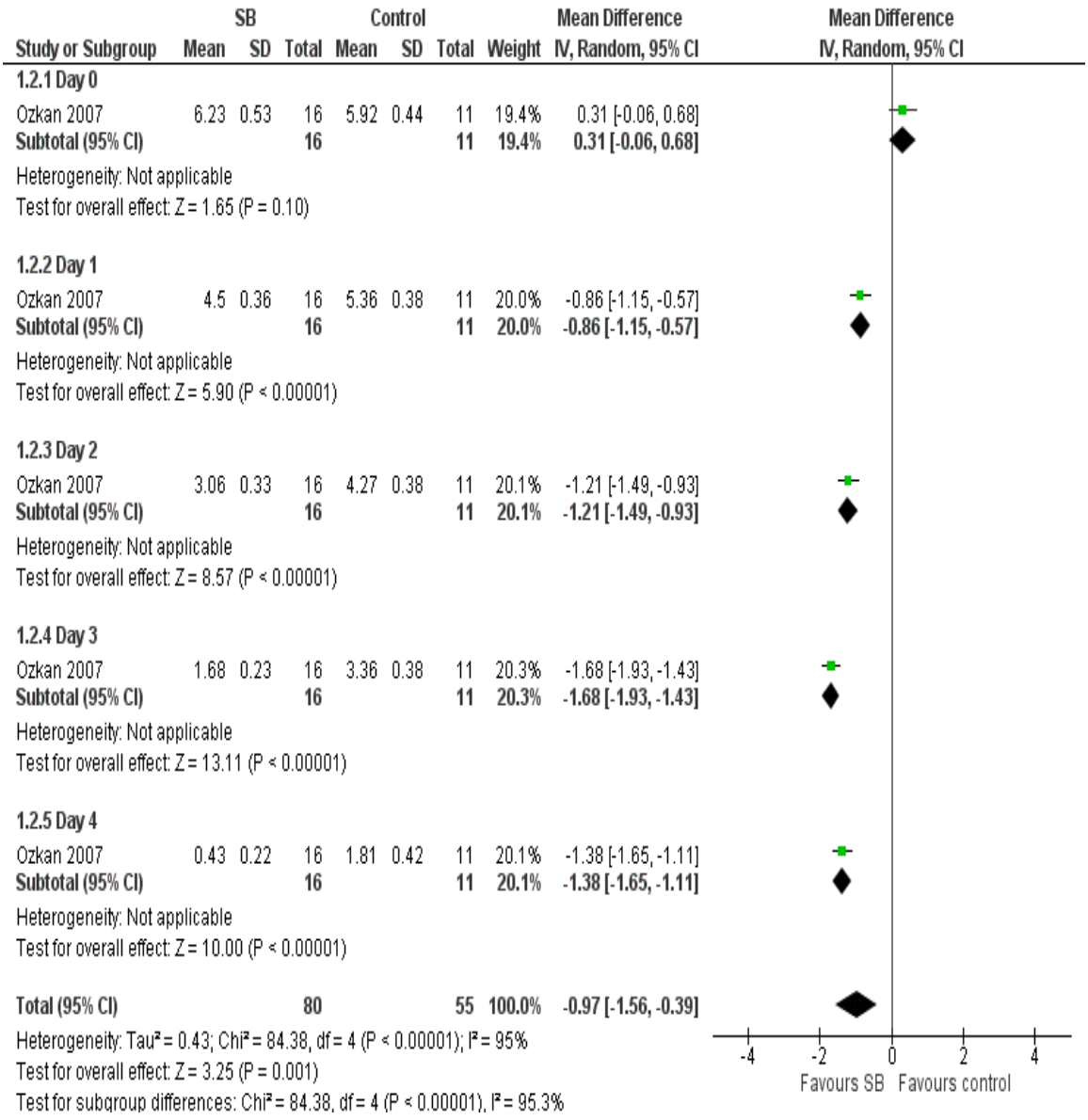
OZKAN 2007

ITEM	Authors judgments	Description
Adequate sequence generation?	Unclear	A general statement was made that patients were randomly allocated to one of two treatment groups, but no further details on how this was done is described.
Adequate allocation concealment?	Unclear	No details on how allocation concealment was guaranteed are provided.
Adequate blinding of participants and personnel?	Yes	The control group (group 2) was given a placebo treatment that had identical characteristics and appearance.
Adequate blinding of outcome assessment?	Unclear	No details on the outcome assessment technique used have been provided.
Incomplete outcome data addressed?	Unclear	No information on any incomplete data.
Free of selective reporting?	Yes	All outcomes were reported on.
Free of other bias?	Yes	

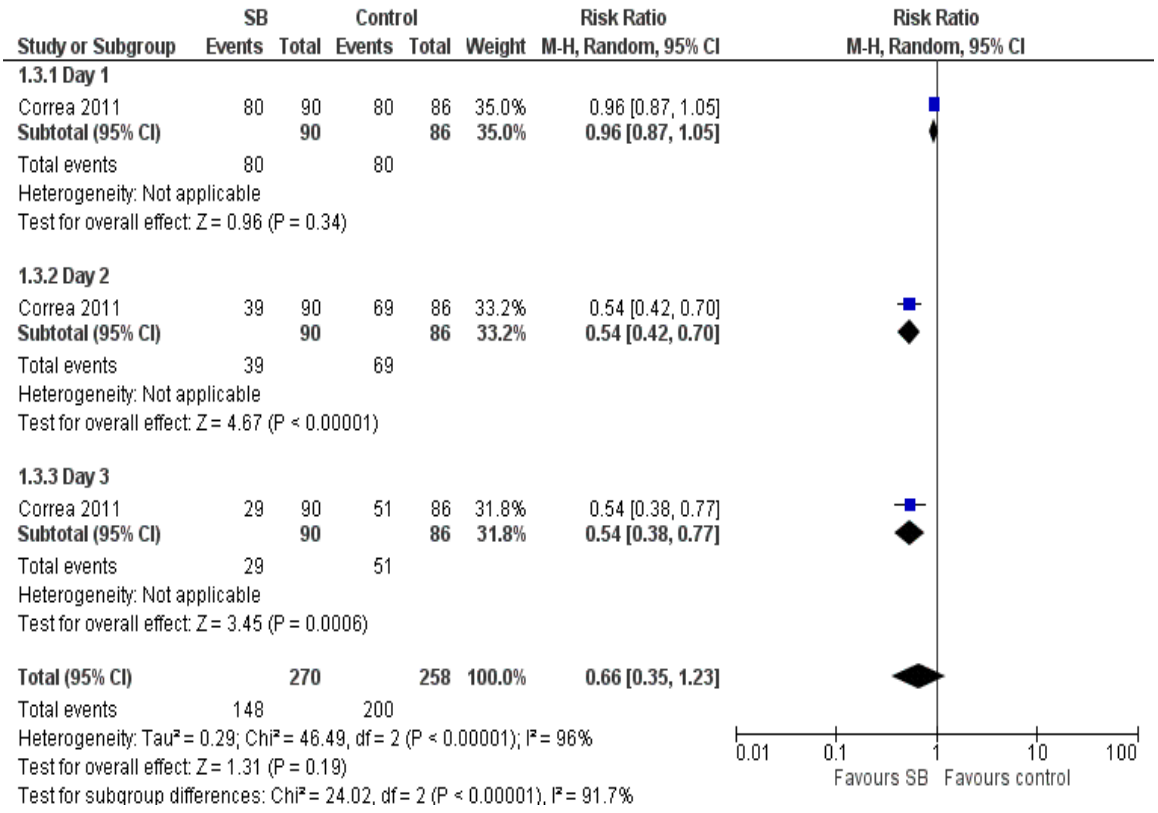
RIAZ 2010

ITEM	Authors judgments	Description
Adequate sequence generation?	Unclear	A general comment with no further detail is given stating that after informed consent was obtained, the participants were *randomly* given wither a placebo or intervention.
Adequate allocation concealment?	Yes	A non-departmental colleague not involved in the study randomized these identical packets of placebo or *Saccharomyces boulardii*.
Adequate blinding of participants and personnel?	Yes	Placebo or *Saccharomyces boulardii* in identical packets mixed with puffed rice powder.
Adequate blinding of outcome assessment?	Yes	Use clear discharge and recovery criteria, observed by the mother (who was blinded) and then personnel.
Incomplete outcome data addressed?	Yes	ITT and PP analysis were done.
Free of selective reporting?	Yes	All stated outcomes were reported on.
Free of other bias?	Yes	

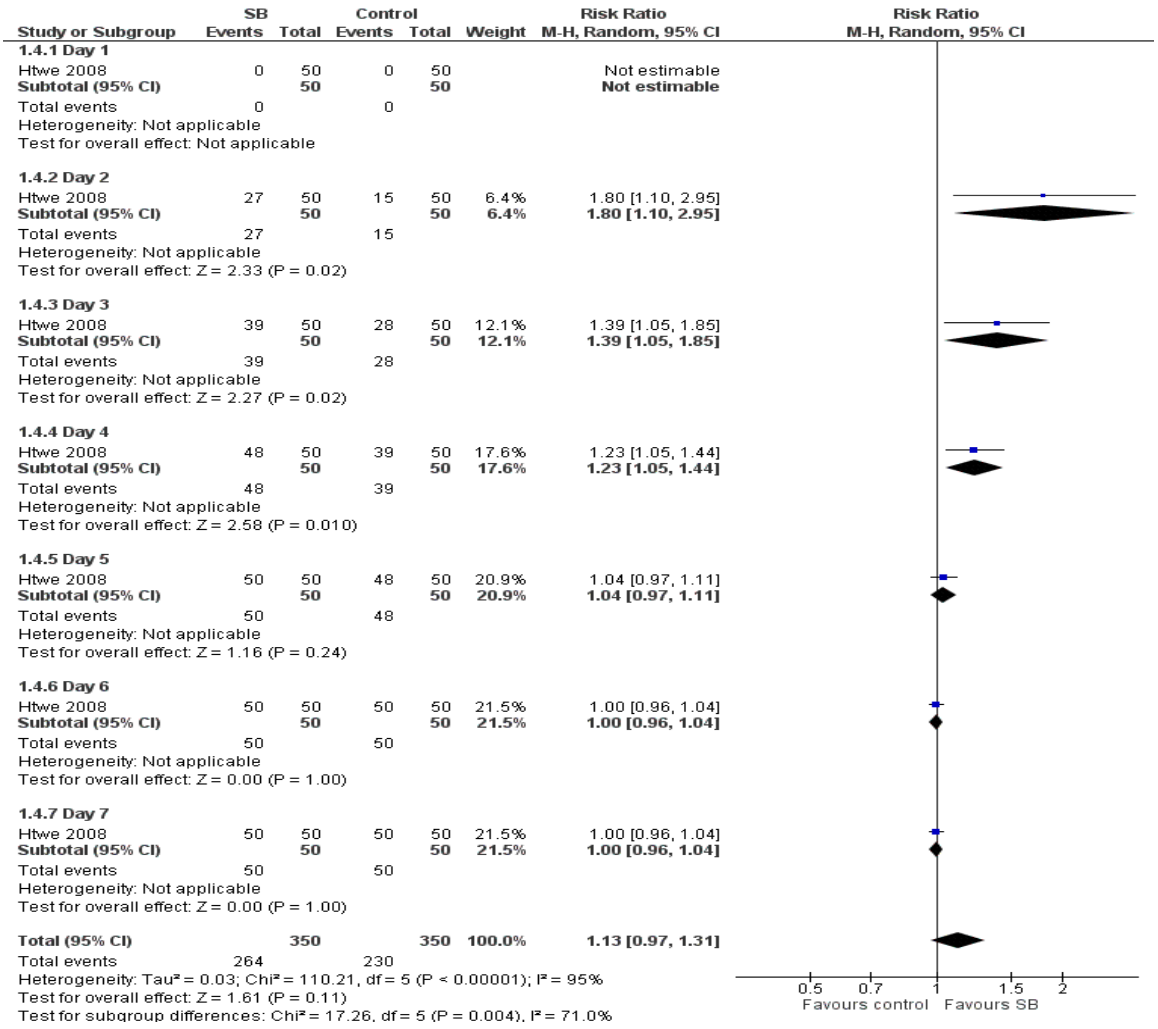
APPENDIX 6.10: Forest plot: mean number of stools per day (*Saccharomyces boulardii* versus control)



APPENDIX 6.11: Forest plot: frequency of diarrhea during the first three days post intervention (*Saccharomyces boulardii* versus control)



APPENDIX 6.12: Forest plot: number having less than three stools per day during the first 7 days after starting intervention (*Saccharomyces boulardii* versus control)



APPENDIX 6.13: Forest plot: number having solid stools during the first 7 days after starting intervention (*Saccharomyces boulardii* versus control)