Unraveling the Clinical Efficacy of Probiotics in Pediatrics

Written by

My Kim Chau

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Department of Pharmacology and Toxicology
University of Toronto

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2016

Abstract

Evidence have shown that manipulation of the intestinal microbiota with probiotics are promising therapeutic agents for restoring health, particularly in pediatrics, as probiotics holds a good safety profile. Focus has been on the treatment of a number of common pediatric gastrointestinal conditions, leading to a rise in the number of clinical trials and systematic reviews being published. Therefore, an overview of systematic reviews (OoSR) was conducted to consolidate the evidence on the clinical efficacy of probiotics as a therapeutic option to treat pediatric gastrointestinal conditions.

Recent attention has been on probiotics for the treatment of infantile colic, as the etiology remains elusive with limited treatment options. Therefore, a 21-day randomized, double-blind, placebo-controlled trial was conducted to determine the efficacy of Lactobacillus reuteri DSM 17938 (10^8 colony-forming units; n = 24) versus placebo (n = 28) to treat colic. Final analyses revealed that colicky infants receiving L. reuteri cried/fussed (min/d) significantly less compared to placebo (Day 21, median [IQR]: 60(64) vs. 102(87); (P=0.045), respectively. Furthermore, more infants in the L. reuteri group showed a 50% crying/fussing time reduction compared to placebo (17 infants vs. 6 (P=0.035); RR: 3.3 [95% CI: 1.55 to
Thus, *L. reuteri* was shown effective at treating colic in breastfed Canadian infants compared to placebo.

However, contradictory results from a larger RCT concluded *L. reuteri* was ineffective. Therefore, a meta-analysis was conducted to determine the effectiveness of *L. reuteri* to treat colic by pooling treatment effects from five RCT (total $n = 335$; *L reuteri* $n = 172$, placebo $n = 163$). Pooled estimates revealed infants receiving *L. reuteri* cried/fussed significantly less (mean difference: 43.5 minutes; 95% CI: 54.3 to 32.6 minutes) and successfully responded to treatment by day 7 ($RR = 2.43$; 95% CI: 1.41 to 4.16; $P < 0.00001$) compared to the placebo group. The quality of evidence of each outcome was assessed using Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. Therefore, evidence shows *L. reuteri* leads to significant improvements in colic symptoms compared to placebo; however, larger trials are needed to definitively endorse *L. reuteri* as part of routine clinical practice.
Acknowledgements

“To get to the full value of joy you must have someone to divide it with.”
~ Mark Twain

Everyone mentioned here is my ‘someone’! Immeasurable and deepest gratitude for the help and support are extended to the following persons whom in one way or another have contributed to making this entire journey possible.

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I dedicate this thesis to my hubs, Dimitri.  

And my beloved boys, Zane & Kade.  

I love you.
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<tr>
<td>AAD</td>
<td>Antibiotic-associated diarrhea</td>
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<tr>
<td>ACCP</td>
<td>American College of Chest Physicians</td>
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<td>AMP</td>
<td>Anti-microbial proteins</td>
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<tr>
<td>BID</td>
<td>Twice daily</td>
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<td>BMJ</td>
<td>British Medical Journal</td>
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<tr>
<td>C. diff</td>
<td><em>Clostridium difficile</em></td>
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<tr>
<td>CADTH</td>
<td>Canadian Agency for Drugs and Technology in Health</td>
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<tr>
<td>CCRBT</td>
<td>Cochrane Collaboration risk of bias tool</td>
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<tr>
<td>CDAD</td>
<td><em>Clostridium difficile</em>-associated diarrhea</td>
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<tr>
<td>CDI</td>
<td><em>Clostridium difficile</em> infection</td>
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<tr>
<td>CFU</td>
<td>Colony forming units</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CMP</td>
<td>Cow’s milk protein</td>
</tr>
<tr>
<td>CMPA</td>
<td>Cow’s milk protein allergy</td>
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<tr>
<td>DC-SIGN</td>
<td>Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin</td>
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<tr>
<td>EBM</td>
<td>Evidence-based medicine</td>
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<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
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<tr>
<td>EPDS</td>
<td>Edinburgh Postpartum Depression Score</td>
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<td>FGIDs</td>
<td>Functional gastrointestinal disorders</td>
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<td>FOXP3+</td>
<td>Forkhead Box P3</td>
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<tr>
<td>GALT</td>
<td>Gut associated lymphoid tissue</td>
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<td>GER</td>
<td>Gastro-esophageal reflux</td>
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<td>GERQ</td>
<td>Gastro-esophageal reflux questionnaire</td>
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<td>GF</td>
<td>Germ-free</td>
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<td>GI</td>
<td>Gastrointestinal</td>
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<td>Abbreviation</td>
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<td>GIT</td>
<td>Gastrointestinal tract</td>
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<td>GPCRs</td>
<td>G-protein coupled receptors</td>
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<tr>
<td>GPP</td>
<td>Good publication practice</td>
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<tr>
<td>GRADE</td>
<td>Grading of Recommendations Assessment, Development and Evaluation</td>
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<tr>
<td>GRAS</td>
<td>Generally recognized as safe</td>
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<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
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<td>IBS</td>
<td>Irritable bowel syndrome</td>
</tr>
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<td>IBS-D</td>
<td>Diarrhea-predominant IBS</td>
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<td>IEC</td>
<td>Intestinal epithelial cells</td>
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<td>IgA</td>
<td>Immunoglobulin A</td>
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<td>IL-12</td>
<td>Interleukin-12</td>
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<td>IL-6</td>
<td>Interleukin-6</td>
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<td><em>L. reuteri</em></td>
<td><em>Lactobacillus reuteri</em> DSM 17938</td>
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<tr>
<td>LAB</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>LGG</td>
<td><em>Lactobacillus rhamnosus</em> GG</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>MD</td>
<td>Mean difference</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor kappa (κ)-light-chain-enhancer of activated B cells</td>
</tr>
<tr>
<td>NICE</td>
<td>National Institute for Health and Clinical Excellence</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>NIH-CTCAE</td>
<td>NIH Common terminology criteria for adverse events</td>
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<td>NNT</td>
<td>Number needed to treat</td>
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<td>OoSR</td>
<td>Overview of systematic reviews</td>
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<td>PBMC</td>
<td>Peripheral blood mononuclear cells</td>
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<td>PI-IBS</td>
<td>Post-infectious IBS</td>
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<tr>
<td>PPI</td>
<td>Proton pump inhibitor</td>
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<tr>
<td>QD</td>
<td>Once daily</td>
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<tr>
<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>QID</td>
<td>Four times daily</td>
</tr>
<tr>
<td>QoE</td>
<td>Quality of evidence</td>
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<tr>
<td>QOL</td>
<td>Quality of life</td>
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<tr>
<td>RAP</td>
<td>Recurrent abdominal pain</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized control trial</td>
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<tr>
<td>REB</td>
<td>Research ethics board</td>
</tr>
<tr>
<td>RoB</td>
<td>Risk of bias</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short-chain fatty acids</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SIDS</td>
<td>Sudden infant death syndrome</td>
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<td>SoF</td>
<td>Summary of Findings</td>
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<td>SRMA</td>
<td>Systematic review and meta-analysis</td>
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<td>TLR</td>
<td>Toll-like receptor</td>
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<td>TNF</td>
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<td>Treg</td>
<td>T-regulatory cells</td>
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<td>WHO</td>
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Chapter 1
Introduction

1.1 Statement of the Problem

For many decades, the use of probiotics was primarily considered alternative medicine; however, a recent shift has now brought probiotics into mainstream medical care. While there has been a steady rise in the number of potential health benefits attributed to probiotic therapies, leading to a proportional increase in probiotic efficacy studies (Kaur, Kuhad, Garg, & Chopra, 2009), there still remains contradictory evidence from well-designed clinical trials of probiotic effects and even fewer consistent studies in the paediatric population (Cruchet et al., 2015). Probiotic research in paediatrics has primarily focused on gastrointestinal diseases and preliminary results have been promising; however, there is difficulty demonstrating that a direct causal relationship exists between the use of probiotics, leading to colonization of bacteria in the intestinal microbiota, and subsequent improvement of clinical symptoms (van den Nieuwboer, Browne, & Claassen, 2016). Furthermore, only a limited number of microbial strains have been studied, and it is important to recognize that the clinical efficacy of different probiotic microorganisms is not equivalent; therefore, the clinical community is faced with limitations in generalizing the available evidence (Goodrich et al., 2014; Tiwari, Tiwari, Pandey, & Pandey, 2012; van den Nieuwboer et al., 2016). Probiotics are considered to be tolerable and safe in otherwise healthy individuals (Sanders et al., 2010; Hempel et al., 2011; van den Nieuwboer et al., 2015), despite the lack of conclusive evidence for their use in most gastrointestinal illnesses and contradictory results proving clinical efficacy (Sanders et al., 2013; Zuccotti, Albani, & Meneghin, 2016), probiotics are commonly used, sometimes inappropriately to prevent and treat various clinical conditions (van den Nieuwboer et al., 2015; Cruchet et al., 2015). This is a serious concern because of the potential risks that may be associated with incorrect use of probiotics, especially among children with co-morbidities or immuno-compromised individuals.
1.2 Purpose of the Study and Objectives

The overall objective was to examine the efficacy of probiotics in the paediatric population. This thesis addresses three specific aims:

1. To critically examine the current available body of evidence on the clinical efficacy of common probiotic species (i.e. *Lactobacillus* spp., *Bifidobacterium* spp., *Sacchromyces boulardii*, *Streptococcus thermophilus*) as a prevention and/or treatment for gastrointestinal conditions in the pediatric population (e.g., birth to 18 years of age);

2. To investigate the efficacy of the specific probiotic strain, *Lactobacillus reuteri* (L. reuteri) DSM 17938, for the treatment of infantile colic in breastfed Canadian infants, compared to placebo;

3. To critically assess the evidence and clarify the effectiveness of *Lactobacillus reuteri* DSM 17938 in reducing crying and fussing in breastfed colicky infants using a systematic review and meta-analysis from all available randomized controlled trials.

1.3 Rationale and Statement of Research Hypotheses

The human gastrointestinal tract (GIT) is ubiquitously colonized by a diverse and an abundant number of microorganisms, referred to as the intestinal microbiota, and shares a mutualistic relationship with the host. With our progress in knowledge, it is now well-established that the intestinal microbiota is intimately involved in various aspects of normal host physiology, as it serves many important functions (Marchesi et al., 2016; Hornef, 2015; McFarland, 2014; Sommer & Bäckhed, 2013; Sanders et al., 2013; Fujimura, Slusher, Cabana, & Lynch, 2010). To further substantiate the importance of the role the gut microbiota plays, most essential bodily functions, such as body temperature regulation, tissue growth, and reproduction, are influenced by the intestinal microbiota (Sommer & Bäckhed, 2013; Fujimura et al., 2010; Sekirov, Russell, Antunes, & Finlay, 2010), strongly suggesting that the gut microbiota may influence health and disease risk.

This has led to the idea that disruption of the composition of the intestinal microbiota leads to disease and that subsequently, the consumption of probiotics may restore intestinal microbial equilibrium (McFarland, 2014). Consequently, the notion that probiotic
supplementation may lead to the modulation of the composition and/or activity of the intestinal microbiota has rapidly advanced probiotics as a novel therapeutic option for various conditions, particularly in the paediatric population, as it carries the notion that it is ‘natural’ and thus, lacks adverse effects (Cruchet et al., 2015; van den Nieuwboer et al., 2015; Hempel et al., 2011; Wallace, 2009; NASPGHAN, Michail, Sylvester, Fuchs, & Issenman, 2006; Ishibashi & Yamazaki, 2001). Accordingly, to date, there is a vast number of published clinical trials and systematic reviews, some of which show conflicting results and others making unsubstantiated health claims, surrounding the use of probiotics to treat paediatric GI conditions, potentially leading to the ineffective use of probiotics (Sanders et al., 2013; Zuccotti et al., 2016). This is of great concern because not all microorganisms exert the same effect on the host; each probiotic species, and even distinct strains within each species, may have different activities (Reid, 2016; Sanders et al., 2013; Gareau, Sherman, & Walker, 2010; Sherman, Ossa, & Johnson-Henry, 2009). This necessitates the urgency to eliminate uncertainty by comprehensively examining the effectiveness and quality of evidence (QoE) for specific species and strain of probiotics for paediatric GI conditions.

Additionally, though several published randomized controlled trials (RCTs) examine *L. reuteri* DSM 17938 for the treatment of colic (Chau et al., 2015; Mi et al., 2015; Savino et al., 2010; Sung et al., 2014; Szajewska, Gyrczuk, & Horvath, 2013a), these trials showed contradictory results and therefore, do not provide definitive answers with respect to the effectiveness of *L. reuteri* DSM 17938 as a treatment option. Furthermore, there is a need to conduct repeat studies in different populations, as there is geographical and cultural variability in the composition of intestinal microbiota (Fallani et al., 2010; Yatsunenko et al., 2012).

Accordingly, the primary aim of this thesis is to examine and understand the potential effect of specific probiotic species and strains in the prevention and treatment of various paediatric GI conditions. The role of a specific probiotic strain, *L. reuteri* DSM 17938, for the treatment of infantile colic was also clinically investigated.

The general hypothesis of this thesis is that, because specific strains of probiotics are capable of modulating the intestinal microbiota composition, probiotics may be clinically efficacious in the prevention and/or treatment of certain paediatric GI conditions. More specifically, it was hypothesized that, due to the link between the intestinal microbiota and the development of infantile colic, supplementation with *Lactobacillus reuteri* DSM 17938 is an effective treatment option to improve colic symptoms.
2.1. Overview of the Human Intestinal Microbiota

2.1.1. Defining the Intestinal Microbiota

In humans, the gastrointestinal tract (GIT) is ubiquitously colonized by various microbial species consisting of viruses, archaea and eukaryote fungi; however, bacteria are the most abundant microbial units in a normal human gut (Kirchfeld, Wade, 1994; Metchnikoff, 1907). For many decades, the term *microflora* has been mistakenly misused interchangeably with *microbiota* to describe the assortment of microorganisms that resides along the entire GIT sharing a symbiotic relationship with the host (Huttenhower, 2012; Ursell, Metcalf, Parfrey, & Knight, 2012). Historically, the term *microflora* is used in reference to plants (Kunz, Kuntz, & Rudloff, 2009) and since bacteria bear little resemblance to plants, scientists in the area of microbial research have recently transitioned to predominantly using the term *intestinal microbiota* to describe the assemblage of microbes in the human gut, replacing the term *gut microflora* (Ursell et al., 2012).

The intestinal microbiota houses as many as $10^{14}$ microorganisms composed of various genera, species and strains of bacteria inhabiting the human gut, outnumbering somatic and germ cells by 10 to 1 and making up 1 to 3 percent of the body’s mass. This shared mutualistic relationship between the host and microbes is most preferable to colonize the GIT, as the human gut constitutes the body’s second largest surface area covering approximately 200–400 m$^2$ and thus, easily accommodating the 1,000 distinct bacterial species (Gebbers & Laissue, 1989). Furthermore, the ubiquitous presence and high density of indigenous bacteria, referred to as *commensals*, within the GIT confirms that microbes play an important role within the host, as commensal bacteria reside in the host and do not cause adverse effects under normal conditions (Eckburg et al., 2005; Fujimura et al., 2010). Rather, commensal bacteria co-evolved with the host and are essential for many physiological processes, such as, enhancement of the intestinal epithelial barrier by providing colonization resistance against invasion by pathogenic bacteria; maturation and development of the immune system; macronutrient metabolism; and synthesis of micronutrients (*e.g.*, vitamins B and K) from food sources (Jandhyala et al., 2015; Krajmalnik-Brown, Ilhan, Kang, & DiBaise, 2012; Sommer &
Bäckhed, 2013). However, under certain circumstances, typically external or environmentally induced (e.g., secondary to antibiotic intake), commensal species can become *pathobionts*, indigenous silent microbes that may become virulent and cause pathology, resulting in the activation of pro-inflammatory and/or allergic responses (Hornef, 2015; Kamada, Chen, Inohara, & Núñez, 2013; Round & Mazmanian, 2009). As such, the host-microbial, microbial-microbial, and microbial-environmental interactions are able to control the distribution of commensals throughout the GIT with the ability to alter the intestinal microbiota balance and influence host health (Table 2.1) (Bäckhed, Ley, Sonnenburg, Peterson, & Gordon, 2005; Hornef, 2015; Jandhyala et al., 2015; Kamada et al., 2013; Round & Mazmanian, 2009; Sommer & Bäckhed, 2013).

**Table 2.1: Definition of symbiotic relationships**

<table>
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<tr>
<th>TERMS</th>
<th>DEFINITION</th>
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<tr>
<td>Symbiont</td>
<td>An organism living in <em>symbiosis</em>, mutually beneficial, with another</td>
</tr>
<tr>
<td>Commensals</td>
<td>A relationship between microbe and host in which one benefits while the other neither derives benefit or harm</td>
</tr>
<tr>
<td>Pathobionts</td>
<td><em>patho-</em>: disease causing; <em>-biont</em>: living organism; an organism that is potentially pathogenic, but lives as a symbiont under normal circumstances</td>
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Although the gut houses a wide diversity of bacterial species, studies have demonstrated that the careful balance of bacterial community composition is responsible for maintaining host health (Bäckhed et al., 2005; Eckburg et al., 2005; Huttenhower, 2012; Sekirov et al., 2010). This suggests the complexity of the host-microbe interface and the influential role the microbial community has on in controlling normal host physiology (Eckburg et al., 2005; Sekirov et al., 2010).

### 2.1.2. Composition of the Intestinal Microbiota

The diversity of the gut microbiota is a result of microbes coevolving with the host over time and thus, reflects the selection pressures and adaptation to environmental elements that determine microbiota stability (Berg, 1996; Gebbers & Laissue, 1989). At birth, a neonate’s GIT is immediately inoculated by microbes that originate from their mother, if delivered
vaginally, or the environment of their birth if delivered by caesarean section (Bezirtzoglou, 1997; Gronlund, Lehtonen, Eerola, & Kero, 1999; Penders et al., 2006). Bifidobacteria and lactobacilli are the two most commonly known beneficial bacteria and both are also two of the early colonizers typically derived from the mother during passage through the vaginal canal. Staphylococcus and Clostridium species, potentially pathogenic bacteria, are also early colonizers; they are prevalent in hospital settings (Bezirtzoglou, 1997) and commonly found in neonates delivered by caesarean section (Fallani et al., 2010; Gronlund et al., 1999; Penders et al., 2006). Thus, the composition of the gut microbiota is quite dynamic during early infancy, as certain components of the bacterial communities are metabolically flexible and influenced by multiple factors (Eckburg et al., 2005; Huttenhower, 2012). It becomes more and more complex over time and is impacted by various factors, including changes in dietary and hygiene practices, environmental exposures, as well as geographical regions. By approximately 3 years of age, the infant microbiota begins to converge towards a more adult-like colonization pattern and is relatively stable and more resistant to change throughout adulthood compared to infancy. However, during different stages of life, it does continue to evolve and shifts in microbial composition may occur due to external factors (e.g. diet, antibiotic exposure), which accounts for microbiota individuality (Berg, 1996; Koleva, Kim, Scott, & Kozyrskyj, 2015).

The composition of gut microorganisms in the human intestinal tract can be categorized into two groups: (1) resident (or essential) bacteria, which are considered the ‘core’ species that stably reside in the gut throughout adulthood with the ability to readily re-establish themselves if disturbed; and (2) transient bacteria, which include non-pathogenic and potentially pathogenic or opportunistic bacteria (Berg, 1996). Transient microbes are derived from the environment and can remain in the human gut for hours, days or even weeks; however, they do not take up permanent residence in the gut (Berg, 1996). In a healthy gut, the number of transient microbes is limited and plays little significance, as they are stringently controlled by normal resident microbes (Bäckhed et al., 2005; Faith et al., 2013; Ursell et al., 2012). However, studies have demonstrated that disturbance of the composition of resident microbes provides an opportunity for transient pathogenic microbes to colonize, proliferate and cause disease (Sekirov et al., 2010). Additionally, ‘opportunistic bacteria’, which can be considered as a sub-category of pathogenic bacteria whereby, under normal conditions, both resident and transient microbes cause no harm to the host. However, if opportunities arise, some of these microbes have the potential to become opportunistic pathogens or pathobiants and cause
disease (Berg, 1996). For example, under conditions in which the immune system is vulnerable, some transient microbes can overpopulate and outnumber resident bacteria or translocate into foreign sites. Additionally, under conditions when the balance of normal resident microbes is disrupted (e.g., antibiotic exposure), surrounding transient microbes are presented with the opportunity to adhere and colonize the GIT and cause harm (Berg, 1996; Kamada et al., 2013; Smith, McCoy, & Macpherson, 2007).

The entire length of the human gastrointestinal tract is densely populated with a broad spectrum of different microbial communities and it is important that the composition of the intestinal microbiota remains diverse (Bäckhed et al., 2005; Eckburg et al., 2005). A study conducted by Frank et al. (2007) demonstrated that, when comparing biopsy samples of proximal to distal sites along the axis of the gastrointestinal tract of inflammatory bowel disease (IBD) subjects to non-IBD subjects, the composition of bacteria in the non-IBD subjects’ intestinal microbiota appeared to be more diverse than the IBD subjects. The IBD subjects’ intestinal microbiota was described as abnormal and characterized by depletion of various species of commensal bacteria (Frank et al., 2007). The authors presented the theory that ‘species-rich communities’ in the intestinal microbiota provides an environment that is less susceptible for pathogenic bacterial invasion because a diverse microbial community limits the available resources for growth of pathogenic microbes (Frank et al., 2007; Huttenhower, 2012; Sekirov et al., 2010). As such, it was theorized that a complete and diverse composition of normal beneficial resident microbes within the intestinal microbiota creates great difficulty for pathogenic microbes to compete and colonize with the established resident bacteria to cause harm to the host.

Due to the presence of gastric acid, bile, and pancreatic secretions in the stomach and proximal small intestine, colonization by most bacteria is limited to fewer than $10^3$ colony-forming units (CFU) per milliliter in these regions of the upper GIT. In contrast, the density, complexity and diversity of bacteria increases distally along the intestinal tract, progressing to $10^4$ to $10^7$ bacteria per gram of jejunum and ileum and approaching approximately $10^{11}$ to $10^{12}$ bacteria per gram of colonic content (Berg, 1996; Gebbers & Laissue, 1989; Huttenhower, 2012; Sekirov et al., 2010). In addition to compositional variation of bacteria longitudinally, there exists radial heterogeneity of bacteria, displaying lower ratios of anaerobes to aerobes at epithelial surfaces compared to that within the intestinal lumen and feces (Figure 2.1) (Berg, 1996; Gebbers & Laissue, 1989; Huttenhower, 2012; Sekirov et al., 2010). Several studies
have demonstrated that alterations or disruptions of bacterial composition along the axis, as well as across the GIT, may play a significant role in causing certain diseases (Fujimura et al., 2010; Hawrelak & Myers, 2004; Jandhyala et al., 2015; Sekirov et al., 2010; Sommer & Bäckhed, 2013). Furthermore, resident microbes share a commensal, and some a symbiotic relationship, with the host and may even be beneficial when residing in their normal location within the intestinal tract. However, disease may result if large portions migrate and are introduced into foreign sites that are normally axenic and take up residence; thus, disruption of the tightly controlled composition of the microbiota may be a risk factor for disease (Bäckhed et al., 2005; Fujimura et al., 2010; Gareau et al., 2010; Hawrelak & Myers, 2004; Sekirov et al., 2010). For example, *Escherichia coli* normally resides in the colon; however, these microbes cause urinary tract infections (cystitis) when they are present in the areas surrounding the bladder (Givens & Wenzel, 1980). Another example of microbes entering atypical sites is during invasive medical procedures in which catheters are introduced or when surgical wounds allow the translocation of microbes into normally sterile sites (Givens & Wenzel, 1980).

**Figure 2.1:** Spatial and temporal depiction of the composition of the intestinal microbiota. (A) The diversity of microbial numbers and composition along the axis of the gastrointestinal tract. (B) Longitudinal cross-section of the variation of the composition of microbes in the intestine. (C) The temporal establishment of the intestinal microbiota and factors influencing the composition of microbes (Reprinted from: Sekirov I, *et al.* Gut microbiota in health and disease. *Physiol Rev* 2010 Jul: 90(3); 859-904) (Sekirov et al., 2010).
2.1.3. Functions of the Intestinal Microbiota

It is now well-established that the gut microbiota is intimately involved in various aspects of normal host physiology. The intestinal microbiota has the ability to function as a ‘virtual organ’, as this microbial ecosystem aids in the acquisition of nutrients from food sources and importantly, maintenance of homeostasis (Cummings & Macfarlane, 1997). It serves many important functions, such as providing a physical barrier against invasion and colonization of pathobionts, stimulating the development and modulation of the immune system (Gebbers & Laissue, 1989), production of vitamins B and K, and exerting important metabolic functions (e.g., extraction of energy from ingested food products, synthesis of nutrients, and digestion and fermentation of non-digestible fibers) (Cummings & Macfarlane, 1997). To further substantiate the important role of the gut microbiota on host health, it has also been found to be involved in many essential bodily functions, such as body temperature regulation, tissue growth and reproduction, which are energy-dependent processes (Cummings & Macfarlane, 1997; Fujimura et al., 2010; Jandhyala et al., 2015; Sommer & Bäckhed, 2013). Thus, mounting evidence from human studies, as well as from gnotobiotic (germ-free; Gf) mouse experiments, supports the theory that the intestinal microbiota bears an important functional role in maintaining normal host health.

As previously mentioned, the intestinal microbiota maintains a symbiotic relationship with the host and confers substantial metabolic, immunological, and protective functions. Importantly is the ability of the intestinal microbes to metabolize macronutrients (e.g., carbohydrates, proteins and fats) from food components, as well as, the intestinal microbiota’s extensive metabolic capabilities and considerable functional plasticity (Bäckhed et al., 2012; Huttenhower, 2012; Sommer & Bäckhed, 2013). As such, this section has been divided into separate functional components of the intestinal microbiota to provide a brief overview of its substantial functional role on host health.

**Structural integrity of the intestinal barrier**

Evidence from numerous studies supports the role of the intestinal microbiota in maintaining the structural integrity and normal function of the GIT (Berg, 1996; Bäckhed et al., 2012; Fujimura et al., 2010; Huttenhower, 2012; Shanahan, 2002; Sommer & Bäckhed, 2013). For example, *L. rhamnosus* GG has been shown to produce and secrete soluble proteins (e.g.,
p40 and p75), which are capable of preventing cytokine-induced apoptosis of the epithelial cells through an epidermal growth factor receptor (EGFR)-dependent mechanism (Yan et al., 2011). Moreover, certain gram-negative bacteria (e.g., Akkermansia muciniphilia) have been shown to increase levels of endocannabinoids, which play a role in controlling barrier functions by decreasing metabolic endotoxemia (Gebbers & Laissue, 1989). Additionally, studies have demonstrated that the gut microbiota contributes to the structural development and integrity of the intestinal mucosa. Studies from GF mice have shown that lack of bacterial colonization reduces the villus capillary network and thereby lowers intestinal surface area, impairs peristalsis, increase cell cycle time, and decreases villous regeneration resulting in thinning of the villi. All of these, in turn, impair nutrient digestion and absorption capabilities of the GIT (Alam, Midtvedt, & Uribe, 1994; Yan et al., 2011).

**Nutrient metabolism**

Nutrients extracted and metabolized by intestinal microbes are primarily derived from dietary carbohydrates, and members of the genus *Bacteroides* (e.g., Oxalobacter formigenes), certain *Bifidobacterium* and *Lactobacillus* species are the most prominent organisms responsible for carbohydrate metabolism (Cantarel, Lombard, & Henrissat, 2012). Furthermore, members of the *Bifidobacteria* and *Bacteroides* genus are involved in the synthesis of vitamins B and K, another major metabolic function of the intestinal microbiota (Bäckhed et al., 2005; Sommer & Bäckhed, 2013; Ursell et al., 2012). It has also been demonstrated that the intestinal microbiota is involved in lipid metabolism by suppressing the inhibition of lipoprotein lipase activity in adipocytes (Hooper et al., 2001). As well, the intestinal microbiota is equipped with the ability to metabolize proteins via microbial proteinases and peptidases. Amino acid transporters on the bacterial cell wall increase the permeability of amino acids from the intestinal lumen into the bacteria, where the amino acids are converted into small signaling molecules and anti-microbial peptides, such as bacteriocin (Corr et al., 2007; Hooper et al., 2001). Bacteriocin can then be secreted by the producing bacteria to kill or inhibit other closely related pathobiont strain without harming themselves due to specific immunity proteins within the producing bacteria (Yang, Lin, Sung, & Fang, 2014). Interestingly, the direction of research in food technology has been moving towards purifying bacteriocin to be used to extend preservation time of perishable foods. As well, there have been preliminary discussions of identifying specific species of bacteriocin-producing microbes that may become potential drug
candidates to clinically supplement antibiotics, as bacteriocin possesses anti-microbial effects (Yang et al., 2014).

**Antimicrobial protection**

One of the main functions of a healthy intestinal microbiota is the ability to maintain host health by preventing overgrowth of pathogenic bacteria. The simplest mechanism by which antimicrobial protection is accomplished is through the presence of a two-tiered mucus layer, which acts a physical barrier to prevent luminal microbes from coming into contact with the epithelium (Johansson et al., 2008). The inner layer is denser and lacks the presence of microbes, while the outer layer provides glycans as a source of nutrition for the surrounding microbes. The mucus, which constitutes a variety of mucin glycoproteins, is able to produce factors, such as trefoil-factor and the resistin-like molecule-β that can stabilize mucin polymers and thus, participates in maintaining barrier integrity (Kim & Ho, 2010). Furthermore, the structural components and metabolites of the intestinal microbiota have been shown to induce the synthesis of anti-microbial proteins (AMP), including pro-defensins and cathelicidins (Hooper, 2009). One of the key species responsible for AMP production is *Lactobacillus innocua*; it provides its antimicrobial effects through the production of lactic acid, which has the ability to affect the antimicrobial activity of host lysozyme by disrupting the outer membrane of the bacterial cell wall (Alakomi et al., 2000).

Another mechanism by which the intestinal microbiota provides antimicrobial protection and inhibits pathogenic bacteria is through its ability to induce local immunoglobulins, in particular through the activities of Gram-negative microbes, such as *Bacteroides* species. These microbes are able to induce the expression of secretory immunoglobulin A (IgA) from plasma cells within the intestinal mucosa, which in turn coat the intestinal microbiota to increase its resistance to degradation by bacterial proteases (Alakomi et al., 2000; Round & Mazmanian, 2009). Altogether, the primary objective of these mechanisms is to protect the host from pathogens by restricting opportunistic microbes within the intestinal lumen from translocating to the systemic circulation, thus preventing a systemic immune response (Berg, 1996; Gebbers & Laissue, 1989; Hooper, 2009; Round & Mazmanian, 2009).
**Immunomodulation**

The immunomodulatory capacity of the intestinal microbiota plays a vital role in maintaining host health, as it serves to eliminate the invasion of pathogenic microbes and aids in sustaining tolerance to commensal bacteria (Berg, 1996; Bäckhed et al., 2005; Gebbers & Laissue, 1989; Hooper et al., 2001; Round & Mazmanian, 2009; Wu & Wu, 2012). The use of germ-free mouse models, whereby mice are reared in a sterile environment completely devoid of microorganism exposure, have been instrumental in delineating the critical role of the intestinal microbiota in the development of both the innate and adaptive immune systems (Smith et al., 2007). The intestinal microbiota participates in various components of the immunomodulatory process, such as the normal development and function of the gut-associated lymphoid tissue (GALT), effector and regulator T cells, IgA-producing B (plasma) cells, lymphoid cells, and resident macrophages and dendritic cells in the lamina propria (Blaut & Clavel, 2007; Gebbers & Laissue, 1989; Hooper, 2009; Round & Mazmanian, 2009; Smith et al., 2007).

The GALT is responsible for the immune properties of the intestinal mucosa and provides a well-regulated network of tolerance-inducing mechanisms. It is composed of lymphoid aggregates, which includes the Peyer’s patches (sites of immune response induction) and the mesenteric lymph nodes (Arpaia et al., 2013; Gebbers & Laissue, 1989; Wu & Wu, 2012). As well, large numbers of immune-competent cells exist in the lamina propria and the mucosal epithelium. Essentially, the GALT works as a containment system, as it is able to prevent potentially pathogenic components from crossing the intestinal barrier through its constant interaction with the intestinal microbiota (Gebbers & Laissue, 1989; Hooper, 2009; Round & Mazmanian, 2009; Wu & Wu, 2012). Furthermore, in the case of potent mediators that prevent the attack of the immune system on healthy self-tissues, such as Forkhead box P3 (FoxP3)+ T-regulatory (Treg) cells, it has been shown that these Tregs are also tightly regulated by the intestinal microbiota; however, the exact mechanism remains unknown. For example, *B. fragilis*, which produces short-chain fatty acids (SCFAs; end products of dietary fiber fermentation shown to exert beneficial effects on energy metabolism), have been implicated in the development and function of Tregs (Smith et al., 2013). The proposed mechanism is that SCFAs, produced by *B. fragilis*’s ability to ferment indigestible food sources, activate G-protein-coupled receptors (GPCRs) expressed by intestinal epithelial cells (IEC) and through increased acetylation of the *FOXP3* locus, regulates Treg expression and activity to eliminate...
or downregulate the expression of self-reactive T and B lymphocytes (Arpaia et al., 2013; Smith et al., 2013).

The fact that the intestinal microbiota possesses more than the above mentioned immunemodulatory capacities, ultimately playing an important role in a variety of signaling pathways that modulates proper immune, inflammatory and allergic responses, affirms the interplay between the intestinal microbiota and the immune system. As the infant microbiota becomes more complex and diverse, the intestinal immune system simultaneously also becomes more mature. This strongly suggests that an aberrant microbiota is a predisposition to disease in infancy and later in life in a variety of intestinal disorders, such as IBD, irritable bowel syndrome (IBS), necrotizing enterocolitis (NEC), obesity and infantile colic, just to identify a few (Adlerberth, 2008; Blaut & Clavel, 2007; Bäckhed et al., 2005; Hooper, 2009; Huttenhower, 2012; Wu & Wu, 2012).

### 2.1.4. Concept of Intestinal Dysbiosis

It is well established that maintaining microbial balance by meticulously controlling the composition of bacterial communities plays an important role in maintaining host health (Huttenhower, 2012; Sekirov et al., 2010). Accordingly, microbial imbalance of the gut, referred to as dysbiosis, a term coined by Nobel Prize winner, Elie Metchnikoff (Kirchfeld, 1994; Metchnikoff, 1907), has been proposed to be a central or contributing cause of various chronic and degenerative disease states (Hawrelak & Myers, 2004). The theory of intestinal dysbiosis describes a state in which an imbalanced microbiota produces adverse effects on the host as a result of: (1) significant alterations of the quantity and quality of the intestinal microbes; (2) consequential modification of microbe metabolic activity; and (3) spatial redistribution of bacterial communities (Fujimura et al., 2010; Hawrelak & Myers, 2004; Sekirov et al., 2010). For example, decreased microbial diversity in infancy has been associated with an increased risk of various allergic and atopic conditions later in childhood, as well as other GI-related conditions, including IBD, IBS, acute and chronic diarrheal conditions, NEC, and infantile colic (De Palma et al., 2010; de Weerth, Fuentes, & de Vos, 2013a; Swidsinski, Loening-Baucke, Verstraelen, Osowska, & Doerffel, 2008; Tannock, 2008). As such, exposure to a wide spectrum of microbes in early infancy, as well as, the proper
Colonization of the intestinal microbiota may profoundly impact the health and well-being of an individual throughout life (Figure 2.2).

Figure 2.2: Schematic representation of the concept of intestinal dysbiosis. (A) Immunological equilibrium is the result of a healthy microbiota, which contains a balanced composition of various classes of symbionts (beneficial microbes), commensals (permanent resident microbes but provide no benefit or harm to the host) and pathobionts (permanent resident microbes but are pathogenic). (B) Intestinal dysbiosis is the result of an unnatural shift in the composition of the microbiota; thus, causing either a reduction in the numbers of symbionts and/or an increase in the numbers of pathobionts. The result is immunological disequilibrium and non-specific inflammation, leading to inflammatory disease, such as IBD, and obesity. (Reprinted from: Round JL, et al. Nat Rev Immunol. 2009 May: 9(5); 313-23) (Round & Mazmanian, 2009).

The two main functions of the intestinal microbiota are: (1) to provide a barrier against pathogenic microbes from entering the systemic circulation, and (2) to extract essential nutrients from ingested food sources (Hawrelak & Myers, 2004). This has led to the hypothesis that modernization of society, referring to the consumption of a more westernized, highly processed diet, sedentary lifestyles, and increased use of antibiotics and other drugs, have all led to the distortion of the composition of the normal intestinal microbiota, thus causing changes in bacterial metabolic activity and overgrowth of pathobionts and subsequently, disease (Hawrelak & Myers, 2004; Sommer & Bäckhed, 2013). There is increasing evidence that substantiates and clarifies this intestinal dysbiosis hypothesis. The disorderly overgrowth invasion of exogenous pathogenic bacteria, caused by altered commensal distribution, is believed to release potentially toxic metabolites and the presence of these toxic byproducts may be the cause of many chronic and degenerative diseases (Hawrelak & Myers, 2004;
Sommer & Bäckhed, 2013), such as celiac disease (De Palma et al., 2010), type 2 diabetes (Larsen et al., 2010), IBS, IBD, Crohn’s disease (Tannock, 2008), and acute and chronic diarrheal disease (Swidsinski et al., 2008). More recently, an association has been established showing intestinal dysbiosis as a contributing factor to obesity (Turnbaugh et al., 2006).

Several recent studies have compared the composition of intestinal bacteria of healthy patients to patients displaying symptoms of diseases and the results revealed a dramatically altered disarray of their microbiota in patients with disease, whereas, healthy patients displayed the typical distinct and species-rich bacterial communities within the appropriate sites of the intestinal microbiota (Blaut & Clavel, 2007). For example, a group of researchers determined a link between obesity and gut microbiota based on the findings of their study that compared the ratio of Bacteroidetes to Firmicutes in lean and obese subjects (Turnbaugh et al., 2006). The results revealed a considerable difference in the composition of the intestinal microbiota of lean subjects compared to obese patients, where a ten-fold increase of Firmicutes to Bacteroidetes (from 3:1 to 35:1) were found in obese subjects compared to the lean subjects, respectively (Turnbaugh et al., 2006). This study is one of many studies presenting the theory that modulation of the gut microbiota may have a significant influence on the host health and disease and that treating and maintaining the normal intestinal microbiota may be a potential therapeutic target (Gareau et al., 2010; Kaur et al., 2009; Marteau, de Vrese, Cellier, & Schrezenmeir, 2001; Turnbaugh et al., 2006).

### 2.1.5. Factors Affecting Microbiota Composition in Infancy

The development of the intestinal microbiota begins in early infancy and a myriad of factors can influence the composition of bacterial colonization during an infant’s first week of life. Factors include route of delivery (vaginal or caesarean section) (Gronlund et al., 1999), infant mucosal maturation, gestational age (prematurity or full term) (Penders et al., 2006), breastfed or bottle-fed (Stark & Lee, 1982), maternal diet (i.e., Westernized versus non-Westernized diets) (Marques et al., 2010), antibiotic use during the perinatal and/or postnatal period (Bennet, Eriksson, & Nord, 2002), and geographical (Yatsunenko et al., 2012) and cultural environments (i.e., developing versus developed countries) (Fallani et al., 2010).
**Mode of Delivery**

Many studies have been conducted comparing the composition of the infant microbiota in vaginally delivered infants to those born by caesarean section and the findings determined, and confirmed, that the earliest determinant of the intestinal microbiota is the mode of delivery (Grönlund, Grześkowiak, Isolauri, & Salminen, 2011). For example, a study conducted by Gronlund et al. (1999) confirmed that fecal samples from infants born vaginally contained bacterial colonies similar to that of fecal and vaginal bacteria of the mother, predominantly consisting of anaerobic bacteria from the *Bifidobacteria* genus (Gronlund et al., 1999). In contrast, the microbiota of infants born by caesarean section is characterized by micro-aerophilic bacteria and sporulate forms, in which the first inoculation is bacteria originating from the hospital environment and staff members (Fallani et al., 2010; Gronlund et al., 1999; Grönlund et al., 2011). Furthermore, some studies have demonstrated that, at one year of life, infants delivered by caesarean section have a lower ratio of obligate anaerobic to facultative anaerobic bacteria, compared to vaginally born infants (Adlerberth, 2008; Grönlund et al., 2011). This has been suggested to be indicative of an underdeveloped anaerobic microbiota, leading to an inability to successfully suppress facultative anaerobic bacterial overgrowth (Gronlund et al., 1999). Therefore, caesarean section-delivered infants oftentimes acquire early colonizers, such as *Bacteroides*, *Escherichia coli*, and, to a lesser extent, *Bifidobacterium* spp., later than the usual inoculation during the first month of life (Fallani et al., 2010; Gronlund et al., 1999), and with respect to colonization of *Bacteroides* and *E. coli*, caesarean section-born infants sometimes do not catch up until their first year of life (Adlerberth, 2008; Gronlund et al., 1999).

**Feeding Type**

Human breast milk contains small amounts of early colonizers, such as *Lactobacillus*, *Bifidobacterium*, *Staphylococcus*, *Streptococcus* and *Micrococcus* species (Bezirtzoglou, 1997) and more recently, in efforts to make infant formula more similar to breast milk, some infant formulas are now fortified with multi-strain probiotics in attempt to mimic the bacterial composition of breast milk (Braegger et al., 2011). It is not surprising that the type of feeding has a weighted effect on influencing infant bacterial colonization pattern. Comparing breast milk to infant formula, the bactericidal properties of breast milk provide an infant’s intestinal tract protection against colonization and proliferation of pathogenic bacteria (Garofalo &
Goldman, 1999) and thus, prior to probiotic-fortified infant formula, studies have reported that formula-fed infants more readily experience acute otitis media (Sabirov, Casey, Murphy, & Pichichero, 2009), gastroenteritis, and diarrhea (Lamberti, Fischer Walker, Noiman, Victora, & Black, 2011) compared to breastfed infants. Interestingly, however, previously several studies were unable to confirm a significant difference in the colonization pattern between breastfed versus formula-fed infants (Penders et al., 2005; Stark & Lee, 1982; Yoshioka, Iseki, & Fujita, 1983).

**Geographical Locations**

Another major determinant of the composition of an infant’s intestinal microbiota is the environment to which the infant is exposed during the first few years of life (Fallani et al., 2010; Suzuki & Worobey, 2014; Yatsunenko et al., 2012). Geographical regions and cultural practices, including different cultural dietary choices and habits, can strongly impact the development and colonization pattern of the gut microbiota during early infancy (Fallani et al., 2010; Marques et al., 2010; Suzuki & Worobey, 2014; Yatsunenko et al., 2012). For example, in a study that compared the fecal microbiota of non-Western children with Western (American) children aged 0–3 years of age, significant differences in the phylogenetic composition of the microbiota were found (Yatsunenko et al., 2012; Yatsunenko et al., 2012). It was proposed that, to some extent, the intestinal microbiota follows ‘Bergmann’s rule’ (Foster & Collard, 2013), where populations in higher latitudes tends to have higher body mass compared to populations in lower latitudes (Roberts, 1953). Suzuki et al. (2014) proposed that the observed differences in colonization pattern of the intestinal microbiota based on geographical regions could be explained by the fact that host genes have the ability to alter food preferences or amount of food intake to regulate the host microbiota to allow for normal microbiota function. Due to the dynamic nature of the intestinal microbiota during infancy, it is able to adapt to environmental and climatic influences. For example, in colder climates, an individual naturally has the propensity to increase food intake (Brobeck, 1948; Roberts, 1953); thus, the intestinal microbiota in those individuals would have a greater ability to more efficiently extract energy and store fat from food to maintain normal host physiology compared to individuals in warmer regions (Suzuki & Worobey, 2014; Yatsunenko et al., 2012). Although data is limited to fully characterize the geographical variation of intestinal microbiota composition, particularly in infants, it is accepted that the colonization pattern of a ‘healthy
intestinal microbiota’ may differ based on different geographical regions (Fallani et al., 2010; Marques et al., 2010; Suzuki & Worobey, 2014; Yatsunenko et al., 2012).

Additionally, even infants residing in the same geographical regions, but with different cultural traditions, such as, differing perspectives and attitudes on keeping companion animals, as close physical contact with animals affect the acquisition and exchange of microbes (Penders et al., 2006; Serpell JA & P, 2011), and maternal dietary habits influence the composition of microbes residing in the infant’s gut microbiota (Suzuki & Worobey, 2014; Yoshioka et al., 1983).

**Antibiotic Exposure**

Maternal antibiotic use during the perinatal period are used to treat infections, such as chorioamnionitis, and to prevent infections in the neonate, which may subsequently temporally alter the composition of maternal microbiota (Persaud et al., 2014; Rautava, Luoto, Salminen, & Isolauri, 2012). Studies have shown that these determinants may have an effect on the development of the infant’s gut microbiota, as it could potentially disturb the host-microbe interaction (Persaud et al., 2014). For example, broad-spectrum maternal antibiotic use in the perinatal period has been associated with an increased risk of neonatal NEC (Kenyon, Taylor, Tarnow-Mordi, & ORACLE, 2001) and cerebral palsy (Kenyon et al., 2008). Furthermore, infant antibiotic use has also been associated with increased risk of childhood IBD (Shaw, Blanchard, & Bernstein, 2010). The potentially detrimental effects on the infant intestinal microbiota associated with antibiotic exposure during the perinatal and postnatal period highlight the importance of the host-microbial interaction during infancy and how this interaction may shape the course of the infant’s health later in life.

**Other Factors**

Additional factors may play a role in the development of an infant’s intestinal microbiota, including gestational age, full- or pre-term births, and long-term separation from their mothers after birth. It has been shown that these factors prominently contribute to the selection of microbes that colonize the GIT in early infancy (Adlerberth, 2008; Penders et al., 2006). For example, the intestinal microbiota of preterm infants have been shown to differ greatly from term infants, as preterm infants are typically hospitalized and cared for in aseptic conditions with minimal maternal contact in the early days of life. Therefore, their main sources of microbes are derived from the hospital environment and because they are kept
separate from their mother, they lack the exposure of maternal microbes through skin-to-skin contact (Bennet et al., 2002; Koleva et al., 2015; Penders et al., 2006; Yatsunenko et al., 2012). Other factors include maternal health, pH in the stomach, and stress (Gebbers & Laissue, 1989; Hawrelak & Myers, 2004).

Overall, it is evident that the initial process of microbial colonization significantly impacts the development of a ‘healthy’ intestinal microbiota in early infancy, which may be a prerequisite in determining host health later in life (Figure 2.3).

**Figure 2.3: Factors affecting intestinal microbiota composition in infancy.** An infant’s GIT is first inoculated with microbes originating from the maternal gut microbiota during vaginal delivery, which is associated with increased maternal intestinal permeability and translocation of gut bacteria into breast milk. Mode of feeding, whether it be formula or breast milk, the latter also provides a direct source of maternal bacteria to the infant. Skin-to-skin contact is another source of direct maternal bacteria transfer. Direct transfer of bacteria from mother to infant enhances healthy immune and metabolic maturation. In contrast, antibiotic exposure results in a significant decrease in the composition and diversity of an infant’s intestinal microbiota. (Adapted and modified from: Kovela PT, et al. The infant gut microbiome: evidence for obesity risk and dietary interventions. *Nutrients.* 7(4); 2237-60.) (Koleva, Bridgman, & Kozyrskyj, 2015).

### 2.2. Overview of Probiotics

#### 2.2.1. Probiotics in the Manipulation of the Intestinal Microbiota

Previous studies have shown that lactic acid-producing bacteria, which cause fermentation of milk products, beneficially alters the intestinal microbial composition by inhibiting growth of putrefactive bacteria in the gut (Metchnikoff, 1907), which is believed to be a contributing etiological factor in many diseases (Blaut & Clavel, 2007; Round & Mazmanian, 2009; Sekirov et al., 2010). This led Metchnikoff to propose the notion that
production of intestinal toxins, a byproduct of overconsumption or intake of nutrient-void and calorically dense foods, and the subsequent fermentation of toxins results in intestinal overgrowth of bacteria, leading to disease (Metchnikoff, 1907). Therefore, to detoxify and prevent accumulation of toxins, he proposed that consumption of fermented milk products, such as yogurt, cheeses, buttermilk and kefir, may result in correcting the “autointoxication” by inhibiting growth of putrefactive bacteria and as a consequence, restore the intestinal microbiota balance (Metchnikoff, 1907). Although this hypothesis was proposed a century ago, it is quite accurate in that the concept of alteration of intestinal microbes leads to disease and subsequently, the consumption of fermented dairy products containing cultured bacteria may lead to resolution of the intestinal microbial aberration (Fujimura et al., 2010; Hornef, 2015; Kaur et al., 2009; Kirchfeld, 1994; Marques et al., 2010). This phenomenon has led to a marked rise in the interest of probiotic supplementation over the past few years, with many food products, such as yogurts and other dairy products, as well as infant formula, to be supplemented with multi-strain beneficial bacteria (Andersson et al., 2001; Braegger et al., 2011; NASPGHAN, 2006).

The most universally accepted definition of probiotics, according to the World Health Organization, is “live microorganisms that, when administered in adequate amounts, have been shown to confer health benefits on the host” (FAO/WHO, 2001). Therefore, a microorganism may classify as a ‘probiotic’ if it exhibits beneficial effects on the host, is non-toxic and non-pathogenic, is able to remain viable and metabolically active within the gut (FAO/WHO, 2001; Reid, 2016). Although there are various species and strains of probiotics currently available, most probiotics belong to the Lactobacillus and Bifidobacterium genera (e.g., Lactobacillus reuteri, L. rhamnosus GG, L. acidophilus, L. gasseri, L. casei, and Bifidobacterium breve, B. bifidum, B. lactis, and B. longum). Additionally, yeasts are also regarded as probiotics, such as Saccharomyces boulardii and Aspergillus oryzae; however, the latter is not commonly used (Schrezenmeir & de Vrese, 2001). Lactobacillus and Bifidobacterium are both Gram-positive, non-motile, non-spore forming bacteria. In particular, lactobacilli are part of the lactic acid bacteria (LAB) group, which produce lactic acid as a byproduct of carbohydrate metabolism, and the genus encompasses more than 120 species. In contrast, Bifidobacterium includes just 29 species and differs from LAB phylogenetically; lactic acid is the end product of bifidobacterial fermentation by a different sugar fermentation pathway (Tannock, 1999).
Probiotic use has been positively associated with symptomatic improvements in patients with various health conditions, such as atopic conditions (Ozdemir, 2010), cardiovascular disease (Saini, Saini, & Sharma, 2010), metabolic conditions (Larsen et al., 2010), gastrointestinal-related conditions (De Palma et al., 2010; Gareau et al., 2010; Koleva et al., 2015) and more recently, obesity (Turnbaugh et al., 2006). Despite the fact that some health claims are unsubstantiated, its use has continually risen with new and potentially effective strains becoming more readily available. The popularity of probiotic supplementation may primarily be attributed to its likely good safety profile, particularly pertaining to probiotics containing species from either *Lactobacillus* or *Bifidobacteria* genus, as both are indigenously present in the human intestinal microbiota (Ishibashi & Yamazaki, 2001; Kaur et al., 2009; Sethi, 2009). However, despite only rare reported instances of adverse outcomes associated with probiotic use, probiotics may theoretically exert a range of adverse effects, including infections, antibiotic resistance, metabolic effects, immune-related effects, sepsis, and meningitis (Antony, 2000; Ishibashi & Yamazaki, 2001). There exist some reported cases of bacteremia; however, in these instances, the authors concluded that the subjects were predisposed to infection due to an underlying health condition and therefore, at increased risk of developing bacteremia with probiotic use (Antony, 2000; Salminen et al., 2004). Another report of adverse events associated with probiotics was from a study conducted to evaluate the safety and efficacy of intra-duodenal administration of a mixture of six probiotic strains in patients with severe pancreatitis (Besselink et al., 2008). The authors reported that there were a significantly higher number of deaths in the probiotic group compared to the control group. Post-mortem examination determined that cause of death was due to necrotizing jejunitis, which may have been a result of high concentrations of microorganisms in the proximal intestinal leading to impaired splanchnic circulation. The authors concluded that the risk of death in this special population was associated with probiotic use (van Minnen et al., 2007). Despite the outcome of this study, probiotics are generally well tolerated and considered to be safe with limited associated adverse events, particularly in otherwise healthy individuals (Boyle, Robins-Browne, & Tang, 2006; Ishibashi & Yamazaki, 2001).

Although reports of adverse events are rare and probiotics are generally considered to confer health benefits, it is important to recognize that not all probiotics are beneficial in all circumstances. Due to the availability of a vast selection of microbes that may be beneficial and recognized as a ‘probiotic’, it is inappropriate to overgeneralize the effects of all probiotics,
particularly when using them as therapeutic agents (McFarland, 2014; van den Nieuwboer et al., 2016; Marchesi et al., 2016). It is widely accepted that there are general benefits ascribed to probiotics, with a shared mechanism of creating a healthy gut and supporting a health GIT and immune system. This conclusion was based on high-quality meta-analyses of studies on activity against infectious diarrhoea, antibiotic-associated diarrhoea, irritable bowel syndrome and other conditions, which lends to the strength of the evidence. However, this does not necessarily imply that the mechanisms of action are the same for each condition, or that the exact mechanisms have been demonstrated in humans, particularly since different strains and product formulations (e.g., differences in the bacteria in various multi-strain probiotics) exist (Goldenberg et al., 2015; Cruchet et al., 2015; Lytvyn et al., 2016). Manipulation and control of the immune system by probiotics is hard to evaluate and make general conclusions about because the same strain of probiotic might enhance antimicrobial activity, while simultaneously increasing anti-inflammatory Th2 responses or regulatory activity depending on the individual host and the delivery method of the probiotic (Govender et al., 2014). The theory is that a specific probiotic strain possesses the ability to assess the niche into which it is placed and is capable of reacting accordingly (Segers & Lebeer, 2014). For example, L. rhamnosus GG (LGG) has been shown to have multiple mechanisms by which it interacts with the host, but also, there are many ways for the host to respond to LGG. LGG has been shown to both relieve inflammation and stimulate Th1 responses, which appear to be contradictory mechanisms (Segers & Lebeer, 2014) (e.g., LGG reduces the duration of diarrhea (Aggarwal et al., 2014), but does not alleviate constipation (Chmielewska & Szajewska, 2010)). Furthermore, certain strains of L. reuteri are significantly different genetically and functionally. Some strains are capable of producing reuterin, which is important for inhibiting pathogens in the gut, while other strains produce biosurfactants that inhibit the attachment of uropathogens (Cadieux et al., 2008; Langa et al., 2014). Collectively, this demonstrates the need to not use probiotics simply for the false sense that it “promotes health”, but rather, the emphasis should be placed on identifying and using specific strains of probiotics based on their mechanisms of action that align with the desired clinical outcome when used as a therapeutic agent to treat specific conditions (Gareau et al., 2010; Sanders et al., 2013; Reid, 2016).
2.2.2. Mechanism of Action of Probiotics

Over the recent years, growing evidence has shown that probiotics elicit beneficial health effects on the host, but more prominently involving gastrointestinal conditions (Wallace, 2009; Johnston et al., 2012; Goldenberg et al., 2015; Cruchet et al., 2015; Lytvyn et al., 2016). For the most part, the main effect of probiotics is their ability to elicit tolerogenic responses to promote harmony between the intestinal microbiota and the immune system (Blaut & Clavel, 2007; Boirivant & Strober, 2007; Gebbers & Laissue, 1989; Marteau et al., 2001). As previously discussed, the state of dysbiosis is thought to potentially be a cause of various GI-related conditions, such as in the case of IBD, whereby the intestinal dysbiosis and immune dysregulation act together with environmental perturbation resulting in inflammation and disease (Kamada et al., 2013; Shaw et al., 2010; Swidsinski et al., 2008; Tannock, 2008). Since probiotics have been shown to possess the ability to suppress intestinal inflammation by preventing the overgrowth of inflammation-inducing microbes and gas-forming coliforms (Blaut & Clavel, 2007; Koleva et al., 2015; Marteau et al., 2001; WHO, 2001), it was proposed that introducing probiotics into an inflamed GI milieu to restore the imbalance within the microbiota may potentially calm or even eliminate inflammation. This supports the use of probiotics as a therapeutic option to treat various GI-related conditions (Blaut & Clavel, 2007; Boirivant & Strober, 2007; Canani et al., 2007; Marteau et al., 2001; Sherman et al., 2009).

Probiotics have been shown to modulate intestinal bacterial patterns by aiding in the colonization of beneficial bacteria to increase the diversity of microbes in the intestine and exert its beneficial action by adhering, colonizing and competitively occupying attachment sites (Boirivant & Strober, 2007; Sherman et al., 2009). More specifically, probiotics have been associated with the regulation of the intestinal microbial homeostasis by enhancing the physical barrier to inhibit pathogenic bacterial adhesion to the mucosa. Additionally, probiotics may indirectly modulate and evoke local and systemic immune responses, induce enzymatic activity that favours efficient nutrient synthesis and energy extraction, stabilize or maintain the GI barrier function, and possibly inhibit pro-carcinogenic enzyme activity (Boirivant & Strober, 2007; Sherman et al., 2009; Gareau et al., 2010; Sanders et al., 2013; Reid, 2016). Furthermore, probiotic microbes also have the unique ability to express specific surface molecules, such as glycolipids and microbe-associated molecular patterns (MAMPs), and secrete byproducts that possess antimicrobial effects. For example, some probiotic strains secrete antibacterial products called bacteriocins, which are able to inhibit the growth and
virulence of enteric pathogenic bacteria (Corr et al., 2007). It is important to note that some of these probiotic effects are only observed with viable probiotic organisms, while other effects are observable even with non-viable organisms. Additionally, each of these mechanisms may be further sub-categorized based on the site of probiotic action. For instance, for strains of probiotics with sites of action at the mucosal barrier, these strains are capable of acting through more general antimicrobial effects. Probiotics can also play a role in epithelial cell inflammatory responses and epithelial cell survival and act to maintain intestinal permeability in the absence of pathogenic bacteria (Figure 2.4) (Boirivant & Strober, 2007; Sherman et al., 2009; Gareau et al., 2010; Sanders et al., 2013; Reid, 2016).

Given the complex functions of the intestinal microbiota on host health, it is unlikely that any one probiotic species or strain is capable of conferring more than a few beneficial effects, but rather, different probiotics have different effects and possibly overlapping mechanisms of action (Marco, Pavan, & Kleerebezem, 2006; Boirivant & Strober, 2007; Sherman et al., 2009; Gareau et al., 2010; Sanders et al., 2013; Reid, 2016). As such, claims of probiotic efficacy should be limited to specific genera, species and strain of bacteria and for symptoms of specific disease entities. For example, a comparative RCT was conducted comparing the effect of different commercial probiotic preparations (e.g., L. rhamnosus GG,
Saccharomyces boulardii, Bacillus clausii, Streptococcus thermophilus, L. acidophilus, and Bifidobacterium bifidum) on the duration of acute diarrhea in children (Canani et al., 2007). In this trial, 571 children (3 to 36 months of age) presenting with acute diarrhea were randomly assigned to receive one of these probiotic preparations or no probiotic for 5 days. The results demonstrated that children receiving L. rhamnosus GG and the probiotic mixture containing LAB were effective at reducing the duration of diarrhea (Canani et al., 2007). As such, the authors concluded that the effect of probiotics is species- and strain-specific. Thus, the selection of probiotics as a therapeutic agent should be based on well-designed clinical trials verifying their efficacy for the indicated condition.

2.3. Probiotics in the Prevention and Treatment of Gastrointestinal Conditions

The complexity of the interaction between the intestinal microbes and host health and disease may not yet be fully understood; however, increasing efforts are underway to elucidate the contribution of probiotics as a therapeutic option for certain pediatric diseases. As alluded to above, given that the GIT houses the largest reservoir of microbes in the human body, it is not surprising that the most studied and well-defined actions of probiotics are their capacity to maintain a healthy GIT, improve its function, and to treat certain intestinal infections and post-antibiotic syndromes. Mechanisms differ according to the specific probiotic strain and exert specific clinical effects (Boirivant & Strober, 2007; Gareau et al., 2010; Reid, 2016). As such, a discussion is warranted on the prevailing theories of the effects probiotics have on common pediatric GI-related conditions, and the potential mechanisms of action in relation to specific conditions.

2.3.1. Irritable Bowel Syndrome (IBS)

Irritable bowel syndrome is a multi-symptom GI condition with unclear etiology and pathogenesis. Symptoms of the condition may overlap other diseases of the GIT and greater use of simple screening tests are being advocated and used prior to the diagnosis of IBS being made (Drossman & Dumitrascu, 2006; Drossman, 2006). Numerous studies have demonstrated that IBS is the result of a disruption of the normal composition of the intestinal microbiota (Berg, 1996; Blaut & Clavel, 2007; Sekirov et al., 2010; Tannock, 2008; Ursell et al., 2012), causing symptoms related to abdominal pain, bloating, flatulence, and changes in bowel
movement habits (e.g., diarrhea and/or constipation) (Andersson et al., 2001; Hawrelak & Myers, 2004). IBS has been shown to be primarily associated with intestinal changes, such as altered microbial colonization, increased intestinal permeability, but no consistent data on altered motility have been identified. Extra-intestinal manifestations, such as depression, anxiety, and insomnia, have also been linked to IBS (Ford, Talley, Schoenfeld, Quigley, & Moayyedi, 2009). Post-infectious IBS may result following an episode of acute enteric infection (commonly Salmonella, Campylobacter jejuni and enterohemorrhagic E. coli O157: H7) indicating that an altered microbial composition or induction of an inflammatory or immune response leads to changes in intestinal functional and resulting in IBS symptoms (Spiller & Garsed, 2009). Lab characteristics of IBS include an increase in pro-inflammatory cytokines, such as IL-6 and TNF-α, and an increase in lymphocyte count (Rana et al., 2012).

Although research results have not been uniformly positive in pediatrics, as the vast majority of clinical trials have been limited to adults, there does exist some, albeit inconsistent, evidence regarding the efficacy for the use of probiotics for the treatment of IBS symptoms in pediatrics. A RCT involving 77 IBS patients was conducted to investigate the efficacy of ingesting probiotic preparations containing either Bifidobacterium infantis 35624 or Lactobacillus salivarius UCC4331 (O’Mahony et al., 2005). The patients were randomly assigned to receive either the probiotics administered at a dose of $1 \times 10^{10}$ CFU in a malted milk drink, or a malted milk drink alone as a placebo for 8 weeks. The results from this study revealed that IBS patients in the probiotic group containing B. infantis 35624, but not the L. salivarius UCC4331 group, reported significantly improved quality of life, as well as reduced abdominal pain and bloating, and biochemical improvements included increased production of IL-10 (immune-modulatory cytokine) in isolated peripheral blood mononuclear cells (PBMCs) in vitro (O’Mahony et al., 2005). In a separate RCT, patients receiving a multi-species and strain probiotic mixture (LGG, L. rhamnosus Lc705, Propionibacterium freudenreichii shermanii JS and B. animalis lactis Bb12) reported reduced abdominal distention and pain, as well as stabilization of the intestinal microbiota (Kajander, Hatakka, Poussa, Färkkilä, & Korpela, 2005).

Similar to the results of the studies in adult IBS patients, a RCT involving children with IBS administered the VSL#3 probiotic preparation (containing a blend of B. breve, B. longum, B. infantis, L. acidophilus, L. plantarum, L. paracasei, L. bulgaricus, and S. thermophilus) demonstrated success in maintaining IBS symptom remission compared to the placebo group.
The reportedly improved symptoms included reduced abdominal pain and distention or bloating, flatulence and frequency of diarrhea episodes (Guandalini et al., 2010).

Possible mechanism of action of probiotics in IBS

Enhancement of epithelial barrier function is one of the proposed mechanisms of action of probiotics in IBS, as evidence has indicated that disruption of epithelial barrier function may precede the onset of low-grade immune activation, inflammation, and intestinal dysfunction in a subset of IBS patients (Piche, 2014), especially in patients presenting with diarrhea-predominant IBS (IBS-D), as well as, post-infectious IBS (PI-IBS) (Barbara, 2006; Marshall et al., 2004). Alteration in intestinal barrier function leading to translocation of bacteria and their by-products was found to be associated with IBS symptoms. For example, lipopolysaccharide (LPS), an endotoxin and a major component of Gram-negative bacteria cell wall, typically does not pass through an intact epithelium; however, upon reduced epithelial integrity, LPS is able to pass the GI barrier, which, with the presence of LPS in the systemic compartment, further contributes to increasing intestinal permeability (Ludidi et al., 2015). As such, patients supplemented with probiotics have shown improvement of IBS symptoms due to preservation of epithelial barrier function and prevention of mucosal damage triggered by pathogens, pro-inflammatory cytokines, food, and drugs (e.g., aspirin) (Ludidi et al., 2015; Piche, 2014). The beneficial effects of probiotics have shown to be modulated by their ability to induce mucin secretion, enhance tight junction protein phosphorylation, restore chloride secretion and change trans-epithelial resistance (Piche, 2014).

Oral administration of VSL#3 has been shown to up-regulate heat shock proteins, which are known for their ability to maintain and enhance cytoskeletal integrity, as well as protect enterocytes from oxidative stress (Ludidi et al., 2015). Results from animal studies have demonstrated that LGG is also capable of exerting mitogenic effects by increasing cell proliferation in the villi of germ-free rats, thus enhancing mucosal regeneration. Additionally, LGG has been shown to inhibit apoptosis in intestinal epithelial cells through its ability to modulate pro- and anti-apoptotic signaling pathways (Barbara, 2006). These protective effects were reproducible with L. acidophilus ATCC 4356 and S. thermophilus ATCC 19258 (Resta-Lenert & Barrett, 2006). Taken together, various probiotics have proven to be effective in improving intestinal barrier function and integrity, which is associated with significant improvements in IBS symptoms, confirming the role of probiotics in the treatment or
management of IBS symptoms. It is noteworthy that, given the heterogeneity of IBS, the use of probiotics should focus on the management and treatment of the specific IBS sub-populations and for specific IBS-related symptoms for which there is quantifiable evidence.

2.3.2. Infectious and Non-Infectious Diarrheal Conditions

*Clostridium difficile infection (CDI)*

Representing the most common nosocomial infection, *Clostridium difficile (C. diff)* infection is highly prevalent and severe and is associated with incidences of morbidity and mortality and high health care costs in the United States (AlGhounaim et al., 2016) and elsewhere. *C. diff* is an anaerobic, spore-forming, toxin-producing, Gram-positive enteric bacteria that has been classified as a true pathogen in pediatrics, as it is responsible for a broad spectrum of diseases in this patient population (e.g., self-limiting secretory diarrhea, colitis, intestinal perforation) (Borali & De Giacomo, 2016). While dormant colonization of the GIT by *C. diff* is common in many healthy individuals, those receiving antibiotics, resulting in a disruption of the intestinal microbiota composition, are at increased risk of developing diarrhea and in severe cases, pseudo-membranous colitis (Bartlett, Chang, Taylor, & Onderdonk, 1979; McFarland, 2008a). An imbalance of commensal microbes following antibiotic exposure allows for *C. diff* to proliferate and produce enterotoxins A and B; toxin A is responsible disrupting colonic mucosal cell adherence to colonic basement membrane and further damages villus tips; whereas toxin B is a potent cytotoxin that enters the cell by endocytosis and induces apoptosis (Bartlett et al., 1979; Borali & De Giacomo, 2016; McFarland, 2008b; McFarland, 2008a). Interestingly, *C. diff* asymptomatic carriage rate among the pediatric population (children less than 3 years of age) was found to be higher than in adults (i.e., 40% carriers in infants versus 14% in adults) (Rousseau et al., 2012; Shim, Johnson, Samore, Bliss, & Gerding, 1998). One study conducted by Matsuki *et al.* (2005) showed that 48% of children 5 years or younger were carriers, with the highest carriage rate (80% to 100%) in infants (Matsuki et al., 2005). However, infant carriers of *C. diff* typically remain asymptomatic, the incidence and severity of *Clostridium difficile* infection (CDI) is generally not a serious issue. The morbidity associated with *C. diff* in adults, particularly those who are hospitalized, is associated with an increased risk of mortality and can have a major impact on quality of life, especially if *C. diff*-associated diarrhea (CDAD) develops and becomes re-current.
In the past decade, probiotics have been used prophylactically to aid in restoring the imbalance of the intestinal microbiota by reseeding the colon with normal commensal bacteria following antibiotic treatment in an effort to reduce the incidence of *C. diff*-associated diarrhea in elderly (Leffler & Lamont, 2009). A meta-analysis was conducted to determine the efficacy of the specific strains of probiotics at reducing the incidence of acute diarrhea in *C. diff*-infected patients. The results revealed that *S. boulardii* showed the most promising protective effect against the development of CDAD and prevention of recurrence. However, LGG and *L. plantarum* were reported to not demonstrate the same effectiveness (Pillai & Nelson, 2008).

With the success of probiotics as a preventative option for CDAD in the elderly, probiotics prophylactically have become increasingly more common practice the pediatric population as well (Leffler & Lamont, 2009).

**Antibiotic-associated diarrhea**

As mentioned above, antibiotic-associated diarrhea (AAD) is caused by treatment with antibiotics, leading to disruption of the intestinal microbiota and causing acute inflammation of the intestinal mucosa, resulting in loose stools and diarrhea (McFarland, 2008b). Most commonly, the microbe associated with ADD is *Clostridium difficile*; however, overgrowth by staphylococci, yeasts and fungi has also been implicated (Lytvyn et al., 2016; McFarland, 2008b; Shim et al., 1998). As such, the rationale for the use of probiotics in AAD is to indirectly restore the intestinal microbiota imbalance, possibly by competitively inhibiting pathogenic bacteria from invasion (McFarland, 2008b). A meta-analysis that included six RCTs, collectively assessing 766 children for the efficacy of several probiotic species (e.g. *L. plantarum*, LGG, *B. lactis*, *S. thermophilus*, and *S. boulardii*) in the prevention of AAD, revealed that concomitant use of probiotics during antibiotic treatment was effective at reducing the risk of AAD in infants and children compared with infants receiving placebo (Szajewska, Ruszczyński, & Radzikowski, 2006). As well, a recent Cochrane review conducted by Goldenberg *et al.* (2015) confirmed the beneficial effects of several probiotic strains (Goldenberg et al., 2015). More specifically, the positive effects were the strongest for *B. lactis* and *S. thermophilus* when fortified in formula milk, whereas LGG showed a significant risk reduction of AAD when administered as a supplement (Goldenberg et al., 2015; Szajewska et al., 2006). Based on the available evidence, various probiotic species and strains have been shown to be effective at providing protection against the occurrence of AAD, with
no adverse events reported in the 23 included RCTs conducted (Goldenberg et al., 2015; McFarland, 2008b; Szajewska et al., 2006).

**Acute infectious diarrhea**

Acute infectious diarrhea is typically classified according to the duration of the bout of diarrhea and is commonly caused by the ingestion of bacterial or viral contaminants in food or water, or by a non-infectious agent (e.g., new medication), leading to diarrhea that may last for up to 14 days (Fewtrell et al., 2005). With infectious diarrhea, the invasion of pathogenic bacteria (e.g. *C. diff*, herpes simplex virus, *Salmonella* spp., *Escherichia coli*) can cause inflammation leading to a cascade of inflammatory responses, whereby the lining of the intestinal epithelium is damaged either by a toxin produced by the pathogen or by the pathogen itself invading the mucosa (Canani et al., 2007). In contrast, there are pathogens that can cause acute diarrhea without eliciting an inflammatory response, such as Norwalk virus, rotovirus, *Staphylococcus aureus*, *Clostridium perfringens*, *Giardia*, or *Cryptosporidium* (Guerrant et al., 2005). In such cases, in addition to episodic diarrhea, patients typically also experience low-grade fever, malaise, nausea, and vomiting (Huang, Bousvaros, Lee, Diaz, & Davidson, 2002). The rationale for using probiotics to prevent and/or treat acute diarrhea is based on the assumption that the invading pathogen leads to disruption of the intestinal microbiota and thus, administration of probiotics can act against the enteric pathogen and ameliorate the disruption by competitively inhibiting the adherence and colonization of the pathogen to restore the imbalanced microbiota (Goldenberg et al., 2015; Huang et al., 2002; Marteau et al., 2001; Szajewska et al., 2006). Probiotics may also aid in the up-regulation of innate protective mechanisms of mucosal defense, such as intestinal cell mucin expression, thus, aiding in the inhibition of viral replication (Ciacci et al., 1993). A total of 63 studies have been conducted (*n*=8,014) in infants and children to assess efficacy of various species and strains of probiotic. The most studied probiotic includes various species of *Lactobacilli* and the most efficacious strain has been LGG (Szajewska et al., 2006). Furthermore, when probiotics are given as a supplement early in the course of diarrhea, it was shown to be most effective at preventing or reducing the duration of diarrhea by 24.76 h (95% CI 15.9 – 33.6 h), the risk of diarrhea lasting ≥ 4 days was reduced (RR 0.41; 95% CI 0.32 – 0.53), and a reduction in stool frequency on day 2 (mean difference 0.80; 95% CI 0.45-1.14), compared to no probiotic supplementation (Huang et al., 2002).
Possible mechanisms of action of probiotics in diarrheal diseases

As discussed, in cases of infectious and non-infectious diarrheal conditions, the assumption is that diarrhea is caused by an invading enteric pathogen, resulting in an imbalanced intestinal microbiota (Bartlett et al., 1979; Marteau et al., 2001). Therefore, one theory of the mechanism of action of probiotics is that certain strains have the capacity to produce anti-bacterial factors, which in turn, aids in their ability to outcompete the undesired organisms (Gareau et al., 2010). Example of such factors are bacteriocins, which are small heat-stable peptides that prevent the growth of various enteric pathogens through its anti-microbial properties against closely related strains of bacteria of the producing microbe (Boirivant & Strober, 2007; Marco et al., 2006). Previous studies have shown that some Lactobacillus strains produce bacteriocins, which provide protection by preventing pathogens from translocating from the intestinal epithelial lumen to the systemic circulation. As well, surface-layer proteins, which are glycoproteins on the cell surface of some lactobacilli (one in particular is L. acidophilus), are capable of binding to the dendritic cell receptor, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), which induces an immune-regulatory response, and thus promotes mucosal homeostasis. Surface-layer proteins on L. helveticus have been shown to prevent the binding of various pathogenic bacteria to intestinal epithelial cells (Johnson-Henry, Hagen, Gordonpour, Tompkins, & Sherman, 2007).

Another mechanism by which probiotics prevent the detrimental effects of infections by various pathogens is by enhancing the intestinal epithelial barrier function (Boirivant & Strober, 2007; Huang et al., 2002; Sherman et al., 2009). For example, both L. helveticus R0052 and LGG possess the ability to prevent the effects of enterohemorrhagic Escherichia coli (EHEC) strain O157:H7, a major foodborne pathogen that is a subset of the pathogenic E. coli known to cause diarrhea and, in more severe cases, hemorrhagic colitis and renal failure in the form of hemolytic uremic syndrome (Sherman et al., 2005). EHEC O157:H7 is capable of producing toxins, known as verotoxin or Shiga toxin (Stxs), which can increase epithelial permeability (Lim, Yoon, & Hovde, 2010). Both L. helveticus R0052 and LGG are able to competitively inhibit attachment of EHEC O157:H7 to adhesion sites, prevent cytoskeletal rearrangements and ultimately, preserving the architecture of intercellular apical tight junctions (Lim et al., 2010; Sherman et al., 2005). Studies have demonstrated that other species and strains of probiotics are also capable of maintaining epithelial barrier function following pathogenic infection and therefore, shortening the duration of infection and indirectly preventing systemic
infection (Boirivant & Strober, 2007; Gareau et al., 2010; Marteau et al., 2001; Sherman et al., 2009).

2.3.3. Necrotizing Enterocolitis (NEC)

Necrotizing enterocolitis is the most severe acquired GIT disease that affects neonates and infants, and is associated with severe morbidity (e.g., intestinal perforation, intestinal stricture, and sepsis) and increased rates of infant mortality (Kosloske, 1984a; Kosloske, 1990). Although 5% to 25% of cases present in term infants, the disease primarily affects preterm infants and mostly very low birth weight (VLBW) infants (weighing <1500 g) (Kosloske, 1990). NEC is the result of necrosis of the intestinal wall lining, which may lead to bowel perforation in one-third of affected infants (AlFaleh & Anabrees, 2014a). Despite decades of research, both the pathogenesis and etiology of NEC remains incompletely understood. However, it is speculated that delayed development of or altered microbial colonization, formula feeding, and neonatal stress are associated with the pathogenesis of NEC, thus leading to the coincidental occurrence of various pathogenic events, such as overgrowth of pathobionts, increase epithelial permeability and excess protein substrate in the intestinal lumen (Kosloske, 1984a; Kosloske, 1990). A study of VLBW preterm infants revealed that early and prolonged exposure to antibiotics significantly increased the risk of developing NEC, and possibly death (Cotten et al., 2009). This study brought forth the notion that an altered intestinal microbiota plays a major role in the pathogenesis of NEC, as 16S rRNA sequencing demonstrated a significant reduction in fecal microbial diversity in NEC infants compared with healthy controls (Cotten et al., 2009). Subsequently, two large clinical trials showed that infants supplemented with probiotics containing Lactobacillus spp. and Bifidobacterium spp. in combination with breast milk exhibited higher Lactobacillus and Bifidobacterium counts in stool and reduced intestinal permeability compared to infants not receiving these probiotics. Furthermore, the probiotic-supplemented infants had significantly reduced NEC incidence and severity compared to control infants (Braga, da Silva, de Lira, & de Carvalho Lima, 2011; Lin et al., 2008a). Another study involved 187 VLBW breast-fed infants who were randomized to groups that were either supplemented with a mixture of L. acidophilus plus B. infantis or received breast milk alone. The results from this study revealed that the incidence of NEC was significantly reduced in the breast-fed infants in the probiotic group compared to the infants.
receiving breast milk alone (Lin et al., 2008a). Additionally, another study investigated the effects of *B. infantis*, *S. thermophilus*, and *B. bifidus* in breast-fed infants compared to infants receiving no probiotics. The findings from this study coincides with previous studies in that the incidence of NEC was significantly lower, 4% in infants in the probiotic supplemented group \((n = 72)\) versus 16% in the no probiotic group \((n = 73)\). Furthermore, it was reported that the severity of NEC was less severe in the probiotic-treated group and no adverse events were observed in this group. However, there were 3 infant deaths in the no probiotic group (Bin-Nun et al., 2005), which led to a meta-analysis to be conducted to determine whether the use probiotics might be an effective therapeutic option for NEC. The results from this study confirmed that probiotic supplementation reduced the incidence of NEC with limited adverse events or long-term effects associated with its use (Deshpande, Rao, Patole, & Bulsara, 2010). However, contrary to the promising findings from these studies, a Finnish study conducted in 2010 failed to confirm the beneficial effect of LGG in preventing NEC (Luoto, Matomäki, Isolauri, & Lehtonen, 2010). Taken together, these findings suggest that more research is necessary to identify the specific probiotic strains that are capable of the specific clinical effects of reducing the risk of developing NEC and its complications.

**Possible mechanism of action of probiotics in NEC**

A number of animal studies have investigated the mechanism by which probiotics decrease NEC incidence and severity (Bergmann et al., 2013; Good et al., 2014; Liu, Tran, Fatereee, & Marc Rhoads, 2014), and it was proposed that probiotics act through its abilities to reduce intestinal permeability (Bergmann et al., 2013), inhibit inflammation (Good et al., 2014), stimulate enterocyte proliferation, reduce apoptosis, modulate T cell activation, and inhibit Toll-like receptor 4 (TLR4) activation, which subsequently leads to nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation (Bergmann et al., 2013; Good et al., 2014; Liu et al., 2014). More specifically, *Bifidobacterium bifidum* OLB 6378 was shown to be associated with reduced mucosal inflammation, improvement of the structural integrity of the intestinal epithelium, and enhanced epithelial barrier function (Khailova et al., 2009). In contrast, supplementation with LGG resulted in up-regulation of cytoprotective gene expression, leading to prevention of the intestinal epithelium from undergoing apoptosis (Lin, Nasr, Berardinelli, Kumar, & Neish, 2008b). Although the exact mechanism of action is uncertain, based on results from animal models, probiotics appear to exert beneficial effects to
reduce the incidence of NEC by limiting the ability of microbes to translocate from the intestinal lumen to the systemic circulation, by enhancing the intestinal epithelial barrier, and by decreasing epithelial apoptosis (Bergmann et al., 2013; Good et al., 2014; Khailova et al., 2009; Lin et al., 2008b; Liu et al., 2014). The exact protective and immunomodulatory mechanisms of these beneficial effects are being increasingly studied and documented.

2.3.4. Infantile Colic

Briefly, infantile colic is a symptom of a condition that is characterized by prolonged crying and fussing episodes that can last approximately 3 hours or more per day, for 3 days or more per week, for 3 weeks or more (Wessel, Cobb, Jackson, Harris, & Detwiler, 1954). Although colic is symptom of a condition of unknown etiology and pathology, a number of studies have implicated an association between intestinal microbiota imbalance and the development of infantile colic (Anabrees, Indrio, Paes, & AlFaleh, 2013; de Weerth, Fuentes, Puylaert, & de Vos, 2013b; Hall, Chesters, & Robinson, 2012). A study was conducted by Lehtonen et al. (1994) to determine whether a difference in the microbial profiles of the intestinal microbiota exists between colicky and non-colicky infants (Lehtonen, Korvenranta, & Eerola, 1994). The results from this study revealed that alterations in the number and species of microbes in the infant gut potentially led to the onset of infant colic in their study participants; more specifically, inadequate levels of various species of bacteria in early infancy influences intestinal fatty acid profiles (Lehtonen et al., 1994). To further substantiate the difference in intestinal microbial composition between colicky and non-colicky infants, mounting evidence indicates that colicky infants have lower counts of lactobacilli, with a parallel increase in anaerobic Gram-negative bacteria in non-colicky infants, and the colonization pattern differed vastly between the two groups (Akbarian-Rad et al., 2013; Savino et al., 2004a; Savino et al., 2005a; Savino et al., 2009). Significantly, colic was proposed to be a consequence of excessive formation of intraluminal gas, causing abdominal discomfort in colicky infants (Akbarian-Rad et al., 2013; Barr, 1998; Hall et al., 2012; Lucassen & Assendelft, 2001; Savino et al., 2009). Appropriately, Savino et al. (2009) conducted a study examining whether a difference exists in the colonization pattern of gas-forming bacteria between colicky and non-colicky infants (Savino et al., 2009). The results from this study confirmed that colicky infants were more frequently colonized with the gas-forming
Clostridium difficile and that its presence temporally coincided with infantile colic. However, several RCTs and meta-analyses have been conducted to investigate possible treatments for infantile colic and the results consistently revealed that colicky infants treated with simethicone, an anti-foaming agent used to reduce or prevent the formation of gas in the GIT (Brecević, Bosan-Kilibarda, & Strajnar, 1994), were no more effective at reducing colic symptoms than placebo (Hall et al., 2012; Kvitvaer, Miller, & Newell, 2012; Lucassen & Assendelft, 2001; Savino, Pelle, Palumeri, Oggero, & Miniero, 2007). Taken together, Savino and colleagues concluded that colic may be the result of the atypical increase presence of gas-forming bacteria, rather than the gas itself, and therefore, the authors suggested that an aberrant intestinal microbiota in early infancy may be the contributing factor in the development of colic, which is consistent with findings from other clinical studies (Anabrees et al., 2013; de Weerth et al., 2013a; Lehtonen et al., 1994; Savino et al., 2005a; Savino et al., 2007). Despite the fact that it remains uncertain whether it is the presence or absence of specific species of microbes that may be the cause of colic, a large body of evidence strongly suggests that an association between the microbiota and infantile colic exists. Therefore, it was proposed that the use of probiotics might be a potential therapeutic intervention for infantile colic (Akbarian-Rad et al., 2013; Chau et al., 2015; Lucassen & Assendelft, 2001; Savino et al., 2007; Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a).

As this area of research is the main focus of this thesis, an in depth overview of infantile colic, the role of probiotics as a treatment for the condition and possible mechanisms of action in which probiotics exerts its beneficial effects in colicky infants are provided in the following section.

In summary, based on the evidence provided above, regarding the mechanisms of action of probiotics in common pediatric GI-related conditions, there are clear clinical rationales for the use of probiotics as a therapeutic option in the pediatric population. As the protective and immunomodulatory effects of probiotics are increasingly being documented, with clinical benefits being demonstrated for specific species and strains of microbes, it is important to focus research on matching the specific probiotic species with the desired clinical effect in a particular clinical condition in order to maximize the effectiveness of probiotics as therapeutic agents.
2.4. Overview of Infantile Colic

In the early days of life, crying is thought to be normal behavior during infancy, as it is an infant’s means of survival to elicit help for their physiologic needs, such as hunger, diaper changes, temperature modulation and discomfort or pain relief (Brazelton, 1962). However, between 5% to 40% of infants cry excessively and inconsolably, sometimes accompanied by frequent bouts of fussiness and gassiness (Anabrees et al., 2013; Lucassen et al., 2001; Wake et al., 2006), which describes symptoms of condition known as “infantile colic”, coined by a pediatrician named Morris A. Wessel (Wessel et al., 1954). More accurately, but seemingly arbitrarily, the widely accepted definition of infantile colic is characterized by Wessel’s “rule of 3s”, which describes an infant with colic as “one who is otherwise healthy and well-fed, had paroxysms of irritability, fussy or crying lasting for a total of three hours a day, occurring on more than three days in any one week for a period of three weeks” (Wessel et al., 1954). There is also a distinction between ‘crying’ and ‘fussing’ behaviour, as fussing is not quite crying but rather describes an infant who is discontented despite being awake, well-fed and comforted (Barr, Kramer, Boisjoly, McVey-White, & Pless, 1988).

A myriad of studies have been conducted in attempts to fully understand the course of the condition associated with colic and the consensus is that colic can manifest as early as 2 weeks of age, with excessive crying and fussy episodes peaking between 6 to 8 weeks of life and showing signs of diminishing in crying and fussing duration times at approximately 4 to 6 months of age (Barr, 1990; Barr, 1998; Brazelton, 1962; Wessel et al., 1954) (Figure 2.5).

![Figure 2.5: Crying pattern in early infancy. Duration of infant crying times of high criers, average criers and low criers. (Reprinted with permission for use from: Barr RG. (2002) The period of PURPLE crying. The National Center on Shaken Baby Syndrome, Ogden, Utah. (Website: http://purplecrying.info/sub-pages/crying/why-does-my-baby-cry-so-much.php)](image)
A colic episode is frequently characterized as excessive, inconsolable, prolonged, high-pitched crying in which infants appear rigid, with tenseness of the abdomen, tightly clenched fists and tightly closed eyes, reddened flushed face, frequent flatulence, with legs drawn up towards the abdomen (Table 2.3) (Brazelton, 1962; Kvitvaer et al., 2012; Wessel et al., 1954).

Table 3: Checklist of colic diagnostic criteria.

<table>
<thead>
<tr>
<th>Characteristics of Infantile Colic</th>
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<tbody>
<tr>
<td>Excessive and prolonged crying/fussing</td>
</tr>
<tr>
<td>Flexing/curling legs</td>
</tr>
<tr>
<td>Clenched fists</td>
</tr>
<tr>
<td>Distended abdomen</td>
</tr>
<tr>
<td>Difficulty/discomfort with bowel movements</td>
</tr>
<tr>
<td>Prolonged irritability post-feeding</td>
</tr>
<tr>
<td>Appears to be in pain</td>
</tr>
<tr>
<td>Changes from happy to crying in an instant</td>
</tr>
<tr>
<td>Family history of asthma/allergy</td>
</tr>
</tbody>
</table>

(Adapted and modified from: Kvitvaer BR, et al. Improving our understanding of the colicky infant: a prospective observational study. JNC. 2012 Jan: 21(1-2); 63-9) (Kvitvaer et al., 2012).

Of note is that a diurnal occurrence pattern has been observed, in that crying or fussing and gassy periods may possibly begin at the same time each day, with infants displaying an increase in anxiousness in the late afternoon, early evening and into the late night, which can last up to 2 to 3 hours (Figure 2.6) (Barr, 1990; Wessel et al., 1954). Moreover, during colic crying and/or a fussing/gassy bout, crying can be continuous, lasting up to 5 minutes per episode; more concerning to parents and caregivers is that the infant appears to be in appreciable pain (Barr, 1998; Kvitvaer et al., 2012; Lucassen et al., 2001). Interestingly, and oftentimes frustratingly, paroxysms of crying and fussy/gassy periods can commence and subside without an obvious trigger or cause, which makes infantile colic difficult to manage or treat (Barr, 1990; Brazelton, 1962; Kvitvaer et al., 2012; Wessel et al., 1954).
Figure 2.6: Diurnal pattern of colic episodes. An hourly incidence of crying and fussing episodes in colicky infants. (Reprinted from: Wessel MA, et al. Paroxymal fussing in infancy, sometimes called “colic”. Pediatrics 1954 Nov: 14(5); 421-35.)

2.4.1. Etiological Theories of Infantile Colic

Despite decades of research, the etiology of infantile colic remains unclear; however, a plethora of theories have been proposed, which suggests that the cause may be multifactorial. A strongly held notion is that colic is a result of underlying organic causes of excessive crying, while other theories have described infant colic as a psychological disorder (Lucassen et al., 2001). Additional possible causes include overproduction of intestinal gas, cow’s milk protein allergy, altered intestinal microbiota, forceful intestinal contraction, transient lactase deficiency, infant’s difficult temperament, inadequate mother-infant communication or insecure attachment, less than optimal parent-infant interaction, and parental overstimulation (Barr, 1998; Hall et al., 2012; Lucassen, 2010; Poole, 1991). Some of these proposed theories have led researchers to conclude that colic may possibly be of gastrointestinal origin; as such, to better understand the etiology of the condition being of GI origin, a more detailed discussion of the most prominent theories follows below.

2.4.1.1. Cow’s milk protein allergy (CMPA) and maternal diet

For many decades, cow’s milk protein allergy (CMPA) has been proposed to be the cause of infantile colic (Hill et al., 1995; Jakobsson & Lindberg, 1978; Jakobsson & Lindberg, 1983; Lothe & Lindberg, 1989b). CMPA refers to an immunologic reaction to one or more of
the proteins present in bovine milk, which may result in an immunological response and GI symptoms (e.g. diarrhea, abdominal discomfort, vomiting, and colic) (Berg, Jakobsson, & Lindberg, 1979; Jakobsson & Lindberg, 1979). Commonly, the first signs of CMPA manifest in early infancy in both formula- and breast-fed infants if the breastfeeding mother consumes dairy products; however, the detection of CMPA is lower in breastfed infants, as less cow’s milk protein (CMP) is transferred through the breast milk (Berg et al., 1979; Jakobsson & Lindberg, 1978; Jakobsson & Lindberg, 1983). There are 2 types of CMPA, (1) an immunoglobulin E (IgE)-mediated type, and (2) a non-IgE-mediated type, which manifest different symptoms. The IgE-mediated CMPA commonly leads to vomiting and/or urticarial immediately following ingestion of CMPA, whereas symptoms of non-IgE-mediated CMPA may develop within a few hours or even several days following exposure and include diarrhea, atopic dermatitis, infantile colic, and general GI discomfort (Berg et al., 1979; Jakobsson & Lindberg, 1978; Vandenplas et al., 2007).

Although a link between dietary food allergies and colic has not been confirmed, many health care professionals often resort to elimination of CMP exposure as a first attempt to alleviate colic symptoms, as it is the least invasive intervention. A study that was conducted by Clifford and colleagues (2002) to determine whether colic may be reduced through dietary interventions revealed that the type of feeding (e.g., breastfed or formula fed) had no significant effect on the prevalence of colic (Clifford, Campbell, Speechley, & Gorodzinsky, 2002b). Furthermore, results from several other studies concluded that the exclusion of cow’s milk by breastfeeding mothers did not reduce the incidence of colic (Critch, 2011; Dreborg, 2016; Lothe & Lindberg, 1989a; Vandenplas et al., 2007). However, several studies also contradict the previously mentioned studies and demonstrate that elimination of cow’s milk protein in the breastfeeding mother’s diet resulted in the complete resolution of or reduced colic crying (Hill et al., 1995; Jakobsson & Lindberg, 1978; Jakobsson & Lindberg, 1983; Lothe & Lindberg, 1989a; Oggero, Garbo, Savino, & Mostert, 1994). Additionally, a well-designed RCT involving 90 colicky infants and their breastfeeding mothers, conducted to determine whether maternal dietary restriction of dairy products, eggs, nuts, soy and fish had an effect on the incidence of colic, revealed a 37% absolute risk reduction of the incidence of colic compared to infants of the no diet-restricted breastfeeding mothers (Hill et al., 1995). Of note is that the authors of this study did not re-challenge the colicky infant by re-introducing the CMP to determine whether relapse in symptoms occurred, which would have definitively
confirmed the association between CMPA and colic. Therefore, it was suggested that CMPA may potentially cause infant colic symptoms, but more studies are needed to confirm the association.

2.4.1.2. Maternal-infant interaction

There is growing evidence indicating the relative importance of personal and environmental influences on infant development (Hunter & Maunder, 2001; Maunder & Hunter, 2001; Maunder & Esplen, 2001; Winnicott, 1960); it has been proposed that newborns and infants innately and intuitively rely on personal interactions and environmental cues for their social and emotional development (Haley & Stansbury, 2003). Additionally, establishing a strong parent-infant connection provides the infant with security and comfort, which encourages normal behavioural and psychosocial development in the infant (Akman et al., 2006). It is thought that an infant can inherently gauge the level of interaction between themselves and their parents or caregivers by the level of parental responsiveness to their physiologic needs (e.g. hunger, diaper change, sleep), which is expressed through crying (Akman et al., 2006; Brazelton, 1962). Therefore, it has been proposed that colicky infants experience less than optimal parent-infant interaction due to the delay or lack of responsiveness to their needs, resulting in prolonged and excessive crying episodes (Akman et al., 2006; Canivet, Jakobsson, & Hagander, 2000; Haley & Stansbury, 2003). The idea is that parents who respond more immediately and reliably to their infant’s social and emotional cues aid in their infants’ ability to regulate stress more efficiently and develop a more secure emotional parent-infant connection, thus developing more secure attachment behaviours; as such, these infants cry significantly less than infants whose parents delay in responding to their needs (Haley & Stansbury, 2003). As a result, it is proposed that the latter infants form insecure attachment behavioural patterns, such as excessive crying, clinging, following, fussing and protesting (Maunder & Hunter, 2001).

Evidence for this theory was examined in a study conducted by Akman et al. (2006) involving 100 mother-infant pairs to investigate the relationship between attachment styles (or parental responsiveness) and infantile colic. The results from this study support the notion that infants who experienced low levels of maternal (or parental) interaction responded by excessive crying to signal unmet needs or distress (Akman et al., 2006). Additionally, results from a separate study confirmed that infants of more responsive parents showed a greater
ability to regulate stress, which was determined by their ability to be soothed and calmed once their needs were met, leading to the development of secure attachment styles with their parents due to the optimal level of parent-infant interaction (Haley & Stansbury, 2003). Interestingly, Akman and colleagues revealed that more mothers suffering from postpartum depression (determined by higher mean Edinburgh Postpartum Depression Score (EPDS) score) had infants diagnosed with colic compared to mothers who did not experience postpartum depression.

Despite the many studies supporting the maternal-infant interaction theory of infantile colic, there are nearly as many contrary studies. The authors of a study conducted to investigate the association between maternal-infant interaction and attachment style and infantile colic suggested that, while excessive crying and fussiness or difficult temperament have been linked to poor maternal-infant interaction and subsequently, insecure infant attachment, the association is typically qualified by other factors (Stifter & Bono, 1998), such as maternal personality and sensitivity to responsiveness (Mangelsdorf, Gunnar, Kestenbaum, Lang, & Andreas, 1990), postpartum depression (Akman et al., 2006) and lack of social support (Crockenberg, 1981). Stifter and colleagues (1998) explained that the development of an insecure attachment is a result of the mother’s lack of sensitive responsiveness to her crying infant, and infantile colic was identified as preceding maternal sensitivity to respond and development of insecure attachment. For instance, a colicky infant who does not respond to maternal attempts to soothe and calm their negative states or one who does not show much positivity in response to their mother’s attempts to interact may, in turn, elicit less sensitive responsiveness from their mother. The lack of responsiveness to an infant’s cues, whether due to postpartum depression or the nature of the mother’s personality, leads to sub-optimal maternal-infant interaction and subsequently, results in the infant’s development of insecure attachment behaviours, such as excessive crying and clinging (Stifter & Bono, 1998).

It is uncertain whether maternal postpartum depression, resulting in inadequate maternal-infant interaction, is a predictor of having a colicky infant or whether an excessively crying infant precede the former remains to be determined. Despite the difficulty determining whether infantile colic is the cause or the effect, the findings from these studies suggest that maternal-infant interactions and responsiveness may play a role in the attachment styles of an infant and importantly, may impact greatly on an infant’s ability to regulate stress and crying.
behaviours despite the conflicting results (Akman et al., 2006; Clifford, Campbell, Speechley, & Gorodzinsky, 2002a; Haley & Stansbury, 2003; Hunter & Maunder, 2001).

2.4.1.3. Feeding difficulties

Feeding difficulties during infancy have been noted to commonly coincide with crying problems, and many studies have been conducted to determine whether mode of feeding (Clifford et al., 2002b; Hemmi, Wolke, & Schneider, 2011; Jakobsson & Lindberg, 1978; Jakobsson & Lindberg, 1983) and formula composition (Berg et al., 1979; Dreborg, 2016; Hill et al., 1995; Lothe & Lindberg, 1989a) may be linked to the onset of colic crying. A study conducted by Millar-Loncar et al. (2004) examined the relationship between infantile colic and infant feeding difficulties, in which the authors hypothesized that colicky infants experience more feeding issues, such as disorganized oral motor skills, gastro-esophageal reflux (GER), and discomfort associated with feedings, compared to non-colicky infants. The findings from this study provided evidence of functional feeding difficulties leading to colic symptoms (Miller-Loncar, Bigsby, High, Wallach, & Lester, 2004). Factors such as underfeeding, overfeeding, inability to latch properly resulting in swallowing of excessive air, or not being adequately burped post-feed have been proposed as contributing to prolonged and excessive colic crying. Furthermore, the authors demonstrated that colicky infants showed: (1) a higher incidence of GER, as determined by ultrasound and on the I-GERQ questionnaire; (2) experienced more difficulties sucking, evidenced by arrhythmic jaw movements and difficulties coordinating sucking, swallowing and breathing; and (3) more frequent episodes of feeding discomfort post-feed (e.g., fussing, constant movement), as reported by mothers in behavioural diaries (Miller-Loncar et al., 2004). The authors concluded that, although there is insufficient evidence of a direct causal relationship between feeding difficulties and infantile colic, functional feeding problems seem to contribute to or exacerbate colic symptoms (Miller-Loncar et al., 2004).

2.4.1.4. Altered intestinal microbiota

Perhaps due to the term colic, derived from the Greek word kolikos, meaning relating to the colon (Wessel et al., 1954), many sources have proposed potential causes of colic to be of gastrointestinal origin (Barr, 1998; Hall et al., 2012; Kvitvaer et al., 2012; Lucassen, 2010; Rhoads et al., 2009; Savino, 2007). More recently, numerous studies have emerged implicating
a potential role for the intestinal microbiota in the etiopathogenesis of infantile colic, as it has been demonstrated by several research groups that the intestinal microbiota in colicky infants drastically differs from non-colicky infants (Akbarian-Rad et al., 2013; de Weerth et al., 2013a; Savino et al., 2005a; Savino et al., 2009). Further supporting this association is the finding that alterations in the colonization pattern of the infant GIT result in significant changes in microbial metabolic activity, leading to the release of potential toxins that may cause intestinal inflammation (de Weerth et al., 2013a; de Weerth et al., 2013b; Lucassen, 2010; Savino et al., 2005a). Although the intestinal microbiota theory may be one of many causes proposed, the growing evidence substantiating an association between intestinal microbes and the onset of colic crying in otherwise healthy infants is too significant to be disregarded. A more detailed discussion of the evidence for the association of the intestinal microbiota and infantile colic is provided in subsequent sections.

With contradictory evidence reported for each of the presented theories, the etiology of infantile colic remains poorly understood. However, it can be concluded that infantile colic presents as a heterogeneous condition and is multifactorial with multiple potential causes.

2.4.2. Consequences of Infantile Colic

Although infantile colic is described as a self-limiting, benign and transient condition, there exists some evidence of short- and long-term consequences associated with a colic diagnosis, such as GI and atopic disorders (Savino et al., 2005b), cognitive and behavioural developmental issues (Rao, Brenner, Schisterman, Vik, & Mills, 2004), feeding and sleeping disturbances (White, Gunnar, Larson, Donzella, & Barr, 2000), and more recently, migraines (Epstein & Zee, 2013).

2.4.2.1. Gastrointestinal consequences

It has been proposed that formerly colicky infants are at increased risk of developing functional gastrointestinal disorders (FGIDs) compared to non-colicky infants, as colic has been previously described as an early manifestation of FGIDs (Benninga et al., 2016; Uc, Hyman, & Walker, 2006). However, limited and contradictory evidence suggest that FGID may have occurred coincidentally in the formerly colicky infants who participated in the trial conducted by Joseph and colleagues, which investigated the relationship between infantile
colic and recurrent abdominal pain (RAP), since only 1 in 30 formerly colicky infants developed RAP in later childhood (Joseph & Lupu, 1984). A more recent prospective study conducted by Savino and colleagues revealed that 33% of formerly colicky infants (compared to 4% of non-colicky infants) were diagnosed with RAP by the age of 10. However, the authors noted that a stronger family history of GI disorders was seen in the former colicky infant group compared to the non-colicky infant group (33% versus 13%), which may potentially contribute to the higher number of RAP diagnoses in the first group (Savino et al., 2005b). Taken together, the evidence of an association between infantile colic and FGID diagnosis remains unclear, as the sparse and conflicting available studies are unable to substantiate this claim.

2.4.2.2. Atopic/Allergic disorders

In a prospective 10-year follow-up study by Savino et al. (2005) involving 48 formerly colicky and 48 non-colicky infants was conducted to determine the long-term effects of colic on children later in life. The results from this study revealed that children in the formerly colicky infants group manifested higher frequency of atopic and allergic conditions compared to non-colicky infants (Savino et al., 2005b). Specifically, the frequency of atopic eczema in formerly colicky infants was 31% (vs. 6%), food allergy was 23% (vs. 6%), allergic rhinoconjunctivitis was 27% (vs. 4%), and asthmatic bronchitis was 23% (vs. 6%) when compared to non-colicky infants. Noteworthy was that a higher number of formerly colicky infants reported a family history of atopic and allergic disease, 48% compared to 25% in the non-colicky infants. The authors concluded that formerly colicky infants were at an increased risk of developing atopic and allergic diseases in later childhood compared to non-colicky infants and that infantile colic may be an early expression of some common childhood disorders (Savino et al., 2005b). However, contrary to these findings, another prospective study of 90 formerly colicky infants reported that colicky infants were not at an increased risk of developing atopic or allergic conditions later in childhood. The authors also found that, at 11 years of age, formerly colicky infants did not have a higher frequency of positive skin prick tests or higher levels of total serum IgE compared the control infants (Castro-Rodríguez et al., 2001). With these contradictory results, it is difficult to conclude that an association exists between infantile colic and the later development of atopic and allergic diseases; therefore, further, larger studies are needed to conclusively confirm this association.
2.4.2.3. Cognitive and behavioural development

It has been suggested that formerly colicky infants experience delayed cognitive and behavioural development as a result of the lack of, or distressed, or sometimes detached parent-infant interaction with formerly colicky infants compared with non-colicky infants. It has been reported that, at 6 months of age, more colicky infants obtained lower scores on the Mental and Psychomotor scales of the Bayley scales of Infant Development compared with non-colicky infants (Sloman, Bellinger, & Krentzel, 1990). In line with these results, a separate prospective five-year follow-up study was conducted with 48 colicky infants to determine whether excessive and prolonged crying leads to lower intelligence, impaired motor abilities, and/or behavioural issues (Rao et al., 2004). The authors reported that formerly colicky infants who cried excessively for more than 3 months of age had significantly lower intelligent quotient (IQ) scores and experienced a delay in developing fine motor skills compared to non-colicky infants. Interestingly, the authors concluded that formerly colicky infants who experienced transient prolonged crying for less than 3 months of age did not display delayed cognitive or behavioural development (Rao et al., 2004). This finding was replicated in a separate study conducted by Rautava et al. (1995), as the authors concluded that no differences in cognitive and behavioural development was observed between formerly colicky and non-colicky infants (Rautava, Lehtonen, Helenius, & Sillanpää, 1995).

With regard to developmental behavioural issues, data from a comprehensive meta-analysis that included 9 RCTs revealed that formerly colicky children were more likely to be aggressive, exhibit destructive behaviour with conduct problems, have a higher frequency of temper tantrums and were more commonly diagnosed with attention deficit hyperactivity disorder (ADHD) in late childhood compared to non-colicky children (Hemmi et al., 2011). Notably, 3 of the 9 RCTs included in the meta-analysis involved infants with other health concerns who were classified as ‘persistent criers’ (Hemmi et al., 2011).

Based on the available studies, it appears that, due to the transient and self-limiting nature of colic, colicky infants were categorized in either a ‘typical colicky infant’ or a ‘persistent colicky infant’ group. As such, the evidence suggests that a possible association exists between colic and delayed cognitive and behavioural development in late childhood in infants who experience ‘persistent and prolonged crying’ (>3 months of age); whereas, in the
‘typical colic crying’ group, no developmental delays were observed. Thus, it is evident that more studies are needed to confirm this association.

2.4.2.4. Feeding and sleep disturbances

Feeding, sleeping and crying patterns are collectively referred to as an infant’s regulatory behaviours, as these behaviours requires an adjustment period and thus, are learned behaviours. As such, it was suggested that feeding and sleep disturbances, as well as prolonged crying, share common etiological causes (Hemmi et al., 2011). A four-year follow-up study involving 50 formerly colicky infants examining feeding and sleep habits in later childhood revealed that these children were more likely to display more negative emotions and moods during feeding and with limited food choices and preferences at 4 years of age compared to non-formerly colicky infants (Canivet et al., 2000). Furthermore, similar to the study mentioned above, long-term feeding and sleep consequences associated with colic were subcategorized into ‘typical colic crying’ (<3 months of age) and ‘persistent colic crying’ (> 3 months of age) patterns and durations. The authors concluded that this association between colic and feeding habits were more highly observed in the ‘persistent colic crying’ group than the ‘typical colic crying’ group (Canivet et al., 2000; Hemmi et al., 2011).

As mentioned, excessive crying has been described as an infant’s inability to readily adjust to varying behavioural states and therefore, with respect to sleeping habits, an inability to quickly adapt to the change between sleep-wake patterns results in interrupted sleep cycles (Visser, 2012). As such, it was reported that colicky infants slept approximately 1-2 hours less than non-colicky controls during the first 2 months of life, and this discrepancy in sleep duration persisted until 6 months of age. However, it was reported that by 2 years of age, the sleep patterns of formerly colicky children were similar to those of non-colicky children (James-Roberts, Conroy, & Hurry, 1997). Additionally, no difference was observed in the total duration of night sleep and the number of awakenings between the two groups by 8 to 12 months of age (Lehtonen, Korhonen, & Korvenranta, 1994). Despite the negative results showing no association between colic and sleep disturbances, a study conducted by Schmid et al. (2010) established that ‘persistent colic crying’ (prolonged crying >3 months of age) is a risk factor for sleeping issues by the age of 4 years (Schmid, Schreier, Meyer, & Wolke, 2010).

Taken together, the available evidence regarding the long-term consequences for developing negative feeding habits and disrupted sleeping patterns in formerly colicky infants
appears to be contradictory, as not enough evidence is currently available to conclusively confirm the association. However, based on the multiple studies, there seems to exist an association between these factors in a subpopulation of colicky infants described as ‘persistent colic criers’ (excessive crying beyond 3 months of age).

2.4.2.5. Migraine

Despite the fact that the association between colic and migraines is not completely understood, several retrospective studies have concluded that in children who experience migraines, a high proportion were reported to be formerly colicky infants (Bruni et al., 1997; Jan & Al-Buhairi, 2001; Romanello et al., 2013). Furthermore, another study conducted with similar objectives to investigate the connection between colic and migraines in later childhood revealed that infants with a maternal history of migraines were 2.6 times more likely to be former colicky infants. The authors proposed the notion that infantile colic may be an early manifestation of migraines in later childhood, which may perpetuate throughout adulthood (Gelfand, Thomas, & Goadsby, 2012). However, since the exact etiology of both migraines and colic are incompletely understood, more long-term studies are needed in order to make a conclusive link between the two disorders.

2.4.3. Management and possible treatments for infantile colic

Due to the multiple origins and complex pathogenesis of infantile colic, a consensus on an effective treatment for lessening the intensity and frequency of crying and fussing episodes still remains elusive. However, because many researchers and physician accept the ‘gut hypothesis’ of infantile colic, current treatments are targeted at maintaining efficient intestinal function and a healthy gut. Therefore, maternal nutritional interventions for breastfed infants included substituting cow’s milk with almond, soy or coconut milk, or a hypoallergenic casein hydrolysate milk, lactose-free milk, or fibre-enriched formula milk (Hall et al., 2012; Kvitvaer et al., 2012; Lucassen & Assendelft, 2001); using herbal remedies, such as herbal teas or gripe water (a concoction of herbal oils); and using pharmacological therapies such as dicyclomine, which reduces forceful intestinal contractions, and simethicone, which diminishes the formation of intestinal intraluminal gas (Hall et al., 2012; Lucassen & Assendelft, 2001; Savino et al., 2015). Contrarily, the behavioral component theory suggests alternative therapies such as modifying parent-infant interaction and stimulation; increase parental attentiveness or
carrying; providing constant motion (e.g., car rides, vibrating chair, swing, etc.) and parental physical touch (Akbarian-Rad et al., 2013; de Weerth et al., 2013a; Rautava et al., 1995; Savino et al., 2005a; Savino et al., 2009; White et al., 2000). More recently, with the potential association between the intestinal microbiota and infantile colic, several studies have investigated the effectiveness of probiotic supplementation on reducing crying and fussing times associated with colic (Akbarian-Rad et al., 2013; Anabrees et al., 2013; Chau et al., 2015; de Weerth et al., 2013b; Hall et al., 2012; Lehtonen et al., 1994; Lucassen & Assendelft, 2001; Mi et al., 2015; Rhoads et al., 2009; Savino et al., 2004a; Savino et al., 2005a; Savino et al., 2007; Savino et al., 2009; Savino et al., 2010; Savino et al., 2015; Sung et al., 2014; Szajewska et al., 2013a).

A systematic review was conducted to distinguish effective treatment options for infantile colic (Hall et al., 2012). Despite the wide range of pharmacological, nutritional, behavioral and psychological interventions to aid in alleviating colic symptoms, the findings from the review revealed that the use of dicyclomine (Savino, Brondello, Credi, Oggero, & Silvestro, 2002) and, to a lesser extent, elimination of cow’s milk protein (Lothe & Lindberg, 1989a) demonstrated efficacy above all other treatments, which were consistent with previous findings. Although dicyclomine has been safely used for the treatment of infantile colic for many years, in one study showing a 53% improvement of crying and fussing times (Oggero et al., 1994), use in infants has diminished due to two reports of sudden infant death syndrome (SIDS) associated with administration of dicyclomine (Randall, Gerry, & Rance, 1986). This led to the recommendation of complete discontinuation of dicyclomine use in infants, as findings from two further studies demonstrated that the used of dicyclomine is associated with acute episodes of apnea, seizures and in one severe case, coma (Hwang & Danielsson, 1985; Randall et al., 1986). Thus, there still remains no effective treatment for reducing paroxysmal crying and fussing in colicky infants.

Families of colicky infants experience tremendous distress and exhaustion, and an inconsolable infant can threaten the psychological and physical well-being of the parents or caregiver (Stifter & Bono, 1998), which has even been suggested as a factor for shaken baby syndrome (Talvik, Alexander, & Talvik, 2008). Surprisingly however, either due to the lack of an effective treatment for the condition or the natural course of infantile colic, only one in six (approximately 17%) families with colicky infants consult a medical professional for treatment of colic symptoms (Savino et al., 2005b; Stifter & Bono, 1998). Despite the frequent episodes
of prolonged inconsolable crying and fussiness and the emotional distress experienced by families of colicky infants, the clinical course of the condition is favorable, as the frequency and duration of crying and fussing episodes significantly decrease by the age of 4 to 5 months in most infants (Savino et al., 2005b; White et al., 2000). As a result of the spontaneous resolution of the condition, independent of treatment, it is not surprising that many parents and caregivers opt to not treat colicky infants (Savino et al., 2005b; Stifter & Bono, 1998).

With the myriad of readily available remedies for infant colic, ranging from drugs to behavioral and psychological therapies, the failure to pinpoint a globally effective treatment results in inadequate care to manage an infant’s colic crying, as well as support for parents, caregivers and health care professionals. Consequently, the recent emergence of clinical studies investigating the link between the intestinal microbiota and the onset of colic crying suggests that the intestinal microbiota may be a potential therapeutic target to treat infants presenting with colicky behavior (Akbarian-Rad et al., 2013; Anabrees et al., 2013; Hall et al., 2012; Kvitvaer et al., 2012; Lehtonen et al., 1994; Lucassen & Assendelft, 2001; Rhoads et al., 2009; Savino et al., 2004a; Savino et al., 2005a; Savino et al., 2015).

2.4.4. Probiotics to treat infantile colic

With the emergence of evidence confirming the role of the intestinal microbiota in the development of infantile colic, treating an aberrant microbiota with probiotics in early infancy may be a potentially effective approach to manage colic symptoms (Chau et al., 2015; de Weerth et al., 2013b; Hall et al., 2012; Mi et al., 2015; Savino et al., 2004a; Savino et al., 2005a; Savino et al., 2010; Savino et al., 2015; Sung et al., 2014; Szajewska et al., 2013a). Appropriately, growing evidence suggests that supplementation with probiotics can modulate intestinal bacterial patterns by aiding in the colonization of beneficial bacteria, and therefore, provides an opportunity for probiotics to treat diseases associated with an aberrant intestinal microbiota. Five randomized controlled trial were conducted to examine the effect of probiotic supplementation, using the strain Lactobacillus reuteri DSM 17938, versus placebo, in breastfed infants, for the treatment of infantile colic (Chau et al., 2015; Mi et al., 2015; Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a). Collectively, the results revealed that infants treated with probiotics showed a statistically significant decrease in the frequency of crying and fussy/gassy episodes. Furthermore, two of the RCTs also examined infant fecal
samples to determine the composition of the microbiota and the findings showed an improvement of the diversity of the microbial colonization pattern from baseline to the end of study period in colicky infants treated with the probiotic, *L. reuteri* DSM 17938 compared to the placebo group (Savino et al., 2010; Sung et al., 2014). The proposed mechanism of action is that colonization with *L. reuteri* DSM 17938 influences intestinal motility and function, colonic contractile activity and colonic sensory nerves, leading to an effect on pain perception (Savino et al., 2010). Additionally, *L. reuteri* DSM 17938 may also exert anti-inflammatory effects by suppressing adhesion of inflammatory-inducing bacteria (Gareau et al., 2010; Marco et al., 2006; Savino et al., 2010; Savino et al., 2015). Therefore, the overall conclusion of these results strongly suggests that probiotic supplementation with *L. reuteri* DSM 17938 may be an effective treatment for infantile colic (Chau et al., 2015; Mi et al., 2015; Savino et al., 2010; Savino et al., 2015; Szajewska et al., 2013a). However, as mounting evidence suggests that geographical, dietary and cultural differences all play a role in the effect of probiotics on the intestinal microbiota, repeat studies are necessary to more broadly generalize the role of probiotics for colic.
CHAPTER 3
MATERIALS AND METHODS

3.1 Overview of Systematic Reviews: Clinical Efficacy of Probiotic Use in Pediatrics

Contents in this section contains current work that is in preparation for publication and has been registered on PROSPERO:


[KC drafted the protocol, co-searched the databases, screened and selected eligible reviews, extracted the data, performed the analysis, and prepared the manuscript for submission].

3.1.1 Formulation of Research Question

This review was guided by the following research question: Are probiotics effective therapeutic interventions used to prevent and/or treat gastrointestinal conditions in the pediatric population?

The Cochrane Collaboration introduced a new type of review called, ‘Overview of Systematic Reviews (OoSR)’, which are essentially reviews aimed to provide a summary of and to compare/contrast findings from more than one systematic reviews of a topic under consideration, rather than the traditional inclusion and assessment of individual RCTs. Thereby, OoSRs provide clinical decision makers with all the available up-to-date evidence pertaining to a particular area of health care, at a variety of different levels, including the combination of different interventions, different outcomes, different conditions, problems or populations, or a summary of evidence on the associated adverse events of an intervention, all in a single, comprehensive and up-to-date review (Becker & Oxman, 2009). Therefore, due to the proliferation of RCTs and systematic reviews relating to the use of probiotics in recent years, this OoSR was conceived with the primary objective of amalgamating all the current available evidence relating to the use of probiotics for the prevention and treatment of GI-related conditions in the pediatric population. The gastrointestinal conditions of interest include:
• Inflammatory bowel disease (IBD)
• Functional Gastrointestinal Disorders (FGIDs)
• Irritable Bowel Syndrome (IBS)
• Diarrheal conditions
  ▪ Clostridium difficile-Associated Diarrhea (CDAD)
  ▪ Antibiotic-Associated Diarrhea (AAD)
  ▪ Acute Infectious Diarrhea (AID)
• Necrotizing Enterocolitis ( NEC)
• Infantile Colic

3.1.1 Eligibility Criteria for Considering Reviews

Types of reviews

All Cochrane and non-Cochrane systematic reviews and meta-analyses that fulfilled the Cochrane Collaboration’s Handbook definition of a systematic review of interventions (i.e. “reviews of clearly formulated questions that use systematic and explicit methods to identify, select and critically appraise relevant research” (Higgins & Green, 2011) were included. Eligible reviews had to include at least one form of quantitative data measuring improvement of condition symptoms, as this allowed for calculation of relative risks (RR) and confidence intervals (CI). Of note, all eligible Cochrane reviews that met the inclusion criteria were included and in the event a non-Cochrane systematic review overlapped in topic area (e.g. covering similar GI conditions and probiotic species and strain) also met the above inclusion criteria, only the most up-to-date review was included.

Types of participants

Reviews that included pediatric participants between 0 to 18 years of age diagnosed with an existing acute or chronic GI condition (see above) were included. Pre-term infants were also included. Reviews with otherwise healthy participants or the inclusion of participants based on their risk level of developing GI-related conditions (e.g. due to family history of GI disorders) were excluded. Furthermore, for reviews that included both pediatric and adult data, pediatric data must have been able to be isolated, extracted and summarized separately.
Types of intervention

Any probiotics, bacterial or yeast, as defined by the World Health Organization’s internationally endorsed definition (e.g. “live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2001)) were included. Only reviews with clearly defined probiotics species, strain and dose were included. Reviews of RCTs that investigated probiotics in combination with prebiotics (e.g. synbiotics) were included if the prebiotic data could be isolated and the prebiotic dose was less than 2.5 grams, as at this dose, it was determined to have a minimal effect on altering the intestinal milieu (Davis, Martínez, Walter, & Hutkins, 2010),

Types of outcomes measures

Outcome measures were decided by consultation amongst the authors (KC, DAK and BCJ) and the following outcomes were identified for analysis.

Primary Outcome: Prevention or improvement of gastrointestinal symptoms, such as:

- Change in frequency of episodes and severity of symptoms (e.g. incidence of diarrhea, duration of diarrhea, abdominal pain, flatulence, abdominal distention)
- Change in occurrence of rumination, functional dyspepsia, cyclic vomiting or general abdominal discomfort relating to condition (e.g. IBS-related pain, bloating, flatulence) (Rashid, Taminiau, Benninga, Saps, & Tabbers, 2015)
- Change in biomarkers that correlate with progression or improvement of condition
- Administration to the hospital and length of stay in the hospital
- Administration of intravenous (IV) therapy
- Quality of life (QOL; e.g. overall well-being, IBS-QOL)

Secondary Outcome: Incidence and severity of mild, moderate and serious adverse events, according to NIH’s Common Terminology Criteria for Adverse Events (CTCAE), associated with probiotic use.

3.1.2 Search Strategy for Identification of Systematic Reviews

A comprehensive search strategy was conducted to identify all relevant systematic reviews. A research librarian (AM) searched the following databases, MEDLINE, EMBASE,
CINAHL, the Cochrane Database of Systematic Reviews (CDSR), and The Database of Abstracts and Reviews of Effects (DARE) and retrieved all reviews published from 2000 onwards. Included reviews were restricted to English-language publications only.

3.1.3 Methodological Quality Assessment of Included Reviews

We sought to include reviews that met the minimum standard of quality of systematic reviews; therefore, using the Assessment of Multiple Systematic Reviews (AMSTAR) tool (APPENDIX A – AMSTAR checklist) (Shea et al., 2007), two reviewers (KC and DAK) independently assessed the methodological quality of the most up-to-date review of overlapping reviews that were eligible and discussed the results of the AMSTAR score to ensure agreement. In brief, 11 items were rated as “Yes”, “No”, “Can’t answer”, or “Not applicable” based on study reports, and the quality of each included review was rated based on the number of AMSTAR criteria that were fulfilled. All items answered ‘Yes’ received a score of 1, while all others options (e.g., “no”, “can’t answer”, or “not applicable”) were given a score of 0 (Shea et al., 2007) (possible scoring range for each review was 0 to 11). Based on AMSTAR score, the overall quality of each review was then assigned a possible rating of ‘high’, ‘moderate’, or ‘low’ quality, which was determined by using the sum of the total AMSTAR score. Low quality is defined as an AMSTAR score of 0 to 4, moderate quality is defined as an AMSTAR score of 5 to 8, and high quality is defined as an AMSTAR score of 9 to 11 (Shea et al., 2007). Any discrepancies were resolved through discussion and when necessary, a third reviewer (LDS) was consulted (APPENDIX A – AMSTAR Checklist).

3.1.4 Data Extraction

Extraction of relevant data from the included systematic reviews was completed independently by two reviewers (KC, DAK) using a customized data extraction form created a priori. The following data were extracted: author names and institutions, language, journal and year of publication, funding sources, conflicts of interest, number of RCTs included in review, eligibility criteria for each review, definition and diagnostic criteria for clinical condition, patient characteristics (e.g. mean age, proportion of females and males), number of patients allotted to intervention and control group, specified probiotic strain, dosage, duration and schedule of probiotic use, outcome measures (e.g. incidence, frequency and severity of
symptoms, number of adverse events), duration of treatment, frequency of administration of intervention, and study limitations noted in the reviews (e.g. methodological quality of included RCTs, such as Cochrane Collaboration’s Risk of Bias [RoB] assessments). Of note, data extraction was limited to the data presented in the included systematic reviews; thus, a detail evaluation of the primary RCTs included in each of the systematic reviews was not pursued. Methodological details and results of any meta-bias assessments (e.g., assessment of publication bias) of all included studies that were conducted were assessed. Reported overall conclusions of the reviews were extracted. Any disagreements during the data extraction processes were resolved through discussion and/or consultation of a third reviewer (SI and/or BCJ).

The data reported in the included reviews were summarized using RRs with 95% CIs, where RRs described the probability of the event occurring in the probiotic treatment group compared to the probability of the event in the control group. For reviews that included a meta-analysis, the pooled effect estimates and the $I^2$ values (representing the degree of statistical heterogeneity between trials) were reported.

### 3.1.5 The GRADE Approach for the Assessment of the Quality of Evidence

As one of the aims of our OoSR is to provide pediatricians, general practitioners and allied clinicians and researchers the most up-to-date evidence for the use of probiotics in the pediatric population for GI-related conditions, we conducted a GRADE assessment to evaluate the quality (certainty) of evidence for the most patient-important outcome of benefit and outcome of harm for each eligible systematic review and meta-analysis (SRMA). As with the GRADE approach for assessing outcomes of SRMAs, each target outcome in this OoSR was rated high, moderate, low and very low quality of evidence. Systematic reviews of RCTs provide the highest level of evidence (Guyatt et al., 2011e) and as such, start as high quality of evidence. We considered rating down the quality of evidence based on the five domains: risk of bias, indirectness, imprecision, inconsistency, and publication bias. We considered rating up based on the two domains: large effect size (RRR < 0.25 or RRI > 1.75), and dose-response (evidence of significantly different treatment effects for studies providing a small or moderate dose versus studies providing a large dose).


**Risk of Bias (RoB)**

As all of the included systematic reviews performed the RoB assessment for all the individual RCTs, an assessment of the overall RoB of each systematic review was made according to the following (Guyatt et al., 2011f):

1. RoB of the systematic review was rated down if, following a high/unclear versus low RoB subgroup analysis, a significant test of interaction was observed between studies at high/unclear versus studies at low RoB. In particular, we looked to determine whether studies at high/unclear RoB had a larger treatment effect than studies a low RoB. If the answer was yes, we rated down for RoB.

2. If the systematic review did not conduct a subgroup analysis of high and low RoB and the majority of included trials in the systematic review were at high/unclear RoB, we rated down for RoB.

**Indirectness**

Consideration was made regarding the extent to which uncertainty exists about the applicability of the evidence to the relevant question of interest of each included systematic review. More confidence is placed on the estimate of effect if the evidence directly compares the intervention of interest among the target population (children) and directly measures patient-important outcomes. (Guyatt et al., 2011b).

1. We rated down for indirect evidence if included SRMA measured the impact of probiotics on outcomes related to, but different from, the outcome deemed of greatest importance to patients.
   
   • E.g. The most important outcome for IBS patients was identified as a reduction or absence of abdominal pain. If a systematic review only conducted a meta-analysis on the number of stools passed (or other surrogates to patient-important outcomes), this would classify as a source of indirectness related to measurement of the target outcome and we would rate down.
Inconsistency

We assessed quantitative assessment of heterogeneity (i.e. $I^2$ or p-value for Cochran Q test) in the systematic reviews for the target patient-important outcomes (benefit and harm), if reported. If not reported, we assessed descriptive results and a table for characteristics of included studies and assessed if inconsistency existed among estimates of effect (Guyatt et al., 2011c).

1. The QoE was rated down for inconsistency if considerable heterogeneity ($I^2>75\%$) (Higgins & Green, 2011) was detected in the pooled estimate for the targeted patient-important outcomes and a subgroup analysis could not explain the observed heterogeneity.

Imprecision

There are three general criteria to consider when determining when results are precise enough: sample size, number of events and wide confidence interval (Guyatt et al., 2011a).

1. For dichotomous outcomes, the QoE was rated down for imprecision if the number of events for each target outcome of interest was less than 400 (Guyatt et al., 2011a).
2. For continuous outcomes, the QoE was rated down for imprecision if the number of total subjects for each target outcome of interest is less than 400 (Guyatt et al., 2011a).

Publication Bias

As publication bias is known to threaten the validity of a meta-analysis, it is imperative that it is considered in the analysis and the conclusions of the review (Guyatt et al., 2011d).

1. If an assessment of publication bias was performed, which may include a combination of graphical aids (e.g. funnel plot) and/or statistical tests (e.g. Egger regression test (Egger, Davey Smith, Schneider, & Minder, 1997)), the QoE was rated down if publication bias was suspected for the pooled estimate of the target patient-important outcomes.
2. If no assessment of publication bias was made, but the inclusion of the statement, “publication bias was not assessed due to inadequate numbers of included trials”, then the QoE was not rated down.
3.2 Probiotics for Infantile Colic: A randomized, double-blind, placebo-controlled trial investigating *Lactobacillus reuteri* DSM 17938

Contents of this chapter have been published in the Journal of Pediatrics:


[KC amended the protocol for SickKids REB and Health Canada approval, recruited the participants, conducted the trial, collected the data, performed the analysis, and prepared the manuscript for submission].

### 3.2.1 Study Design

This randomized, blinded (participants, parents, clinicians, data collectors and analysts), placebo-controlled trial was carried out between February 2012 and April 2014 at The Hospital for Sick Children and in pediatric care practices in Toronto, Ontario, Canada. Prior to initiation of the study, approval from The Hospital for Sick Children research ethics board (REB) and Health Canada were obtained. Eligible infants were included in the study after a written informed consent was obtained from the parents/guardian (APPENDIX B – Informed Consent form).

We registered our clinical trial on the National Institutes of Health ClinicalTrials.gov registry (NCT01541046).

### 3.2.2 Eligibility Criteria

Study participants met the following *inclusion criteria*: 1) Diagnosis of infantile colic (i.e. crying or fussy/gassy episodes ≥3 hrs/days for ≥3 days/7 days, as defined by a modified definition of Wessel’s criteria) at time of study commencement; 2) Age between 3 weeks to 6 months at the time of study commencement; 3) Exclusively breastfed; 4) Term delivery (≥37 weeks gestation at birth); 5) Apgar score of ≥7 at 5 minutes; and 6) Birth weight of ≥ 2500 g. *Exclusion criteria* included: 1) Major medical problems or infants with acute illness, including gastrointestinal disorder, as determined by pediatrician; 2) A history of antibiotic treatment prior to or during the study; 3) A history of probiotic or *L. reuteri* supplementation; 4) A history of any allergies to any of the ingredients in the probiotic, *L. reuteri DSM 179938* (freeze-dried, 1x10⁸ per 5 drops; sunflower oil, medium chain triglyceride oil and silicon
dioxide) or placebo (sunflower oil, medium chain triglyceride oil and silicon dioxide); and 5) Concurrent participation in another clinical trial (Table 3.1).

**Table 3.1: Participant eligibility criteria.**

<table>
<thead>
<tr>
<th>INCLUSION CRITERIA</th>
<th>EXCLUSION CRITERIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis of infant colic (≥3hrs/d, ≥3d/wk, 1wk of crying/fussy)</td>
<td>Allergies to study product ingredients: probiotic (<em>L. reuteri DSM 17938</em>) or placebo (excipient ingredients – sunflower oil, medium chain triglyceride oil, SiO₂)</td>
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<td>10 ± 5 days before enrollment</td>
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<tr>
<td>Term (≥ 37 weeks of age) infants between 3 weeks to 6 months of age</td>
<td>Major medical problems or acute/chronic illnesses (e.g. cystic fibrosis, diabetes)</td>
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<tr>
<td>Exclusively breastfed</td>
<td>Infants with gastrointestinal disorder</td>
</tr>
<tr>
<td>Birth weight ≥ 2500 g</td>
<td>History of antibiotic and/or probiotic use</td>
</tr>
<tr>
<td>Apgar score ≥7 at 5 minutes</td>
<td>Participation in another clinical trial</td>
</tr>
</tbody>
</table>

### 3.2.3 Study Outcome Measures

Table 3.2 shows a timeline of the primary and secondary outcome measures and the method of data collection. As proposed by Wessel et al. (Wessel et al., 1954), the primary outcome was defined as a reduction in the duration of average crying and fussing times, from baseline (day 0) to end of treatment (day 21), to <3 hours per day. The secondary outcome measure was the number of participants who responded to treatment on days 7, 14 and 21. Responders were defined as infants who experienced a reduction in daily average crying and fussing time of ≥50% from baseline.

Parents were instructed to record any adverse events daily, and weekly measurements of weight gain, changes in bowel movements and stool characteristics (frequency and consistency), frequency of digestive discomfort and/or intolerance (regurgitation or vomiting). These were collected for the duration of treatment period.
Table 3.2: Timeline and method of data collection for outcome measures of interest.

<table>
<thead>
<tr>
<th>Construct</th>
<th>Timeline (D = days)</th>
<th>Measure</th>
<th>Additional Information</th>
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<tbody>
<tr>
<td></td>
<td>D0</td>
<td>D7</td>
<td>D14</td>
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<tr>
<td><strong>Outcome Measures</strong></td>
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<tr>
<td>1. Change in the mean infant daily minutes of crying and fussing times</td>
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<td>(mins/day)</td>
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<tr>
<td>2. Number of responders vs. non-responders</td>
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<td>■</td>
<td>■</td>
</tr>
<tr>
<td><strong>Questionnaire Data Collection</strong></td>
<td></td>
<td>■</td>
<td>■</td>
</tr>
<tr>
<td>1. Number of crying and fussing episodes per day (mins/day)</td>
<td>■</td>
<td>■</td>
<td>■</td>
</tr>
<tr>
<td>2. Infant breastfeeding daily schedule (time/day)</td>
<td>■</td>
<td>■</td>
<td>■</td>
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<tr>
<td>3. Infant daily stool frequency &amp; characteristics</td>
<td>■</td>
<td>■</td>
<td>■</td>
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</tbody>
</table>

**3.2.4 Study Protocol**

All participants were randomized into one of two treatment arms: (i) *L. reuteri* DSM 17938 group or (ii) placebo group. Independent Research Support Pharmacy (RSP) personnel, not participating in the study prepared a computer-generated randomization schedule with a random block of varying size to ensure balance in the allocation of participants between treatment arms. Additionally, a RSP pharmacist prepared the treatment and placebo study products in identical packaging to ensure the drops were indistinguishable to all study investigators and participants. All study investigators and participants remained blinded to treatment allocation at every phase of the trial, including the final analysis of the data. The randomization code was only revealed upon completion of all outcome analyses.
Upon enrolment (day 0), maternal guardians were interviewed to obtain the following information on a Maternal/Infant Intake Questionnaire Form (APPENDIX C): (1) gestational age; (2) type of delivery; (3) personal medical history; (4) smoking status; (5) a history of gastrointestinal disease; (6) infant birth weight; and (7) description of colic symptoms. The referring paediatrician performed a medical examination on day 0 and infant growth parameters were recorded on day 0 and all subsequent follow up visits (day 7, 14 and 21). Caregivers were instructed to administer 5 drops orally, once daily for 21 days, preferably at the same time each day. Parents and/or legal guardians were instructed to refrain from other modes of therapy or methods to console their colicky infant. The active study product contained $1 \times 10^8$ (100 million) CFU/5 drops of *L. reuteri DSM 17938* suspended in sunflower oil, medium chain triglyceride oil and silicon dioxide. The placebo contains the same excipient ingredients without the live bacteria. All study products remained refrigerated until use. Parents were instructed to complete a structured 21-day maternal diary (APPENDIX D – 21-Day Maternal Diary) to record the frequency of colic episodes and the daily crying and fussing time (in minutes), feeding schedule, stool frequency and characteristics and any adverse events experienced (i.e. constipation, vomiting, erythema) and the frequency and duration of the adverse event. At all phases of the trial, parents and caregivers were encouraged to contact the referring paediatrician and study team investigators as necessary.

3.2.5 Follow-up Visits

To monitor the progress of study participants, follow up visits were conducted on study days 7, 14 and 21 by the same referring pediatrician and a study investigator. During each study visit, the following data were collected using the Maternal/Infant Follow-up Form (APPENDIX E): (1) change in infant colic symptoms (e.g. no change, mild improvement, significant improvement); (2) infant weight; (3) maternal perception of stress was assessed using a 10-point visual analog scale (VAS) with a score of 0 indicating no stress and a score of 10 indicating very stressed; and (4) study associated adverse events. On study day 21, a medical examination again performed by the same referring pediatrician and a study investigator collected the remainder of unused study product and the completed maternal diary. The diaries were reviewed for completion independently by the pediatrician and 2 study team investigators. Two study team investigators independently entered extracted data and a third
study team member reviewed the entered data for accuracy to ensure accuracy of data transferred from the diaries.

### 3.2.6 Statistical Analysis

To provide 80% power to detect an effect size of 0.5 and a detectable difference in mean crying and fussing times between groups of 50 minutes, a minimum of 22 participants were needed per arm.

Statistical analyses were performed using IBM® SPSS® Statistics v. 2.0 (SPSS Inc, Chicago, IL) using an intention-to-treat (ITT) approach. To compare mean values of continuous variables approximating a normal distribution, the Student’s t test was used, whereas, the Mann-Whitney U test was used for non-normally distributed variables. Proportions were compared by the Chi-square test or Fisher exact test, as appropriate. All reported statistical tests were 2-sided.

### 3.3 Systematic Review and Meta-Analysis of *Lactobacillus reuteri* DSM 17938 for the Treatment of Infantile Colic

Contents in this section contains current work that is in preparation for publication:


[KC designed the protocol, co-searched the databases, screened and selected eligible RCTs, extracted the data, performed the meta-analyses and GRADE assessments, and prepared the manuscript for submission].

#### 3.3.1 Systematic Review of the Literature

The objective of this systematic review is to update the evidence and determine the efficacy and safety of *Lactobacillus reuteri* DSM 17938 (*L. reuteri*) for the treatment of infantile colic by systematically amalgamating and meta-analyzing the available studies and critically assessing the overall quality of evidence.

The *Cochrane Handbook for Systematic Review of Interventions* was used as a guideline to conduct this systematic review and meta-analysis (Higgins & Green, 2011).
3.3.2 Formulation of Research Question

This review was guided by the following research question: Among children between 3 weeks to 6 months of age, is supplementing with probiotics containing \textit{Lactobacillus reuteri} DSM 17938 (100 million CFU/day) safe and does it reduce colic symptoms as measured by duration of crying and/or fussing times?

Data generated from qualifying studies were meta-analyzed and disseminated into a distinct quantitative approximation. Subsequently, the overall quality of evidence of the clinical outcomes was assessed using the GRADE approach.

3.3.3 Search Strategy for Identification of RCTs

We conducted comprehensive searches of the five primary databases EMBASE, MEDLINE, Cochrane Central Register of Controlled Trials (CENTRAL), Cumulative Index to Nursing and Allied Health Literature (CINAHL) and Web of Science (WoS) from inception to August 2015. Database-specific Medical Subject Headings (MeSH) and keywords were searched using the terms “colic” OR “infant colic” OR “crying” OR “fussy” OR “gassy” OR “irritable” AND “lactobacillus reuteri” OR “lactobacilli reuteri” limiting the results to “infants (birth to 6 months)” only. A search for ongoing clinical trials on the National Institutes of Health ClinicalTrials.gov registry was also completed. The results of the search from all databases were imported into a bibliography tool (e.g., Bookends v. 12.6.3).

3.3.4 Selection of Studies to be Included in Review

\textit{RCT Inclusion Criteria}

RCTs investigating the effectiveness of \textit{L. reuteri} DSM 17938 versus placebo involving term infants, from birth to 6 months of age diagnosed with colic (defined by the modified Wessel’s “\textit{rule of threes}” criteria of crying/fussing for 3 hours or more per day, for 3 days or more per week, for one week or more were included in this review. Studies were included if they reported on the following outcomes of interest: (1) infant crying/fussing time recorded by the parents in a daily maternal diary in minutes (min) and assessed weekly (i.e., days 7, 14, 21, or 28); (2) the number of treatment responders, defined as the number of infants who experienced a reduction in average daily crying/fussing time of \(\geq 50\%\), and (3) any adverse events.
Studies were excluded if the specific probiotic species, strain and dose administered were not explicitly specified. No restrictions were applied on language or country of study.

3.3.5 Cochrane Collaboration’s Risk of Bias Quality Assessment

We independently applied the Cochrane Collaboration’s RoB instrument to assess the quality of RCTs included in this systematic review (Higgins et al., 2011). The instrument addresses six components of bias, including random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and investigators (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias), and any other sources of bias, such as industry-initiated or industry-funded studies (Higgins et al., 2011). Publication bias was intended to be assessed using funnel plot techniques, as appropriate; however, less than 10 studies were included in this review and therefore, publication bias was not able to be adequately assessed.

3.3.6 Data Extraction

Blinded to the journal of publication, location of research and results, two reviewers (KC and DAK) independently assessed titles, abstracts and full text studies to determine eligibility. Discrepancies were resolved by discussions and, when necessary, additional input from a third reviewer (LDS). Retrieval of potentially relevant RCTs was performed by a separate reviewer (LDS) not responsible for reviewing the studies.

Data were collected using a standardized extraction form and carried out independently by two reviewers (KC, DAK) and subsequently cross-referenced by a third independent reviewer (LDS) for accuracy. The information extracted and tabulated from the eligible studies included: (1) infant characteristics, such as sex, gestational age at birth, age at enrollment, criteria used to determine colic diagnosis, feeding method, consistency and frequency of bowel movements, type of delivery, and medical history; (2) duration of the study; (3) type, frequency, and duration of intervention; (4) the daily dose and duration of probiotic and placebo supplementation; and (5) primary and secondary outcome measures data (see Inclusion Criteria above). For articles published in abstract-form only, we endeavoured to obtain further information from the corresponding authors.
3.3.7 Descriptive and Statistical Analysis

Following the selection of the studies, a summary of study characteristics was tabulated and data were pooled using the random-effects model and completed using the Review Manager (RevMan) statistical package (version 5.3). Continuous data were reported as a mean difference with 95% confidence intervals (CI). In order to estimate the probability of treatment responders, we report the relative risk (RR) with a 95% CI. Since 2 of the 5 studies reported median and interquartile range (IQR) of daily crying/fussing time, (Savino et al., 2010; Szajewska et al., 2013a), the mean and standard deviation from these studies were estimated using a published conversion equation (Higgins & Green, 2011).

Heterogeneity of the included studies was assessed using the I-square statistic ($I^2$), which calculates the proportion of variation due to heterogeneity among studies rather than due to chance (Higgins & Green, 2011). Thresholds for interpreting $I^2$ were assigned as 0% to 40% for low heterogeneity, 40% to 60% for moderate heterogeneity, and 60% to 100% for considerable heterogeneity (Higgins, Thompson, Deeks, & Altman, 2003; Higgins & Green, 2011).

To investigate potential sources of heterogeneity, a subgroup analysis was performed with respect to the primary outcome of daily crying/fussing times stratified by treatment duration of 21 days versus 28 days. Furthermore, to assess the robustness of the effect and to consider the impact of bias in the pooled estimate, a subgroup analysis of high risk versus low RoB trials was performed.

3.3.8 GRADE Assessment of the Overall Quality of Evidence

For each pooled estimate for each measured outcome of interest, the overall quality of evidence was independently assessed (KC, BCJ) using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach (Guyatt et al., 2008). In brief, a body of evidence based on RCTs is initially considered high quality evidence; however, the evidence may be downgraded by 1 or more points based on 5 categories of limitations: RoB, inconsistency, indirectness, imprecision and publication bias. Based on each GRADE domain, we assigned each estimated outcome as high, moderate, low, or very low quality evidence (Guyatt et al., 2008)
CHAPTER 4
RESULTS

4.1 Overview of Systematic Reviews: Clinical Efficacy of Probiotic Use in Pediatrics

Contents in this section contains current work that is in preparation for publication and has been registered on PROSPERO:


[KC designed the protocol, co-searched the databases, screened and selected eligible reviews, extracted the data, performed the analysis, conducted the GRADE analysis, and prepared the manuscript for submission].

4.1.1 Summary of Results of the OoSR

Following the initial database search, 4,412 reviews were retrieved investigating probiotics for the treatment of common pediatric GI-related conditions. We sought to identify and include only reviews that met the definition of a systematic review, as well as, the most up-to-date reviews if there were more than one overlapping reviews pertaining to the same topic. A total of 768 narrative reviews were excluded, as this type of review is subject to high levels of bias (Collins & Fauser, 2005). As well, since the aim of this OoSR is to provide the current evidence on the use of probiotics in pediatrics, several eligible reviews were excluded, as they were updated by subsequent reviews (n= 203) or were conducted prior to the year 2000 (n=340). As such, we identified a total of 9 published reviews and 1 unpublished review for this OoSR (Figure 4.1).
Figure 4.1: Schematic diagram of the search strategy and review selection for the OoSR
4.1.1 Summary of Review Characteristics

Pertinent data from each systematic review were extracted independently by 2 reviewers (KC and DAK) and summarized in Table 4.1 below. Of note, data extraction was limited to the data presented in the included systematic reviews; thus, a detail evaluation of the primary RCTs included in each of the systematic reviews was not pursued.

Table 4.1: Summary of characteristics of included systematic reviews in OoSR

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of included RCTs in review; sample size (n)</th>
<th>Probiotic Intervention and Dose range (CFU)</th>
<th>GI Condition</th>
<th>Age Range</th>
<th>Outcome Measures: (1) Primary Outcomes; (2) Secondary Outcomes;</th>
<th>Results and Authors’ Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahmadi et al. (2015)</td>
<td>14 RCTs; n = 1149</td>
<td>(1) <em>Lactobacillus rhamnosus</em> GG (LGG); (2) Non-LGG; (3) Other species (e.g. <em>Bifidobacterium</em>, <em>Saccharomyces boulardii</em>)</td>
<td>Acute infectious diarrhea (AID)</td>
<td>1 to 72 months</td>
<td>(1) Duration of diarrhea (2) Frequency of diarrhea</td>
<td>All probiotics pooled estimate showed reduced duration diarrhea MD: 0.41; 95% CI: -0.56 to -0.25; P&lt;0.001 with $I^2=39.9$%; P=0.046; LGG alone MD: 0.47 (95% CI: -0.80 to -0.4; P=0.02 with $I^2=57.8$%; P=0.020; Probiotics showed significant effect at reducing the duration of acute rotavirus diarrhea compared to no treatment or placebo.</td>
</tr>
<tr>
<td>Alfaleh et al. (2014)</td>
<td>24 RCTs; n = 5529</td>
<td>Any species and strain at any dose for more than 7 days duration (<em>Lactobacillus spp.</em>, <em>Bifidobacterium spp.</em>, <em>Saccharomyces boulardii</em>, and a combination of multi-strain probiotics).</td>
<td>Necrotizing enterocolitis (NEC)</td>
<td>Preterm infants &lt;37 weeks gestation</td>
<td>(1) Severity of NEC (stage II or more) as per Bell’s criteria, diagnosed prior to discharge (2) Nosocomial sepsis, defined as positive blood or cerebrospinal fluid cultures (3) All cause mortality</td>
<td>Probiotics reduced the incidence of severe NEC (stage II or more) (RR: 0.43; 95% CI: 0.33 to 0.56; P&lt;0.0001; NNT=30); in 20 trials (n=5592), mortality (RR: 0.65; 95% CI: 0.52 to 0.81; P=0.00017; NNT=41); in 17 trials (n=5112) compared to control (placebo or no treatment). No significant reduction in incidence of nosocomial sepsis (RR: 0.91; 95% CI: 0.80 to 1.03;</td>
</tr>
<tr>
<td>Study</td>
<td>Dose Range</td>
<td>Probiotic Strains</td>
<td>Outcomes</td>
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<tr>
<td>Goldenberg et al. (2013)</td>
<td>500 million (5x10⁸) CFU QD to 100 million (10⁹) CFU BID</td>
<td>LGG, L. acidophilus, L. casei, L. paracasei, Clostridium butyricum, L. bulgaricus, L. plantarum, B. bifidum, S. boulardii, S. thermophilus and multi-strain, such as VSL#3</td>
<td>Probiotics prevents severe NEC and all cause mortality in preterm infants; however, no effect on reducing the incidence of nosocomial sepsis was shown.</td>
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<tr>
<td>Goldenberg et al. (2015)</td>
<td>100 trillion to 960 billion (10.2x10⁹ to 9.6x10¹¹) CFU QD</td>
<td>Bacillus spp., Bifidobacterium spp., Clostridium butyricum, Lactobacilli spp., Leuconostoc cremoisor, Saccharomyces spp., Streptococcus spp., and multi-strain probiotics</td>
<td>Significant reduction in the incidence of AAD in the probiotic group (RR=0.46; 95% CI: 0.35 to 0.61; P=0.05) compared to other control groups; I²=52%; P=0.01 GRADE assessment revealed moderate quality of evidence.</td>
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</table>

Overall, moderate quality of evidence strongly suggests a protective effect of probiotics in the prevention of AAD. No adverse serious events were reported in probiotics group.
<table>
<thead>
<tr>
<th>Study</th>
<th>RCTs</th>
<th>Participants</th>
<th>Intervention Details</th>
<th>Comparator</th>
<th>Efficacy Measures</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Korterink et al. (2014)</td>
<td>8</td>
<td>741</td>
<td><em>L. reuteri</em>, LGG, VSL#3 (multi-strain containing: <em>B. breve</em>, <em>B. longum</em>, <em>B. infantis</em>, <em>L. acidophilus</em>, <em>L. plantarum</em>, <em>L. paracasei</em>, <em>L. bulgaricus</em>, <em>S. thermophilus</em>) Dose range: 100 million to 10 billion (10⁸ to 10¹⁰) CFU QD</td>
<td>Placebo</td>
<td>FGID</td>
<td>(1) Treatment success for abdominal pain-related FGID or defecation-related FGID of the probiotics, defined as the absence of, or a reduction of, abdominal pain (measured as a decrease in severity and frequency of pain) or an improvement in the frequency of bowel movements (i.e. stool pattern measured by defecation at least 3 times per week, or no fecal incontinence or episodes of fecal incontinence occurring less than once over a 2-week period); (2) Occurrence of bloating, flatulence and adverse events</td>
</tr>
<tr>
<td>Moayyedi et al. (2010)</td>
<td>18</td>
<td>1650</td>
<td><em>Bifidobacterium spp.</em>, <em>Lactobacilli spp.</em>, <em>Streptococci spp.</em>, and a mixture of <em>Bifidobacterium</em> and <em>Lactobacilli</em></td>
<td>Placebo</td>
<td>FGID</td>
<td>Probiotics, compared to placebo, significantly reduced overall IBS symptoms (as dichotomous outcome); RR of symptoms persisting in the probiotic-treated group 0.71; 95% CI 0.57 – 0.88; P=0.002 with a NNT of 4 (95% CI:</td>
</tr>
</tbody>
</table>
Dose range: 1.5 million \((1.5 \times 10^6)\) CFU QD to 100 billion \((10^9)\) CFU QID

Substantial heterogeneity detected \((I^2=68\%; P=0.001)\).

No distinctions between the effects of the various species and strain of probiotics were observed; all strains included in review demonstrated a trend towards improvement in IBS symptoms.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Probiotic</th>
<th>Dose range</th>
<th>Acute gastroenteritis (AGE)</th>
<th>Duration</th>
<th>Outcome Measure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Szajewska et al. (2013a)</strong></td>
<td>5 RCTs</td>
<td>(Lactobacillus reuteri) (DSM 17938 and ATCC 55730)</td>
<td>Dose range: 10 million to 100 billion ((10^7) to (10^{11})) CFU QD</td>
<td>Acute gastroenteritis (AGE)</td>
<td>3 to 6 month</td>
<td>(1) Stool volume and duration of diarrhea measured until permanent cessation</td>
<td>(L. reuteri) DMS 17938 compared to placebo or no treatment, showed a significant reduction in the duration of diarrhea of 32.4 h ((\text{MD}: -32.4 \text{ h}; 95% \text{ CI}: -41 \text{ to } -24; P&lt;0.00001)) compared to placebo, with no significant heterogeneity detected ((I^2=0%)) (L. reuteri) ATCC 55730 showed a significant reduction in the duration of diarrhea of 18.8 h ((\text{MD}: -18.8 \text{ h}; 95% \text{ CI}: -30.9 \text{ to } -6.75; P&lt;0.002)) compared to placebo, with no significant heterogeneity detected ((I^2=0%)) Treatment with (L. reuteri) (DSM 17938 and ATCC 55730) for children with AGE showed reduced duration of diarrhea and increased chance of cure on day 3; however, unclear whether this translates to decreased hospital stay compared to no treatment or placebo-treated infants.</td>
</tr>
<tr>
<td><strong>Szajewska et al. (2013b)</strong></td>
<td>15 RCTs</td>
<td>(Lactobacillus rhamnosus) GG (LGG)</td>
<td>Dose range: 100 million to 1 trillion</td>
<td>Acute gastroenteritis (AGE)</td>
<td>1 month to 7 years of age</td>
<td>(1) Stool volume and duration of diarrhea measured until permanent cessation</td>
<td>Probiotic-treated group showed significant reduction in the duration of diarrhea of 1.05 days ((95% \text{ CI}: -1.7 \text{ to } -0.4; P = 0.002)). Substantial heterogeneity was detected ((I^2=)</td>
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<tr>
<td>Study</td>
<td>Participants</td>
<td>Intervention</td>
<td>Outcomes</td>
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<tr>
<td>Szajewska et al. (2014)</td>
<td>4 RCTs; n= 304 (probiotic group, n = 157; control group, n = 147)</td>
<td><em>Lactobacillus acidophilus</em> LB Dose range: 20 billion to 30 billion (10^10 to 3 x 10^10) CFU QD</td>
<td>Acute gastroenteritis (AGE) 1 to 48 months  (1) Stool volume and duration of diarrhea measured until permanent cessation  (2) Cure on day 3, cure on day 4, and adverse effects</td>
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<tr>
<td>Tiequn et al. (2015)</td>
<td>6 RCTs; n= 440 (probiotics, n=224; placebo, n=216)</td>
<td><em>Lactobacilli</em> spp. Dose range: 10 million to 3 billion (10^7 to 3 x 10^8) CFU BID</td>
<td>Irritable bowel syndrome (IBS) 0 to 18 years of age (1) Relieve abdominal pain and/or discomfort with alterations in bowel habits, as well as other features, such as abdominal distention, bloating, and excessive flatulence  (2) Adverse events</td>
<td></td>
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<tr>
<td>Chau et al. (2016)</td>
<td>5 RCTs; ( n=335 ) (probiotic, ( n=172 ); placebo, ( n=163 ))</td>
<td><em>Lactobacillus reuteri</em> DSM 17938</td>
<td>Infantile colic</td>
<td>0 to 6 months</td>
<td>(1) Average daily reduction in crying and fussing times (2) Treatment responders, defined as number of infants showing a ≥50% reduction in crying/fussing time from baseline (3) Adverse events</td>
<td></td>
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</tr>
</tbody>
</table>

\[ L. reuteri \] significantly reduced the average daily crying/fussing times (day 21) by 49.7 min (95% CI: 37.9 to 61.5). Substantial heterogeneity was detected at all time points (e.g. day 21: \( I^2 = 65\% \); \( P=0.002 \)). GRADE assessment revealed an overall low quality of evidence due to serious inconsistencies and imprecision of results.

Higher number of colicky infants supplemented with *L. reuteri* successfully responded to treatment (RR 2.33; 95% CI: 1.71 to 3.17; \( P<0.00001 \)). Substantial heterogeneity was observed (e.g. \( I^2 = 80\% \), \( P = 0.002 \)). GRADE assessment, the quality of evidence for the treatment responders outcome was moderate for day 7; however, it was downgraded to low for day 21 and very low on days 14 and 28 due serious inconsistencies and very serious imprecision.

Supplementing with *L. reuteri* shows a beneficial effect at improving colic symptoms compared to placebo. However, the low to very low quality of evidence suggests more evidence is needed to recommend its routine use.

\[ QD = quaque die, \text{ once daily; BID = bis in die, twice daily; QID = quater in die, four times daily} \]
4.1.1 Primary Outcome Measures

The primary objective of this review is to determine the efficacy of probiotics as a therapeutic option for GI-related conditions in the pediatric population. Therefore, the primary outcome measures are defined for each GI condition in Table 4.2.

Table 4.2: Description of the primary outcomes for the GI conditions analyzed in the OoSR

<table>
<thead>
<tr>
<th>Gastrointestinal Disease</th>
<th>Outcome Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional gastrointestinal disorder (FGID) (Korterink et al., 2014)</td>
<td>The primary outcome of treatment success, defined as the absence or reduction of abdominal pain (intensity or frequency) or an improvement in the frequency of bowel movements (defecation at least 3 times per week, or no fecal incontinence or episodes of fecal incontinence occurring less than once over a 2-week period).</td>
</tr>
<tr>
<td>Irritable bowel syndrome (IBS) (Moayyedi et al., 2010; Tiequn, Guanqun, &amp; Shuo, 2015)</td>
<td>The primary outcome is defined as improvement of overall IBS symptoms, either by the absence or presence of physical symptoms (e.g. abdominal pain and/or discomfort, bowel habits (e.g. constipation or diarrhea), excessive flatulence, bloating and distention) or change in symptom scores from baseline.</td>
</tr>
<tr>
<td>Necrotizing enterocolitis (NEC) (AlFaleh &amp; Anabrees, 2014b)</td>
<td>The primary outcome is defined as reduction of the rate severity progression (stage II or more), all-cause mortality and cultured-proven sepsis.</td>
</tr>
<tr>
<td>Antibiotic-associated diarrhea (AAD) (Goldenberg et al., 2015)</td>
<td>The primary outcome is defined as AAD as defined by the authors. The most common definition of AAD was 3 or more loose stools/day for a minimum of 2 days.</td>
</tr>
<tr>
<td><em>Clostridium difficile</em>-associated diarrhea (CDAD) (Goldenberg et al., 2013)</td>
<td>Primary outcome of CDAD is defined as an episode of diarrhea following a positive culture or toxin (A or B) assay within 1 month of exposure to antibiotics.</td>
</tr>
<tr>
<td>Acute Gastroenteritis (AGE) (Szajewska, Skórka, Ruszczyński, &amp; Gieruszcza-Białek, 2013b; Szajewska, Ruszczyński, &amp; Kolaček, 2014a)</td>
<td>Primary outcome measures of AGE in the included reviews were defined as stool volume and the duration of diarrhea (time until complete cessation).</td>
</tr>
<tr>
<td>Infantile Colic (Chau et al., 2016)</td>
<td>The primary outcome is defined as a reduction of average crying and fussing time, as well, treatment success (i.e. number of infants with ≥50% reduction in crying and fussing time from baseline).</td>
</tr>
</tbody>
</table>
4.1.1 Methodological Quality of Included Reviews – AMSTAR Assessment

Based on the AMSTAR assessment of systematic review quality, all included reviews were rated as ‘moderate to high’ quality. Of note, there were methodological limitations in a few of the included reviews. In 7 reviews, it could not be determined whether an ‘a priori’ design was provided, as reference to a protocol was not made and PROSPERO was searched. As well, for all reviews, it was not explicitly stated whether all the included RCTs in the particular review addressed conflicts of interest, and therefore, all received a ‘no’. Only 1 review did not provide details of the RCT selection process (Szajewska, Urbańska, Chmielewska, Weizman, & Shamir, 2014b). Despite the limitations, all included reviews were deemed moderate quality and methodologically sound (Table 4.3).

Table 4.3: Summary of the AMSTAR Quality Assessment (Reviews evaluated by 2 reviewers – KC and DAK)

<table>
<thead>
<tr>
<th>Reviews</th>
<th>(1) ‘a priori’ design</th>
<th>(2) Two Reviewers</th>
<th>(3) ≥2 databases</th>
<th>(4) Publication type</th>
<th>(5) Selection summary</th>
<th>(6) Study summary</th>
<th>(7) RCT quality</th>
<th>(8) Address quality</th>
<th>(9) Meta-analysis</th>
<th>(10) Publication bias</th>
<th>(11) Conflict of interest</th>
<th>TOTAL AMSTAR SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahmadi et al. (2015)</td>
<td>Can’t answer</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Alfaleh et al. (2014)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Goldenberg et al. (2013)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Goldenberg et al. (2015)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Yes</td>
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<tr>
<td>Korterink et al. (2014)</td>
<td>Can’t answer</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Moayyedi et al. (2010)</td>
<td>Can’t answer</td>
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<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Szajewska et al. (2013a)</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Szajewska et al.</td>
<td>Can’t answer</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>(2013b)</td>
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<tr>
<td>Szajewska et al. (2014)</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>9 (High)</td>
</tr>
<tr>
<td>Tiequn et al. (2015)</td>
<td>Can’t answer</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>7 (Moderate)</td>
</tr>
<tr>
<td>Chau et al. (2016)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>9 (High)</td>
</tr>
</tbody>
</table>

1. *a priori* design refers to whether the research question and inclusion criteria were established before the conduct of review (e.g. refers to a protocol or registration of review in PROSPERO).
2. Two independent reviewers screened, selected and extracted data from the included reviews.
3. At least 2 databases (e.g. Cochrane and Central) and 1 supplementary source (e.g. a grey literature) were used to search for relevant reviews with a statement of the date the search was conducted.
4. Types of reports (e.g. publication status, non- and grey literature) was used as an exclusion criteria or the exclusion of reviews based on language or any other restrictions, review receives a ‘no’ rating. If review includes a statement indicating a search in ‘unpublished literature’ or conference proceedings, indicate ‘yes’ for the review.
5. A list of included and excluded reviews should be provided (e.g. schematic diagram of the excluded RCTs and brief rationale for exclusion).
6. A characteristics summary from the original studies (e.g. summary of characteristics of included trials table) should be provided on the participants, interventions and outcomes.
7. An assessment of the scientific quality of the individual included studies should be conducted and documented (e.g. Jadad Scale, Cochrane Collaboration RoB tool).
8. Results of the methodological rigor and scientific quality should be considered appropriately and explicitly stated in formulating conclusions. A statement such as, “the results should be interpreted with caution due to poor quality of included studies” should be provided to receive a ‘yes’.
9. The inclusion of an appropriate test method to assess the homogeneity of included studies (e.g. Chi-squared test, $I^2$).
10. As assessment of publication bias should be included; either the use of a funnel plot and/or statistical tests (e.g. Egger regression test). If no tests or funnel plot was mentioned, the review receives a ‘no’ score; the review may receive a ‘yes’ if a statement was made that publication bias could not be assessed because there were fewer than 10 studies.
11. Potential sources of support should be clearly acknowledged in both the systematic review and each of the included trials.

### 4.1.2 Summary of Main Findings

**Functional Gastrointestinal Disorders (FGIDs)**

*Summary of review* (Korterink et al., 2014)

To assess the effect of probiotics, versus placebo, in the treatment of abdominal pain- and defecation-related functional gastrointestinal disorders (FGID) in children, a review was conducted that included 8 RCTs (n=741 children between 0 to 18 years of age). The primary outcome of interest was treatment success of the probiotics (*i.e.*, *L. reuteri*, LGG, VSL#3 (multi-strain containing: *B. breve*, *B. longum*, *B. infantis*, *L. acidophilus*, *L. plantarum*, *L. paracasei*, *L. bulgaricus*, *S. thermophilus*) at doses ranging from 100 million to 10
billion ($10^8$ to $10^{10}$) CFU QD (quaque die; once daily), which was defined as the absence of, or a reduction of, abdominal pain (measured as a decrease in severity and frequency of pain) or an improvement in the frequency of bowel movements (i.e., stool pattern measured by defecation at least 3 times per week, or no fecal incontinence or episodes of fecal incontinence occurring less than once over a 2-week period). Secondary outcomes included occurrence of bloating and flatulence, as well as any adverse events. Of note, the meta-analyses for abdominal pain- and defecation-related FGID were conducted separately, as abdominal pain-related FGID were categorized into functional abdominal pain (FAP), IBS, functional dyspepsia (FD) and abdominal migraine. Among the included studies, 5 trials represented patients with abdominal pain-related FGID and therefore, the pooled results, using a random effects model, revealed that treatment success with children receiving *Lactobacillus rhamnosus* GG (LGG), *L. reuteri* DSM17938, and VSL#3 was significantly higher than children receiving placebo (RR = 1.50; 95% CI: 1.22 to 1.84; *P* < 0.0001) (Figure 4.2). Heterogeneity between studies was not shown to be significant (*P* = 0.24) with minimal inconsistencies ($I^2$ = 28%).

![Figure 4.2: Forest plot comparing probiotic versus placebo on treatment success for abdominal pain-related FGID.](Reprinted from: Korterink et al. Probiotics for childhood functional gastrointestinal disorders: a systematic review and meta-analysis. *Acta Paediatr.* 2014 Apr; 103(4): 365-72 (Korterink et al., 2014)).
Furthermore, with respect to defecation-related FGID, the pooled estimates showed no significant effect of probiotics compared to placebo (RR=1.16; 95% CI: 0.83 to 1.62; \(P=0.37\)). The test for heterogeneity was of borderline significance according to criteria suggested in the Cochrane Handbook (\(P=0.10\)); while moderate inconsistency was detected (\(I^2=57\%\)) (Figure 4.3).

![Figure 4.3: Forest plot comparing probiotic versus placebo on treatment success for defecation-related FGID. (Reprinted from: Korterink et al. Probiotics for childhood functional gastrointestinal disorders: a systematic review and meta-analysis. Acta Paediatr. 2014 Apr; 103(4): 365-72 (Korterink et al., 2014)).](image)

Based on the overall pooled estimates of effects, Korterink and colleagues concluded that the use of LGG, \(L.\ reuteri\) and VSL#3 significantly increased treatment success in children suffering from abdominal pain-related FGID. More specifically, \(L.\ reuteri\) and LGG were shown to significantly reduce the intensity of abdominal pain, whereas, the combination of probiotics in VSL#3 and LGG were more effective at reducing pain associated with abdominal distention. However, LGG and VSL#3 was not shown to improve stool consistency or frequency in children diagnosed with abdominal pain-related FGID (Korterink et al., 2014). Of note, substantial heterogeneity detected that remained unexplained through subgroup analyses.

In contrast, for defecation-related FGID, a trend towards a beneficial effect was observed in stool pattern and frequency with the use of \(Lactobacillus\ casei\); however, the results were not significant when compared to placebo. Furthermore, the authors concluded that probiotics had minimal effect on children suffering from constipation-related FGID and with the scarce amount of data surrounding the use of probiotics for constipation (Korterink et al., 2014). No serious adverse events associated with the use of
any of the probiotic strains or combinations were reported in the included studies (Korterink et al., 2014).

**Irritable Bowel Syndrome (IBS)**

*Summary of review* (Tiequn et al., 2015)

Six RCTs ($n = 440$) investigating the efficacy of *Lactobacillus* therapy versus placebo in pediatrics (0 to 18 years of age) with IBS were included in Tienqun *et al.* meta-analysis (Tiequn et al., 2015). The primary objective of this review was to evaluate the potential of probiotics in the relief of abdominal pain and/or discomfort with alterations in bowel habits, as well as other features, such as abdominal distention, and excessive flatulence. Since *Lactobacillus* is one of the most widely studied probiotics, the focus of this review was to accumulate and analyze the data pertaining to the efficacy of *Lactobacillus* supplementation at doses ranging from $10^7$ to $3 \times 10^9$ CFU BID (*bis en die*; twice daily). Although this review included both pediatric and adult data, pediatric data were isolated. The quality of each included RCTs was assessed using the Jadad score, which evaluates trials based on randomization, allocation concealment, blinding and description of withdrawals (Jadad, Cook, & Browman, 1997). The quality of RCTs is ranked from 0 to 5; a low quality score is indicated by a score of $\leq 2$ and a high quality is indicated by a score of $\geq 4$. Therefore, based on the authors' assessment, the results revealed that three of the included RCTs were of high quality scoring 4 and the other three RCTs scored 3, indicating moderate quality, one RCT did not provide a description of blinding and the other two RCT did not include a description of withdrawals.

The results from the pooled estimate using a random-effects model revealed that *Lactobacillus* therapy in children showed a positive effect in pediatric IBS, as reported by a reduction of abdominal pain and/or discomfort, changes in bowel habits (e.g. constipation or diarrhea), frequency of bowel movements and flatulence, abdominal distention and change in frequency of bloating (OR: 3.71 (95% CI: 1.05 to 13.11; $p = 0.04$). However, substantial heterogeneity was observed ($I^2 = 68\%; p = 0.04$) (Figure 4.4). A limitation of this review is that no subgroup analysis was performed to account for the heterogeneity observed. As well, a description of the strain of *Lactobacillus* used in each included RCT was not included and therefore, the authors made a generalized conclusion.
of the efficacy of *Lactobacillus* species. Overall, the authors concluded that compared to placebo, children on *Lactobacillus* therapy demonstrated a beneficial effect on pediatric IBS symptoms (Tiequn et al., 2015).

**Figure 4.4: Effect of *Lactobacillus* therapy in pediatric IBS patients.** Subgroup analysis of studies investigating probiotic use in pediatric population with the removal of Ducrotte et al. (2012), Sinn et al. (2008) and Zeng et al. (2008) (Reprinted from: Tiequn B et al. Intern Med 2015. 54; 243-249 (open access) (Tiequn et al., 2015)).

**Summary of review** (Moayyedi et al., 2010)

A separate meta-analysis of 18 clinical trials investigating probiotics in the treatment of IBS, which included different RCTs than Tiequn et al. (2015) review, involving 1,650 patients diagnosed with IBS, was conducted by Moayyedi et al (2010) (Moayyedi et al., 2010). In this review, the probiotics examined included *Lactobacillus* (6 studies), *Bifidobacterium* (3 studies), *Streptococcus* (1 study) at doses ranging from 1.5 million (1.5x10⁶) CFU QD to 100 billion (10⁸) CFU QID (*quater in die*; four times daily), and other studies investigating probiotic combination products (9 studies). Of note, 1 trial reported on both *Lactobacillus* and *Bifidobacterium*. Ten (n = 918) of the 18 studies reported on the effect on pediatric populations and the results revealed that probiotics, compared to placebo, significantly reduced IBS symptoms (e.g. abdominal pain, flatulence, bloating and frequency of bowel movements), relative risk: 0.71; 95 % CI 0.57 – 0.88. Significant heterogeneity was detected among studies ($I^2$=68%, $P$=0.001). Overall, consistent with the findings from Tiequn *et al.* review, Moayyedi and colleagues concluded that, although no distinctions between the effects of the various species and strains of probiotics were observed, the included trials involving *Lactobacillus* (3 trials;
n=140), Bifidobacterium (2 trials; n=422), Streptococcus (1 trial; n=54), and multi-strain probiotic (4 trials; n=302) all demonstrated a trend towards improvement in IBS symptoms (Figure 4.5) (Moayyedi et al., 2010).

<table>
<thead>
<tr>
<th>Study of subcategory</th>
<th>Treatment n/N</th>
<th>Control n/N</th>
<th>RR (random) 95% CI</th>
<th>Weight %</th>
<th>RR (random) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>03 combination</td>
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<td>1.08 (0.60 to 1.95)</td>
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<td>1.02 (0.59 to 1.74)</td>
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<td>1.01 (0.51 to 1.96)</td>
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Figure 4.5: Forest plot of RCTs on the effect of probiotics versus placebo in the treatment of IBS. (Reprint from: Moayyedi et al. The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. Gut 2010 Mar; 59(3): 325-32 (Moayyedi et al., 2010)).

Gastroenteritis

Summary of reviews (Szajewska et al., 2013b; Szajewska et al., 2014a; Szajewska et al., 2014b)

Following the search of the literature, we identified three separate meta-analyses conducted by Szajewska and colleagues, all of which reviewed the efficacy of different probiotic strains for the treatment of pediatric gastroenteritis. Therefore, as the scope of each review covered the efficacy of probiotics, all three reviews were included in this OoSR.
The review conducted in 2013 investigating the efficacy of *L. reuteri* for the treatment acute gastroenteritis (AGE) in children included 2 RCTs (n =196) that evaluated *L. reuteri* DSM 17938 and 3 RCTs (n = 156) that evaluated *L. reuteri* ATCC 55730 (Szajewska et al., 2014b). In this review, hospitalized children (aged 3 to 60 months) were administered *L. reuteri* at doses between the ranges of 10 million to 100 billion (10⁷ to 10¹¹) CFU QD in addition to rehydration therapy consisting of an oral rehydration solution and/or intravenous rehydration. The primary outcome measures of interest were stool volume and the duration of diarrhea, which was measured until the time of permanent cessation. In 4 of the 5 trials, the probiotic group was compared to a placebo group and in 1 RCT, there was no additional therapy in the control group. Following review of the RCTs, the authors noted that none of the included trials evaluated the effects of *L. reuteri* on stool volume and therefore, no data was provided. In the context of duration of diarrhea, the pooled results revealed that infants treated with *L. reuteri* DMS 17938 compared to placebo or no treatment, showed a significant reduction in the duration of diarrhea of 32.4 h (MD: -32.4 h; 95% CI: -41 to -24; \( P<0.00001 \)) with no significant heterogeneity detected (\( I^2=0\% \)). Similarly, the pooled analysis of the infants treated with *L. reuteri* ATCC 55730 showed a significant reduction in the duration of diarrhea of 18.8 h (MD: -18.8 h; 95% CI: -30.9 to -6.75; \( P<0.002 \)) compared to placebo, with no significant heterogeneity detected (\( I^2=0\% \)) (Szajewska et al., 2014b). Importantly, only two studies addressed adverse effects and in both trials, it was reported that there were no differences in adverse effects between the probiotic and control groups. Therefore, the authors concluded that both *L. reuteri* DSM 17938 and ATCC 55730 are safe and effective at reducing the duration of diarrhea and accordingly, reduced the length of hospitalization by 3 days compared to infants not receiving probiotic therapy. A limitation of this review was the precision of the estimates due to the limited number of trials, and the methodological quality varied, as the Cochrane RoB assessment revealed unclear or high RoB in 4 of the 5 trials. Risk of bias among trials was due to inadequate allocation concealment, no blinding in one trial, and no or unclear intention-to-treat analysis (Szajewska et al., 2014b).

In the review conducted to investigate the efficacy of *Lactobacillus rhamnosus* GG for the treatment of pediatric gastroenteritis, 15 RCTs (n = 2963; probiotic group, n = 1606; control group, n = 1360) were included (Szajewska et al., 2013b). Similar to the
Szajewska 2014b review, the primary outcomes of interest were stool volume and duration of diarrhea. The children in the selected RCTs were between the age of 1 month to 7 years and the dose of LGG administered ranged from 100 million to 1 trillion ($10^8$ to $10^{12}$) CFU QD. The authors noted that there was clinical heterogeneity among the included with respect to the type of treatment, such that 10 RCTs were carried out among in-patients, 3 RCTs were in outpatients and 2 RCTs were in both in- and/or outpatients. Therefore, following the pooled estimates from 11 ($n = 2444$) of the 15 RCTs (4 RCTs did not provide data in a format that did not allow for pooling) revealed a significant reduction in the duration of diarrhea of 1.05 days (95% CI: -1.7 to -0.04; $P = 0.002$) for children treated with LGG compared with the placebo or no treatment groups. However, substantial heterogeneity was detected ($I^2 = 98\%$; $P<0.00001$), which pre-planned subgroup analyses (e.g. high vs. low dose, in-patients vs. outpatients) were unable to explain (Figure 4.6). Therefore, the authors acknowledged that a major limitation of this review was the inclusion of trials that were determined to be of high or unclear RoB for unclear or inadequate allocation concealment and no blinding in some of the included trials, which can introduce systematic bias by overestimating the effect and skewing the results in favour of the treatment. Additionally, Szajewska and colleagues performed subgroup and sensitivity analyses in attempt to explain the substantial heterogeneity detected; however, statistically significant between-study heterogeneity persisted and thus, the authors concluded that the differences in outcomes between studies were a result of factors unrelated to methodological quality. As such, the authors concluded that their findings confirmed the effectiveness of LGG to treat acute gastroenteritis in children by concluding that administering LGG in conjunction to standard rehydration therapy reduced the duration of diarrhea by approximately 1 day compared to placebo or no additional treatment (Szajewska et al., 2013b).
In a third review conducted to investigate efficacy of *Lactobacillus acidophilus* LB for the treatment of acute gastroenteritis in children (aged 1 to 48 months), 4 RCTs (*n* = 304; probiotic group, *n* = 157; control group, *n* = 147) were included (Szajewska et al., 2014a). Again, the primary outcome was stool volume and duration of diarrhea and the range of dosing of LB was between 2x10^{10} to 3x10^{10} CFU QD. The results from the pooled analysis revealed that children administered the probiotic showed a significant reduction in diarrhea compared to the placebo group (MD: -21.6 h; 95% CI: -26.5 to -16.6; *P*<0.00001) and no significant heterogeneity among the included trials were detected (*I^2=24%). As such, Szajewska and colleagues concluded that administering *L. acidophilus* LB, in addition to the standard rehydration therapy, improves AGE symptoms by reducing the duration of diarrhea of approximately 20 hours and complete cure within 4 days of therapy compared to infants not receiving probiotics. Of importance is that the reported adverse events were similar in both treatment and placebo group, indicating a good safety profile of *L. acidophilus* LB based on a relatively small number of patients observed (Szajewska et al., 2014a).
Diarrheal conditions

**Clostridium difficile-Associated Diarrhea (CDAD)**

*Summary of review* (Goldenberg et al., 2013)

The effect of any strain of probiotics (i.e., LGG, *L. acidophilus*, *L. casei*, *L. paracasei*, *Clostridium butyricum*, *L. bulgaricus*, *L. plantarum*, *B. bifidum*, *S. boulardii*, *S. thermophilus* and multi-strain, such as VSL#3) at doses ranging from 100 trillion to 960 billion (10.2×10⁹ to 9.6×10¹¹) CFU QD for any treatment duration on the incidence of *Clostridium difficile*-associated diarrhea was assessed in a Cochrane review that included 23 RCTs (20 adult RCTs and 3 children RCTs; n=4,213 participants). A Cochrane RoB assessment revealed that 7 of the 23 trials were at low RoB, and the other 16 trials were determined to be of high or unclear RoB. In this review, a complete case analysis (i.e. participants who completed the study) was conducted on the trials investigating the effect of probiotics on the incidence of CDAD and the findings suggests that probiotics significantly reduced the risk of CDAD by 64% (RR=0.36; 95% CI: 0.26 to 0.51; \(P<0.00001\)), as the incidence of CDAD was 2.0% in the probiotic group compared to 5.5% in the placebo or no treatment group (Goldenberg et al., 2013).

Focusing on the isolated data for the pediatric population, data from 3 pediatric trials (n=605 children) revealed similar findings in that children receiving probiotics showed a reduced risk of CDAD by 60% (RR=0.40; 95% CI: 0.17 to 0.96; \(P<0.00001\)) compared to those receiving placebo or no treatment control. Adverse events were assessed in 26 studies (3964 participants) indicating that probiotics reduced the risk of adverse events by 20% (RR 0.80; 95% CI 0.68 to 0.95). The commonly reported adverse events in both the treatment and control groups included abdominal cramping, fever, nausea, soft stools, and flatulence. Interestingly, with respect to the secondary outcome of the incidence of *C. difficile* infection, the results from the pooled analysis from 13 trials (n=961 participants) did not show a statistically significant reduction when comparing the treatment group to the control group, as the incidence of CDI was 12.6% in the probiotics group compared to 12.7% in the placebo or no treatment group (RR=0.89; 95% CI: 0.64 to 1.24).

Following a GRADE assessment, it was determined that the quality of evidence provides moderate confidence in the efficacy of probiotics to provide a protective effect to prevent the incidence of CDAD. The major limitation when assessing the quality of evidence was precision, with only 154 events among 23 RCTs. The results from this Cochrane
review are consistent with a previously conducted systematic review (Dendukuri, Costa, McGregor, & Brophy, 2005).

Of note is that limitations of this review include significant missing data from multiple trials both due to patients lost to follow-up and the investigators’ success in the fecal sample analysis. In spite of the minor limitations, Goldenberg and colleagues conclude that this report supports the use of probiotics is effective at reducing the incidence of CDAD, particularly since the reported cases of adverse events were higher in the control groups and thus, indicating a good safety profile of probiotics for the short-term use in children that are not immuno-compromised or severely debilitated (Goldenberg et al., 2013).

**Antibiotic-Associated Diarrhea (AAD)**

*Summary of review* (Goldenberg et al., 2015)

A Cochrane review was conducted that included 23 RCTs involving 3,938 children between 1 month and 18 years of age, receiving antibiotics to assess the efficacy of probiotics for the prevention of antibiotics-associated diarrhea. The comparator groups included children receiving placebo, active alternative prophylaxis or no treatment and the treatment period of the trials were between 3 to 30 days of antibiotic therapy. Of the included trials, the probiotics examined included *Bacillus spp.*, *Bifidobacterium spp.*, *Clostridium butyricum*, *Lactobacilli spp.*, *Leuconostoc cremois*, *Saccharomyces spp.*, or *Streptococcus spp.*, and multi-strain probiotics (11 studies) at doses ranging from 100 million to 40 billion (10⁸ to 4×10¹⁰) CFU QD. Following the Cochrane RoB assessment (Higgins et al., 2011), 13 studies were considered to be high or unclear RoB and 10 trials were reported as low RoB. The definition of diarrhea was heterogeneous across the included trials, wherein the authors’ defined the primary outcome as the presence or absence of diarrhea; and reported on the incidence of AAD in 22 trials (n=3,898). The summary of the results from the individual trials revealed that 6 RCTs demonstrated a statistically significant reduction in the incidence of AAD (P<0.05), one trial demonstrated significant reduction in AAD in the active alternative therapy, and 3 trials demonstrated the ‘no treatment group’ showed a statistical significant reduction in AAD. The other remaining RCTs did not show a statistically significant in treatment effect in terms of the incidence of AAD. The overall pooled results from all included trials...
revealed that the use of probiotics produced a statistically significant reduction in the incidence of AAD (RR=0.46; 95% CI: 0.35 to 0.61; \(P=0.05\)) compared to the control groups, such that the incidence of AAD in the probiotics group was 8% compared to 19% in the active alternative, placebo or no treatment control groups. Substantial heterogeneity was detected (\(I^2=55\%; \ P=0.0009\)). A priori subgroup analysis investigating this heterogeneity identified one potential cause of heterogeneity, the definition of diarrhea. However, subgroup results were similar across trials administering different probiotic species including single versus multi-strain probiotic, different probiotic doses, children with different diagnoses, studies at higher versus lower RoB, trials enrolling inpatients versus outpatients, and trials with and without industry sponsorship. Goldenberg and colleagues also performed a GRADE assessment and the analysis indicated that the overall quality of evidence for the efficacy of probiotics to reduce the incidence of AAD was moderate, owing to the level of generally unexplained heterogeneity present. Among 16 trials (n = 2455) that reported on adverse events, none reported serious adverse events attributable to probiotics. Meta-analysis demonstrated a non-significant difference in adverse events between probiotic and control groups (RD 0.00; 95% CI -0.01 to 0.01).

The overall conclusion put forth by the authors of the Cochrane review was that the moderate quality of evidence suggests a large protective effect of probiotics in the prevention of AAD. Among the various strains of probiotics evaluated, it appears that \textit{Lactobacillus rhamnosus} GG or \textit{Saccharomyces boulardii} (given at doses of 5 to 40 billion CFU/day) showed the greatest incidence reduction may be appropriate given the the likelihood that adverse events are very rare (Goldenberg et al., 2015).

**Acute Infectious Diarrhea (AID)**

\textit{Summary of review} (Ahmadi, Alizadeh-Navaei, & Rezai, 2015)

In this systematic review investigating the effect of probiotics on the duration of acute rotavirus diarrhea in children compared to a control group, a total of 14 RCTs (n=1149 children between the age of 1 to 72 months) were included in this review. Since the main strain of probiotic used in the majority of the included trials was \textit{Lactobacillus rhamnosus} GG, the authors categorized the children into separate groups according to the
type of probiotics received: (1) *Lactobacillus rhamnosus* GG, (2) non-LGG, and (3) the other group, which included single and multi-strain probiotics, at doses ranging from 10 million to 50 billion ($10^7$ to $5 \times 10^{10}$) CFU QD. The administered probiotics were provided as capsules, powders, and granules, which were mixed into a selection of food vehicles, such as yogurt or milk.

Following the analysis of the included studies, the pooled estimate of efficacy of probiotics in the prevention of acute diarrhea showed a mean difference of 0.41 (95% CI: -0.56 to -0.25; $P<0.001$) compared to control with a moderate level of heterogeneity ($I^2=39.9\%; P=0.046$). In the context of the specific probiotics strain, the pooled estimate of efficacy of LGG demonstrated a significant reduction in the duration of diarrhea, with 2 trials showing positive point estimates, but were not statistically significant and 6 trials attaining statistical significance with an overall reduction of 0.47 (95% CI: -0.80 to -0.4; $P=0.02$). However, substantial heterogeneity was detected ($I^2=57.8\%; P=0.020$).

Ahmadi and colleagues concluded that, although the efficacy of probiotics, more specifically, LGG, in the treatment of acute rotavirus diarrhea in children yielded contradictory results, the overall trend from the review showed that probiotics had a positive effect in reducing the incidence of acute rotavirus-induced diarrhea compared to the control group (Ahmadi et al., 2015). The findings from this review were consistent with the reported results from a previous systematic review, which reported that the use of probiotics reduced the duration of diarrhea by 20 to 24 hours compared to children not receiving probiotics (Sazawal et al., 2006). Furthermore, Sazawal and colleagues reported that *Bifidobacterium lactis* had a complementary role to LGG to reduce the duration of diarrhea compared to LGG alone. LGG also demonstrated a significant effect at reducing the duration of diarrhea in a dose-dependent manner in that LGG at a dose of $3 \times 10^9$ CFU/g administered twice a day for 6 days was more effective at reducing diarrhea as compared to a lower dose (Sazawal et al., 2006). Taken together, in this present review by Ahmadi et al., the evidence suggests that there is a significant effect of probiotics for reducing the duration of acute rotavirus diarrhea compared to not receiving treatment or placebo (Ahmadi et al., 2015).
**Necrotizing Enterocolitis (NEC)**

*Summary of review (AlFaleh & Anabrees, 2014b)*

In this updated Cochrane review, the primary objective was to compare the efficacy and safety of the use of probiotics for the prevention of severe (stage II or more) NEC versus placebo or no treatment. The total number of preterm infants (<37 weeks gestation and birth weight <2500 g) enrolled in twenty-four RCTS was 5,529 (probiotics group n=2761; control group n=2768). The initiation of probiotic treatment ranged from the first 24 to 72 hours of life or during their first feed and preterm infants were administered various species and strains of probiotics (*e.g.*, *Lactobacillus* spp., *Bifidobacterium* spp., *Saccharomyces boulardii*) or multi-strain probiotics for varying treatment periods, ranging from 2 to 6 weeks or until the date of hospital discharge (<30 days). The patient important outcomes were severe stage II-III NEC (20 RCT; n=5529), all-cause mortality (17 RCTs; n=5112) and any culture proven sepsis (19 RCTs, n=5338).

Following complete analysis of the patient important outcomes, the results revealed that preterm infants administered prophylactic probiotics showed significant reduction in the incidence of severe stage II to III NEC (RR: 0.43; 95% CI: 0.33 to 0.56; \( P<0.00001 \); NNT=30; in 20 trials) and significantly lowered mortality rate (RR: 0.65; 95% CI: 0.52 to 0.81; \( P=0.00017 \); NNT=41; in 17 trials) compared to preterm infants in the control group (placebo or no treatment). Although a positive trend toward the probiotics was observed, the probiotics did not show a significant difference in the incidence of cultured-proven sepsis between the preterm infants given the probiotics compared to the control group (RR: 0.91; 95% CI: 0.80 to 1.03; \( P=0.16 \); in 19 trials).

Based on the reported results, AlFaleh and colleagues concluded that prophylactic use of probiotics in preterm infants (<37 weeks gestation and birth weight of <2500 g) significantly reduced the severity progression of NEC to stage II or more and mortality rate compared to preterm infants not administered probiotics. Furthermore, while the authors acknowledged that more precise data is needed to determine the most effective preparation and dosing of probiotics to use, they strongly recommend a change in practice and adoption of probiotics.
prophylaxis in the management of NEC in preterm infants, as there exists convincing evidence of probiotic safety and efficacy compared to other available interventions (AlFaleh & Anabrees, 2014a).

**Infantile Colic**

A summary of the current evidence regarding the use of probiotics, specifically *L. reuteri* DSM 17938, for infantile colic is provided in section 4.3.

### 4.1.6 GRADE Assessment of the Overall Quality of Evidence

The GRADE assessments of all the included systematic review and meta-analysis (SRMA) are summarized in Table 4.4. In brief, the quality of evidence was evaluated for the most patient-important outcome of benefit and outcome of harm for each included SRMA. The overall quality of evidence for the outcome of benefit was most affected by inconsistency (heterogeneity) and imprecision (limited number of participants or events) for the included reviews (Goldenberg et al., 2015; Korterink et al., 2014; Moayyedi et al., 2010; Szajewska et al., 2013b; Szajewska et al., 2014a; Szajewska et al., 2014b; Tiequn et al., 2015). For the outcome of harm (adverse events), the overall quality was most affected by selective reporting bias (Ahmadi et al., 2015; AlFaleh & Anabrees, 2014a; Goldberg et al., 2015; Korterink et al., 2014; Moayyedi et al., 2010; Szajewska et al., 2013b; Szajewska et al., 2014a; Szajewska et al., 2014b; Tiequn et al., 2015), indirectness (Ahmadi et al., 2015; Goldberg et al., 2013; Goldberg et al., 2015; Korterink et al., 2014; Szajewska et al., 2013b; Szajewska et al., 2014b; Tiequn et al., 2015) due to varying definition of adverse events or lack of reporting, as it is considered a patient-important outcome and imprecision (limited number of events) (Ahmadi et al., 2015; AlFaleh & Anabrees, 2014a; Goldberg et al., 2013; Goldberg et al., 2015; Korterink et al., 2014; Szajewska et al., 2014a; Szajewska et al., 2014b; Tiequn et al., 2015). As such, the overall GRADE assessment of relating to the outcome of benefit for the GI-related conditions evaluated ranged from very low (Moayyedi et al., 2010; Szajewska et al., 2013b; Tiequn et al., 2015), low (Korterink et al., 2014; Szajewska et al., 2014a) to moderate (Ahmadi et al., 2015; AlFaleh &
Anabrees, 2014a; Goldenberg et al., 2013; Goldenberg et al., 2015). Additionally, the overall quality relating to the outcome of harm ranged from very low (Ahmadi et al., 2015; AlFaleh & Anabrees, 2014a; Goldenberg et al., 2015; Korterink et al., 2014; Szajewska et al., 2013b; Szajewska et al., 2014b; Tiequun et al., 2015) to low (Ahmadi et al., 2015; AlFaleh & Anabrees, 2014a; Goldenberg et al., 2013; Moayyedi et al., 2010; Szajewska et al., 2014a), as the majority of the included studies did not evaluate adverse events as a patient-important outcome.
Table 4.4: GRADE Summary of Findings (SoF) profile outlining quality of evidence for the use probiotics in GI-related conditions in pediatrics

**Author(s):** Chau K, Kennedy DA, Smy L, Marson A, Dugoua JJ, Johnston BC, Ito S  
**Question:** Are probiotics effective therapeutic interventions used to prevent and treat gastrointestinal conditions in the pediatric population?

<table>
<thead>
<tr>
<th>Review No. of RCTs</th>
<th>GI Conditions</th>
<th>Patient-Important Outcomes</th>
<th>Risk of Bias (RoB)</th>
<th>Inconsistency</th>
<th>Indirectness</th>
<th>Imprecision</th>
<th>Other Considerations (publication bias, large effect, plausible confounder, dose response gradient)</th>
<th>Relative Effect (95% CI)</th>
<th>Heterogeneity (I²)</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ahmadi et al. (2015)</strong> 14 RCTs; n = 1149</td>
<td>Acute infectious diarrhea (AID)</td>
<td>Duration of diarrhea</td>
<td>Serious ¹</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Not serious</td>
<td>None ²</td>
<td>MD 0.41 (-0.56 to -0.25, p=0.001) F = 39%; P=0.046</td>
<td>MODERATE ³, ⁵, ⁶</td>
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<td></td>
<td>Adverse events</td>
<td>Serious ⁴ N/A Serious ⁴ N/A N/A N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td><strong>Alfaleh et al. (2014)</strong> 24 RCTs; n = 5529</td>
<td>Necrotizing enterocolitis (NEC)</td>
<td>NEC severity progression (stage II or more)</td>
<td>Not serious ¹</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Rated down for publication bias ⁴</td>
<td>RR 0.43 (0.33 to 0.56, p&lt;0.00001) F = 6%, P=0.68</td>
<td>MODERATE ⁵, ⁶</td>
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<td></td>
<td>All-cause mortality</td>
<td>Serious ⁵ Not serious</td>
<td>Not serious</td>
<td>Serious ⁸</td>
<td>Serious ⁸</td>
<td>Serious ⁸</td>
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<td>RR 0.65 (0.52 to 0.81) F = 4%, P=0.41</td>
<td>LOW ⁷, ⁸</td>
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<tr>
<td><strong>Goldenberg et al. (2013)</strong> 31 RCTs; (3 children; n = 665)</td>
<td>Clostridium difficile-associated diarrhea (CDAD)</td>
<td>Incidence of CDAD</td>
<td>Not serious ⁵</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Not serious</td>
<td>None ¹¹</td>
<td>RR 0.40 (0.17 to 0.96; p=0.00001) F = 0%; P=0.76</td>
<td>MODERATE ³, ⁵, ⁶</td>
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<td></td>
<td>Adverse events</td>
<td>Not serious ⁸</td>
<td>Not serious ¹¹</td>
<td>Serious ¹¹</td>
<td>Serious ¹¹</td>
<td>Serious ¹¹</td>
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<td>RR 0.80 (0.68 to 0.95) F = 37%, P=0.06</td>
<td>LOW ⁷, ⁸</td>
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<tr>
<td><strong>Goldenberg et al. (2015)</strong> 23 RCTs; n = 3938</td>
<td>Antibiotic-associated diarrhea (AAD)</td>
<td>Incidence of diarrhea</td>
<td>Not serious ¹⁵</td>
<td>Serious ¹⁸</td>
<td>Not serious</td>
<td>Not serious</td>
<td>None</td>
<td>RR 0.46 (0.35 to 0.51; P&lt;0.05) F = 55%; P&lt;0.00094</td>
<td>MODERATE ³, ⁵, ¹⁶</td>
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<td></td>
<td>Adverse events</td>
<td>Serious ¹⁵</td>
<td>Not serious</td>
<td>Serious ¹⁸</td>
<td>Serious ¹⁸</td>
<td>Serious ¹⁸</td>
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<td>RD 0.00 (-0.01 to 0.01)</td>
<td>VERY LOW ¹⁴, ¹⁷</td>
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<tr>
<td><strong>Korterink et al. (2014)</strong> 8 RCTs; n = 741</td>
<td>Functional gastrointestinal disorders (FGIDs)</td>
<td>Probiotics treatment success (i.e. absence or reduction of abdominal pain)</td>
<td>Not serious ¹⁵</td>
<td>Not serious</td>
<td>Serious ²¹</td>
<td>Not serious</td>
<td>None ²⁴</td>
<td>RR 1.50 (1.22 to 1.84) F = 28%; P=0.24</td>
<td>LOW ²⁰, ²¹, ²₂, ²₃</td>
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<td></td>
<td>Adverse events</td>
<td>Serious ¹⁵</td>
<td>Not serious</td>
<td>Serious ²⁵</td>
<td>Serious ²⁵</td>
<td>Serious ²⁵</td>
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<td></td>
<td>VERY LOW ²₄, ²₅, ²₆</td>
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<td>Study</td>
<td>Condition</td>
<td>Outcomes</td>
<td>RR</td>
<td>CI</td>
<td>GRADE</td>
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<tr>
<td>Moayyedi et al. (2010)</td>
<td>Irritable bowel syndrome (IBS)</td>
<td>Overall IBS symptoms (as a dichotomous outcome)</td>
<td>0.71</td>
<td>(0.57 to 0.88)</td>
<td>VERY LOW</td>
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<td>Szajewska et al. (2013a)</td>
<td>L. reuteri for Acute gastroenteritis (AGE)</td>
<td>Duration of diarrhea (time until permanent cessation)</td>
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<td>Szajewska et al. (2013b)</td>
<td>LGG for Acute gastroenteritis (AGE)</td>
<td>Duration of diarrhea</td>
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<td>Szajewska et al. (2014)</td>
<td>L. acidophilus LB for Acute gastroenteritis (AGE)</td>
<td>Duration of diarrhea</td>
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<td>Tiequn et al. (2015)</td>
<td>Irritable bowel syndrome (IBS)</td>
<td>Overall IBS symptoms (e.g. abdominal pain, bloating or flatulence)</td>
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<td>Chau et al. (2016)</td>
<td>Infantile colic</td>
<td>Day 21 - Treatment responder (e.g. &gt;50% reduction of crying/fussing time from baseline)</td>
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CI: Confidence interval; MD: Mean difference; RR: Risk ratio; N/A: Not applicable.
1. Rated down due to the lack of RoB assessment of included RCTs.
2. The sample size for the target outcome was more than the threshold rule-of-thumb value of 400; as such, the quality of evidence was deemed precise.
3. No publication bias detected, as funnel plot inspection was not suggestive publication bias or other small study effects.
4. No report or discussion of any adverse events in the review, an outcome that would have been documented in the included RCTs; therefore, downgraded for indirectness for the target outcome of adverse events and selective reporting bias.
5. Thirteen of twenty-four trials were rated as unclear/high RoB; however, no statistically significant subgroup difference was detected following a subgroup analysis of high/unclear versus low RoB studies.
6. No assessment of publication bias (e.g. funnel plot or Egger regression test); therefore, rated down for publication bias.
7. None of the included RCTs reported any adverse events, which would have been documented; therefore, downgraded for selective reporting bias.
8. The overall number of events (events=296) was less than the threshold rule-or-thumb value of 400 (assuming α of 0.05, and β of 0.2, which represents a small effect). Therefore, the quality of evidence for the target outcome was downgraded for imprecision.
9. A test for subgroup differences revealed no statistically significant difference in the estimate of the incidence of diarrhea (P = 0.16). Of note, the 2 of the 3 pediatric trials were at low RoB.
10. Effect sizes were consistent across all included RCTs (I²=0%; P = 0.76).
11. Funnel plot inspection and Harbord’s linear regression test (P=0.11) were not suggestive of publication bias.
12. Minimal heterogeneity was detected between all 23 trials (I²=37%; P=0.06).
13. An assessment of adverse events of all included trials was conducted, but no subgroup analysis was performed for adult versus pediatric trials; therefore, downgraded for indirectness of evidence.
14. The overall number of events for pediatric data was less than the threshold rule-of-thumb of 400; therefore, QoE was downgraded for imprecision.
15. A test for interaction between low versus high/unclear RoB was not statistically significant. Additionally, the low RoB trials showed a more favourable probiotic effect compared to the trials deemed high/unclear RoB.
16. Substantial heterogeneity was detected (I²=55%; P=0.0009). While subgroup analyses were conducted, heterogeneity remained unexplained; therefore, QoE was downgraded for inconsistency.
17. Of the 22 trials, only 16 RCT reported adverse events, suggesting selective reporting bias and therefore, QoE was downgraded for RoB.
18. Varying definitions of adverse events were used across included trials and therefore, QoE was downgraded for considerable indirectness in terms of outcomes.
19. Evidence was considered very sparse, as only 81 events were reported; therefore, QoE was downgraded for imprecision.
20. All studies were reported to be at low RoB.
21. Varying diagnostic criteria of FGIDs were used (e.g. Rome II, Rome III or defined by authors) across included trials and therefore, QoE was downgraded for considerable indirectness with respect to reported outcomes.
22. Total number of events (from 5 of 8 RCTs) for treatment success was 263 representing sparse data, as it is less than the threshold rule-of-thumb value of 400 (assuming α of 0.05, and β of 0.2, which represents a small effect); therefore, QoE was downgraded for imprecision of results.
23. Publication bias was not assessed due to inadequate number of RCTs included in review.
24. Adverse events were reported in 2 of the 8 trials and the remainder of the trials did not provide extractable data, which would be documented; therefore, downgraded for selective reporting bias.
25. The definition of adverse events differed between the 2 trials; therefore, QoE was downgraded due to considerable indirectness with respect to outcomes.
26. The overall number of events relating to adverse events (events = 10) reported was less than the threshold rule-of-thumb of 400; therefore, QoE was downgraded for imprecision.
27. Rated down due to the lack of RoB assessment of included RCTs; performed quality assessment using Jadad Scale.
28. Substantial heterogeneity was detected (I²=68%; P=0.001) and subgroup analysis was still unable to explain; therefore, rated down for inconsistency.
29. Varying definition of overall improvement of IBS symptoms (e.g. 10 studies reported absence or reduction of abdominal pain, 8 trials reported bloating and 6 trials reported on flatulence) across all included trials; therefore, QoE was downgraded for considerable indirectness with respect to reported outcomes.
30. Total number of events of overall IBS symptom was 918, which is more than the threshold rule-of-thumb value of 400; therefore, the QoE was deemed precise.
31. Some asymmetry in the funnel plot was detected and Egger test = -2.97; 95% CI: -5.54 to -0.41, P=0.028, suggesting publication bias or other small study effect; therefore, downgraded for publication bias.
32. Nine of the 18 included trials provided extractable data; therefore, QoE was downgraded for selective reporting bias.
33. Total number of events of adverse events from the nine trials that reported was a minimum of 407, which is more than the threshold rule-of-thumb value of 400; therefore, the QoE was deemed precise.
34. No significant difference in adverse events between the probiotics and placebo group was reported (RR of AE on probiotics: 0.93; 95% CI: 0.64 to 1.36).
35. Three of the five trials were rated as unclear/high RoB and no subgroup analysis was conducted; therefore, rated down for high/unclear RoB.
36. Two different strains of *L. reuteri* (ATCC 55730 and DSM 17938) were used; therefore, QoE was downgraded for considerable indirectness with respect to intervention.
37. The total number of participants (*n*=352) is less than the threshold rule-of-thumb value of 400 (assuming α of 0.05, and β of 0.2, which represents a small effect). Therefore, the quality of evidence for the target outcome was downgraded for imprecision.
38. Publication bias was not assessed due to inadequate number of RCTs included in review.
39. Adverse events were reported in 2 of the 5 trials and the remainder of the trials did not provide extractable data, which would be documented; therefore, downgraded for selective reporting bias. Of note, no significant difference in AEs was observed between probiotics (*L. reuteri* DSM 17938) and control group.
40. Two of the five RCTs reported adverse events in the particular strain, *L. reuteri* DSM 17938, which may not be applicable to *L. reuteri* ATCC 55730; therefore, QoE was downgraded from indirectness with respect to intervention.
41. The overall number of events relating to adverse events reported from the two of five trials was less than the threshold rule-of-thumb of 400; therefore, QoE was downgraded for imprecision.
42. Eight of the fifteen included trials were deemed as high/unclear RoB and no subgroup analysis was performed to explore any potential subgroup differences; therefore, QoE was downgraded.
43. Considerable heterogeneity was detected (*I*=98%; *P*<0.00001) and *a priori* subgroup analyses were unable to explain; therefore, QoE was rated down for inconsistency.
44. Publication bias was formally assessed and no significant funnel plot asymmetry (Egger test -5.6; 95% CI: -11.5 to 0.3; *P*=0.06).
45. Adverse events were not reported in review, which would be documented in the original RCTs; therefore, downgraded 2 levels for selective reporting bias
46. Adverse events were deemed the most patient-important outcome of harm; therefore, QoE was downgraded for considerable indirectness with respect to target outcome.
47. Variation in methodological quality between studies, as 2 of the 4 RCTs included were deemed to be at high/unclear RoB; therefore, QoE was downgraded.
48. The overall number of participants (*n*=224) was less than the threshold rule-or-thumb value of 400 (assuming α of 0.05, and β of 0.2, which represents a small effect).
49. Therefore, the quality of evidence for the target outcome was downgraded for imprecision.
50. A statement of adverse events was made, “adverse effects were similar between groups”, but no report of the events were documented; therefore, downgraded for selective reporting bias
51. Small number of studies included and total number of participants (*n*=224) was less than the threshold rule-or-thumb value of 400 (assuming α of 0.05, and β of 0.2, which represents a small effect). Therefore, the quality of evidence for the target outcome was downgraded for imprecision.
52. Rated down due to the lack of RoB assessment of included RCTs; performed quality assessment using Jadad Scale, which only assessed randomization, blinding and loss-to-follow up.
53. Considerable heterogeneity was detected (*I*=68%; *P*=0.04) and no *a priori* subgroup analyses were performed; therefore, QoE was rated down for inconsistency.
54. Varying definition of overall improvement of IBS symptoms (e.g. outcomes measure included absence or reduction of abdominal pain, bloating and flatulence) across all included trials; therefore, QoE was downgraded for considerable indirectness with respect to reported outcomes.
55. Of the 6 included trials, only 3 trials were pediatric patients; therefore, the QoE was downgraded for imprecision, as the total events (events = 96) was less than the threshold value of 400.
56. Although only a few trials were included, Begg’s (*P*=1.000) and Egger’s tests (*P*=0.932) were conducted and no publication bias was detected.
57. Adverse events were not reported in review, which would be documented in the original RCTs; therefore, downgraded for selective reporting bias
58. Adverse events were deemed the most patient-important outcome of harm; therefore, QoE was downgraded for considerable indirectness with respect to target outcome.
59. The overall number of events relating to adverse events reported would be less than the threshold rule-of-thumb of 400; therefore, QoE was downgraded for imprecision.
60. Using the threshold rule-of-thumb of value of 400 events, QoE was rated down, as the total number of events was small (events = 135).
61. The effect size was considered large, as RR is greater than the threshold rule-of-thumb, RR >2; therefore, the quality of evidence was upgraded.
62. Definitions of adverse events were not provided in any of the included trials; therefore, QoE was downgraded for indirectness relating to outcomes.
63. The QoE was downgraded for imprecision, as the total events (events = 135) was less than the threshold rule-of-thumb value of 400.
4.2 Probiotics for Infantile Colic: A randomized, double-blind, placebo-controlled trial investigating *Lactobacillus reuteri* DSM 17938

Contents of this chapter have been published in the Journal of Pediatrics:


[KC amended the protocol for SickKids REB and Health Canada approval, recruited the participants, conducted the trial, collected the data, performed the analysis, and prepared the manuscript for submission].

4.2.1 Study Participant Enrolment

A total of 186 infants were screened for eligibility based on the inclusion criteria. Although eligible, parents of 123 infants declined to participate and 8 infants did not meet the inclusion criteria. Overall, 55 eligible infants were randomized; 27 were assigned to receive placebo and 28 were assigned to receive *L. reuteri*. Three participants randomized to the *L. reuteri* group were withdrawn from the study and therefore, were not included in the analysis for the following reasons: started antibiotic treatment (*n*=1); failure to complete diary (*n*=1); and discontinued intervention due to difficulty dispensing drops (*n*=1). Fifty-two infants (28 in the placebo group and 24 in the *L. reuteri* group) completed the study and were included in the analyses. There were no adverse events reported in either treatment groups (Figure 4.7).
4.2.2. Summary of Baseline Characteristics

Baseline characteristics, including gender, delivery type, entry age, growth parameters, family history of gastrointestinal diseases or atopy, and maternal history of smoking, were similar between the groups (Table 4.4). Additionally, safety and tolerability of both the probiotics and placebo were generally both well tolerated, as no adverse events associated with
the administration of either study products were reported. There were no differences between the \textit{L. reuteri} and placebo groups in changes in mean weight (45.3±0.7 g and 56.7±0.3 g, respectively; \(P=0.28\)), height (3.8±0.5 cm and 3.7±0.2 cm, respectively; \(P=0.99\)) and head circumference (3.2±0.8 cm and 2.9±0.7 cm, respectively; \(P=0.86\)) from baseline to the end of the study (Table 4.4).

Table 4.5: Baseline characteristics of study participants in the placebo and \textit{L. reuteri} DSM 17938 group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Placebo ((n=28))</th>
<th>\textit{L. reuteri} ((n=24))</th>
<th>\textit{p}-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wk), mean ± SD,</td>
<td>39.6 ± 0.36</td>
<td>39.9 ± 0.14</td>
<td>0.467\textsuperscript{b}</td>
</tr>
<tr>
<td>Age at entry (days), mean ± SD,</td>
<td>41.1 ± 9.4</td>
<td>42.1 ± 8.9</td>
<td>0.703\textsuperscript{c}</td>
</tr>
<tr>
<td>Male/Female, (n) (%),</td>
<td>14(50.0)/14(50.0)</td>
<td>11(45.8)/13(54.2)</td>
<td>0.788\textsuperscript{a}</td>
</tr>
<tr>
<td>Type of delivery, vaginal/cesarean, (n) (%),</td>
<td>23(82.1)/5(17.9)</td>
<td>18(75.0)/6(25.0)</td>
<td>0.735\textsuperscript{c}</td>
</tr>
<tr>
<td>Birth weight (g), mean ± SD</td>
<td>3541.7 ± 467.7</td>
<td>3377.2 ± 391.5</td>
<td>0.179\textsuperscript{b}</td>
</tr>
<tr>
<td>Entry weight (g), mean ± SD</td>
<td>4787.0 ± 849.3</td>
<td>4633.9 ± 669.3</td>
<td>0.479\textsuperscript{b}</td>
</tr>
<tr>
<td>Entry length (cm), mean ± SD</td>
<td>54.2 ± 2.5</td>
<td>53.8 ± 2.1</td>
<td>0.999\textsuperscript{b}</td>
</tr>
<tr>
<td>Entry head circumference (cm), mean ± SD</td>
<td>35.5 ± 1.1</td>
<td>35.9 ± 1.6</td>
<td>0.385\textsuperscript{b}</td>
</tr>
<tr>
<td>Family history of gastrointestinal diseases (yes), (n) (%),</td>
<td>3(11)</td>
<td>4(17)</td>
<td>0.754\textsuperscript{a}</td>
</tr>
<tr>
<td>Family history of atopy (yes), (n) (%),</td>
<td>11(39)</td>
<td>9(38)</td>
<td>0.883\textsuperscript{a}</td>
</tr>
<tr>
<td>History of maternal smoking (yes), (n) (%),</td>
<td>3(11)</td>
<td>3(13)</td>
<td>0.854\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Fisher's Exact Test
\textsuperscript{b}Student's \textit{t} Test
\textsuperscript{c}Mann-Whitney U Test

4.2.3. Primary Outcome – Duration of Crying/Fussing Times

There was no baseline difference in median crying and fussing time (minutes/day) between the placebo (122; IQR: 75) and \textit{L. reuteri} group (131; IQR: 65). By the end of treatment period (day 21), the total average crying and fussing times (minutes) for the duration of treatment (day 0 to day 21) was significantly shorter in the \textit{L. reuteri} group compared to the placebo group: 1719±750 min and 2195±764 min, respectively, (\(P=0.028\)). Overall, there was a significantly larger reduction in daily crying and fussing time observed among colicky infants administered the probiotic, \textit{L. reuteri} DSM 17938, at the end of treatment period (day 21) compared to colicky infants who received the placebo: 60 min. (IQR: 64) and 102 min. (IQR: 87), respectively, (\(P=0.045\)) (Table 4.5).
Table 4.6: Primary outcome - Duration of crying and/or fussing times in the placebo and *L. reuteri* DSM 17938 group

<table>
<thead>
<tr>
<th>Duration of Crying Time (min/day), Median (IQR)</th>
<th>Placebo (n=28)</th>
<th><em>L. reuteri</em> (n=24)</th>
<th>Median Difference (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>122(88-163)</td>
<td>131(84-149)</td>
<td>9(-29 to 46)</td>
<td>0.804*</td>
</tr>
<tr>
<td>Day 7</td>
<td>120(91-149)</td>
<td>90(53-129)</td>
<td>-30(-65 to 5)</td>
<td>0.032*</td>
</tr>
<tr>
<td>Day 14</td>
<td>103(78-140)</td>
<td>75(54-103)</td>
<td>-28(-55 to 0)</td>
<td>0.018*</td>
</tr>
<tr>
<td>Day 21</td>
<td>102(61-148)</td>
<td>60(35-99)</td>
<td>-42(-74 to -10)</td>
<td>0.045*</td>
</tr>
<tr>
<td>Mean Difference (95% CI); RR (95% CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Crying/21 Days (mins), mean ± SD</td>
<td>2195 ± 764</td>
<td>1719 ± 750</td>
<td>477(53 to 900); RR = 0.78(0.58-0.98)</td>
<td>0.028*</td>
</tr>
</tbody>
</table>

*Mann-Whitney U Test  
Student’s *t* Test

### 4.2.4. Secondary Outcome – Number of Responders to Treatment

A tendency toward higher proportion of infants in the *L. reuteri* group responded to treatment, thus, showing a reduction in crying and fussing times of ≥50% from baseline compared to infants in the placebo group (significance was only achieved on day 21): day 7: 4 vs. 1 (P=0.375), day 14: 11 vs. 3 (P=0.057), day 21: 17 vs. 6 (P=0.035), respectively (Table 4.6).

Table 4.7: Secondary outcome - Number of responders to experience decrease in daily average crying time of ≥50% from baseline

<table>
<thead>
<tr>
<th>Number of responders</th>
<th>Placebo (n=28)</th>
<th><em>L. reuteri</em> (n=24)</th>
<th>RR (95% CI)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 7</td>
<td>1</td>
<td>4</td>
<td>4.7</td>
<td>0.375a</td>
</tr>
<tr>
<td>Day 14</td>
<td>3</td>
<td>11</td>
<td>4.3</td>
<td>0.057a</td>
</tr>
<tr>
<td>Day 21</td>
<td>6</td>
<td>17</td>
<td>3.3(1.55-7.03)</td>
<td>0.035a</td>
</tr>
</tbody>
</table>

*Fisher’s Exact Test

### 4.2.5. Safety and Tolerability

The probiotics, as well as the placebo, were generally both well tolerated, as no adverse events associated with the administration of either study products were reported. There were
no differences between the \textit{L. reuteri} and placebo groups in changes in mean weight (45.3±0.7 g and 56.7±0.3 g, respectively; \(P=0.28\)), height (3.8±0.5 cm and 3.7±0.2 cm, respectively; \(P=0.99\)) and head circumference (3.2±0.8 cm and 2.9±0.7 cm, respectively; \(P=0.86\)) from baseline to the end of the study.

4.3 \textbf{Systematic Review and Meta-Analysis Investigating Lactobacillus reuteri DSM 17938 for the Treatment of Infantile Colic}

Contents in this section contains current work that is in preparation for publication:

\textbf{Chau K., Smy L, Kennedy DA, Johnston BC, Ito S. Systematic Review and Meta-Analysis of the Evidence on Lactobacillus reuteri DSM 17938 for the Treatment of Infantile Colic.}

[KC designed the protocol, co-searched the databases, screened and selected eligible RCTs, extracted the data, performed the meta-analyses and GRADE assessments, and prepared the manuscript for submission].

4.3.1 \textbf{Search Results and Study Selection}

From the comprehensive electronic database searches, 176 potentially relevant studies were identified. A total of 38 duplicate studies were removed leaving 139 study titles and abstracts to be screened for eligibility. Following the initial title and abstract screen, 122 studies were removed since they did not meet our inclusion criteria leaving 17 studies that were retrieved and assessed in full-text. Our full-text review identified 5 eligible RCTs (Chau et al., 2015; Mi et al., 2015; Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a). A summary of the selection process is provided in \textbf{Figure 4.8}. 

Figure 4.8: Schematic diagram of the search strategy and RCT selection for the meta-analysis
4.3.2 Description of Included Trials

A total of 335 colicky infants were included in the systematic review and meta-analysis (172 received *L. reuteri*; 163 received placebo). In 4 of the 5 RCTs (Chau et al., 2015; Mi et al., 2015; Savino et al., 2010; Szajewska et al., 2013a), the mode of feeding included exclusively breastfed infants, while 1 trial included both breast- and formula-fed infants (Sung et al., 2014). In a trial by Savino and colleagues, mothers of colicky infants were requested to follow a cow’s milk-free diet (Savino et al., 2010). The average infant age upon enrolment was similar between the probiotic and placebo groups across all included studies. All included trials excluded infants with chronic illnesses and a history of GI disorders. Of note is that 1 trial did not exclude infants receiving proton pump inhibitors (Sung et al., 2014; Sung et al., 2014). Baseline crying/fussing times (day 0) were recorded and similar in all trials with the shortest baseline crying/fussing times of 122 min/day (Chau et al., 2015; Chau et al., 2015) and the longest baseline crying/fussing times of 370 min/d (Savino et al., 2010). At onset of the clinical trial (day 1), all participants were administered 5 drops daily of either *L. reuteri* (at a total daily dose of $10^8$ CFUs [100 million CFUs]) or the placebo until the completion of the trial, which was either after 21 days (Chau et al., 2015; Savino et al., 2010) or 28 days (Mi et al., 2015; Sung et al., 2014; Szajewska et al., 2013a). Parents of all infants in all 5 RCTs were requested to keep a daily diary of the infants' colic episodes (*i.e.* number and duration of crying and/or fussing episodes) for the duration of the trial (21- or 28-day). Only 1 trial made a distinction between crying time from fussing time (Sung et al., 2014). A summary of study characteristics of the 5 included RCTs is provided in Table 4.7.
Table 4.8: Summary of characteristics of included RCTs.

<table>
<thead>
<tr>
<th>Reference; Country</th>
<th>Sample size</th>
<th>Study Duration</th>
<th>Inclusion Criteria: (1) Study Participants; (2) Age; (3) Mode of Feeding</th>
<th>Probiotic Intervention Dose (CFU)</th>
<th>Comparison Formulation</th>
<th>Outcome Measures: (1) Primary Outcomes; (2) Secondary Outcomes; (3) Method of Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chau et al. (2014) Canada</td>
<td>24</td>
<td>21-day treatment period</td>
<td>(1) Term infants diagnosed with colic by the modified Wessel’s definition*; (2) 3 wks – 6 mo; (3) exclusively breastfed infants</td>
<td>$10^8$ CFU/5 drops given OD</td>
<td>Placebo composed of sunflower oil, medium-chain triglyceride oil, silicon dioxide</td>
<td>(1) Reduction in average daily crying/fussing time from baseline to end of treatment (day 21); (2) treatment responders on days 7, 14 &amp; 21; (3) 21-day Paternal diary (modified version of Barr et al. Baby Day Diary(Barr et al., 1988))</td>
</tr>
<tr>
<td>Mi et al. (2015) China</td>
<td>20</td>
<td>28</td>
<td>21-day treatment period</td>
<td>(1) Term infants diagnosed with colic by the modified Wessel’s definition (2) ≥4 mos; (3) predominately or exclusively breastfed infants</td>
<td>$10^8$ CFU/5 drops given OD</td>
<td>Placebo formulation not stated</td>
</tr>
<tr>
<td>Savino et al. (2010) Italy</td>
<td>$n = 25$</td>
<td>21-day treatment period</td>
<td>(1) Term infants diagnosed with colic (modified Wessel’s definition ≥3hr/d; (2) 2 wk – 4 mo; (3) exclusively breastfed infants &amp; mothers on a dairy-free restricted diet</td>
<td>$10^8$ CFU/5 drops OD</td>
<td>Placebo (sunflower oil, medium-chain triglyceride oil); 5 drops OD</td>
<td>(1) Reduction in average crying time (min/d) to &lt;3 hrs/d on day 21; (2) responders to treatment (no. of infants showing reduction of daily crying duration of ≥50% on days 7, 14 &amp; day 21 from baseline); (3) structured daily diary</td>
</tr>
<tr>
<td>Diet</td>
<td>Diet</td>
<td>Diet</td>
<td>Diet</td>
<td>Diet</td>
<td>Diet</td>
<td></td>
</tr>
<tr>
<td>------</td>
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<td>------</td>
<td>------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>Szajewska et al. (2013)</td>
<td>Poland</td>
<td>40</td>
<td>40</td>
<td>21-day treatment period; 1 week follow up on day 28</td>
<td>(1) Term infant with diagnosed with colic by the modified Wessel’s definition; (2) &lt;5 mo; (3) predominately breastfed infants</td>
<td>10⁶ CFU/5 drops given OD</td>
</tr>
<tr>
<td>Sung et al. (2014)</td>
<td>Australia</td>
<td>85</td>
<td>82</td>
<td>28 day; 6-month follow-up</td>
<td>(1) Term infants diagnosed with colic; (2) ≥ 13 wks; (3) breastfed and formula-fed infants</td>
<td>10⁶ CFU/5 drops given OD</td>
</tr>
</tbody>
</table>

### 4.3.1 Risk of Bias Assessment of Included Studies

Overall, 4 of the 5 trials were determined to have a low RoB (Chau et al., 2015; Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a). One trial (Mi et al., 2015) was rated to be at high RoB because random sequencing, allocation concealment, blinding of participants and investigators and blinding during outcome assessment were not stated. A summary of our RoB assessment can be found in Figure 4.9 and 4.10. Savino et al. and Szajewska et al. trials were supported by BioGaia with all 5 trials having received the probiotic drops and placebo from BioGaia, Lund, Sweden, at no cost.
Publication bias was not assessed as there were inadequate numbers of included trials to properly assess a funnel plot or more advanced regression-based assessments (Higgins & Green, 2011).

Figure 4.9: Cochrane Collaboration’s Risk of Bias summary of reviewers’ assessment based on methodological quality of included studies

Figure 4.10: The percentage of Cochrane Collaboration’s Risk of Bias summary based on reviewer’s assessment of methodological quality of included studies
4.3.1 Effect of *L. reuteri* DSM 17938 on Crying and Fussing Time

The results of the random-effects model analysis for mean daily crying/fussing times, inclusive of four subgroups determined by the weekly endpoints (day 7, 14, 21 and 28), are shown in Figure 4.11. The administration of *L. reuteri* significantly reduced the average daily crying/fussing times on day 7 by a mean of 34.4 min (95% CI: 7.8 to 60.9) (Chau et al., 2015; Mi et al., 2015; Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a); day 14 by 50.5 min (95% CI: 28.2 to 66.5) (Chau et al., 2015; Mi et al., 2015; Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a); day 21 by 49.7 min (95% CI: 37.9 to 61.5); and day 28 by 46.9 min (95% CI: 14.1 to 79.6) (Mi et al., 2015; Sung et al., 2014; Szajewska et al., 2013a). The overall pooled results of the weekly endpoints revealed that colicky infants treated with *L. reuteri* demonstrated a significant reduction in average daily crying/fussing times of 43.5 min (95% CI: 32.6 to 54.3). However, there was considerable heterogeneity among the included studies (day 7: $I^2 = 96\%$; $P < 0.00001$; day 14: $I^2 = 89\%$; $P < 0.00001$; day 21: $I^2 = 65\%$; $P = 0.02$; day 28: $I^2 = 96\%$; $P < 0.00001$; sum of days 7, 14, 21 and 28: $I^2 = 95\%$; $P < 0.00001$) (Figure 4.11). The GRADE assessment revealed an overall low quality of evidence for the crying/fussing time outcome due to serious inconsistencies and imprecision of results (Table 4.8).
4.3.2 Effect of *L. reuteri* DSM 17938 Measured as Treatment Responders

All five RCTs compared the number of treatment responders in the *L. reuteri* and placebo groups (Chau et al., 2015; Mi et al., 2015; Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a). The results of random-effects model analysis, inclusive of four weekly endpoints (day 7, 14, 21 and 28), are shown in Figure 4.12. The results demonstrated a significantly higher number of colicky infants supplemented with *L. reuteri* successfully responded to treatment (RR 2.33; 95% CI: 1.71 to 3.17; *P*<0.00001). Infants supplemented with *L. reuteri* showed a significant response to treatment.
compared with the placebo group each week until day 28. It should be noted that substantial heterogeneity was observed for the day 14, 21 and 28 endpoints ($I^2 = 72\%, P = 0.01; I^2 = 80\%, P = 0.002; I^2 = 87\%, P = 0.0004$; respectively) (Figure 4.12). Based on the GRADE assessment, the quality of evidence for the treatment responders outcome was moderate for day 7; however, it was downgraded to low for day 21 and very low on days 14 and 28 due serious inconsistencies and very serious imprecision (Table 4.8).

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Favour L. reuteri</th>
<th>Favour Placebo</th>
<th>Risk Ratio IV (Random, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>7.1.1 Day 7 Responders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chau K et al., 2014</td>
<td>4</td>
<td>1</td>
<td>$4.67 {0.56, 38.97}$</td>
</tr>
<tr>
<td>Mi CL, et al., 2015</td>
<td>2</td>
<td>0</td>
<td>$4.78 {0.24, 93.19}$</td>
</tr>
<tr>
<td>Savino F, et al., 2010</td>
<td>20</td>
<td>8</td>
<td>$2.10 {1.18, 3.75}$</td>
</tr>
<tr>
<td>Sajewska H, et al., 2013</td>
<td>6</td>
<td>0</td>
<td>$13.00 {0.76, 223.33}$</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>109</td>
<td>108</td>
<td>$2.45 {1.41, 4.48}$</td>
</tr>
<tr>
<td><strong>Total events</strong></td>
<td>32</td>
<td>9</td>
<td>120%</td>
</tr>
<tr>
<td>Heterogeneity: $\tau^2 = 0.00; Ch^2 = 2.14, df = 3 (P = 0.54); I^2 = 0%$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: $Z = 3.22 (P = 0.001)$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>7.1.2 Day 14 Responders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chau K et al., 2014</td>
<td>11</td>
<td>3</td>
<td>$4.28 {1.35, 13.57}$</td>
</tr>
<tr>
<td>Mi CL, et al., 2015</td>
<td>10</td>
<td>1</td>
<td>$9.50 {1.34, 67.27}$</td>
</tr>
<tr>
<td>Savino F, et al., 2010</td>
<td>24</td>
<td>13</td>
<td>$1.55 {1.10, 2.19}$</td>
</tr>
<tr>
<td>Sajewska H, et al., 2013</td>
<td>10</td>
<td>7</td>
<td>$4.29 {2.14, 8.60}$</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>109</td>
<td>108</td>
<td>$3.23 {1.47, 7.12}$</td>
</tr>
<tr>
<td><strong>Total events</strong></td>
<td>75</td>
<td>24</td>
<td>260%</td>
</tr>
<tr>
<td>Heterogeneity: $\tau^2 = 0.41; Ch^2 = 10.74, df = 3 (P = 0.01); I^2 = 72%$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: $Z = 2.91 (P = 0.004)$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>7.1.3 Day 21 Responders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chau K et al., 2014</td>
<td>17</td>
<td>6</td>
<td>$7.4% {1.55, 7.03}$</td>
</tr>
<tr>
<td>Mi CL, et al., 2015</td>
<td>18</td>
<td>1</td>
<td>$2.2% {1.49, 115.86}$</td>
</tr>
<tr>
<td>Savino F, et al., 2010</td>
<td>24</td>
<td>15</td>
<td>$1.34 {1.01, 1.78}$</td>
</tr>
<tr>
<td>Sajewska H, et al., 2013</td>
<td>39</td>
<td>15</td>
<td>$2.80 {1.74, 3.89}$</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>109</td>
<td>108</td>
<td>$2.57 {1.36, 4.86}$</td>
</tr>
<tr>
<td><strong>Total events</strong></td>
<td>98</td>
<td>37</td>
<td>322%</td>
</tr>
<tr>
<td>Heterogeneity: $\tau^2 = 0.28; Ch^2 = 15.12, df = 3 (P = 0.002); I^2 = 80%$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: $Z = 2.92 (P = 0.004)$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>7.1.4 Day 28 Responders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mi CL, et al., 2015</td>
<td>20</td>
<td>3</td>
<td>$5.9% {2.15, 14.48}$</td>
</tr>
<tr>
<td>Sung V, et al., 2014</td>
<td>27</td>
<td>29</td>
<td>$0.83 {0.56, 1.23}$</td>
</tr>
<tr>
<td>Sajewska H, et al., 2013</td>
<td>40</td>
<td>40</td>
<td>$1.59 {1.25, 2.02}$</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>127</td>
<td>119</td>
<td>$1.69 {0.83, 3.46}$</td>
</tr>
<tr>
<td><strong>Total events</strong></td>
<td>87</td>
<td>57</td>
<td>28.9%</td>
</tr>
<tr>
<td>Heterogeneity: $\tau^2 = 0.32; Ch^2 = 15.83, df = 2 (P = 0.0004); I^2 = 87%$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: $Z = 1.44 (P = 0.15)$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>454</td>
<td>443</td>
<td>100.0%</td>
</tr>
<tr>
<td><strong>Total events</strong></td>
<td>292</td>
<td>127</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Figure 4.12: Forest plot of pooled analysis comparing the number of responders to treatment between *L. reuteri* versus placebo group
4.3.3 Subgroup Analyses

Based on the subgroup analysis stratified by post-treatment period (e.g., 21-versus 28-day), the subgroups do not have different effects ($P = 0.80$), such that, in both treatment periods, 21-day or a 28-day, infants receiving $L$. reuteri showed a greater reduction in crying/fussing time compared to infants in the placebo group [mean difference: 51.94 min (95% CI: 30.20 to 73.67) versus 46.88 min (95% CI: 14.13 to 79.63), respectively]. The $p$-value for the interaction between the 21-day versus the 28-day subgroups suggests that there is no significant difference in treatment effect between subgroups ($P=0.80$). We did, however, note less heterogeneity observed in the 21-day subgroup compared to the 28-day subgroup ($I^2 = 27\%, P = 0.24$ versus $I^2 = 96\%, P < 0.00001$, respectively) (Figure 4.13).

![Figure 4.13: Subgroup analysis stratified by 21- versus 28-day treatment days](image)

As shown, four of the five studies (Chau et al., 2015; Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a) were reported as low RoB, with one study, Mi et al. (Mi et al., 2015) suspected of high RoB. The $p$-value for the test of interaction between the high and low RoB subgroups revealed that no significant subgroup effect on any treatment days ($P = 0.5$ on Day 7; $P = 0.37$ on Day 14; $P = 0.32$ on Day 21; $P = 0.12$ on Day 28) (Figures 4.14 to 4.17).
Figure 4.14: Day 7 subgroup analysis stratified by low versus high risk of bias

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>L. reuteri</th>
<th>Placebo</th>
<th>Mean Difference</th>
<th>Mean Difference</th>
<th>Risk of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Total</td>
<td>IV, Random, 95% CI</td>
<td>A B C D E F G</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IV, Random, 95% CI</td>
<td></td>
</tr>
<tr>
<td>Low Risk of Bias</td>
<td></td>
<td></td>
<td></td>
<td>IV, Random, 95% CI</td>
<td></td>
</tr>
<tr>
<td>Chau K et al., 2014</td>
<td>92.04</td>
<td>44.97</td>
<td>24</td>
<td>118.07, 44.46</td>
<td>-26.03 [-50.42, -1.64]</td>
</tr>
<tr>
<td>Sevila F., et al., 2010</td>
<td>98.2</td>
<td>24.65</td>
<td>25</td>
<td>187.1, 43.03</td>
<td>-21.03 [-39.68, -12.12]</td>
</tr>
<tr>
<td>Sung V., et al., 2014</td>
<td>264</td>
<td>128</td>
<td>75</td>
<td>261, 104</td>
<td>15.13 [3.36, 44.42]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>161</td>
<td>150</td>
<td>77.3%</td>
<td>-30.86 [10.81, 72.42]</td>
<td></td>
</tr>
<tr>
<td>High Risk of Bias</td>
<td></td>
<td></td>
<td></td>
<td>IV, Random, 95% CI</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>181</td>
<td>169</td>
<td>100.0%</td>
<td>-34.45 [-61.05, -7.85]</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 1644.45; Chi² = 55.41, df = 3 (P < 0.000001); I² = 95%
Test for overall effect: Z = 1.45 (P = 0.15)

Figure 4.15: Day 14 subgroup analysis stratified by low versus high risk of bias

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>L. reuteri</th>
<th>Placebo</th>
<th>Mean Difference</th>
<th>Mean Difference</th>
<th>Risk of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Total</td>
<td>IV, Random, 95% CI</td>
<td>A B C D E F G</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IV, Random, 95% CI</td>
<td></td>
</tr>
<tr>
<td>Low Risk of Bias</td>
<td></td>
<td></td>
<td></td>
<td>IV, Random, 95% CI</td>
<td></td>
</tr>
<tr>
<td>Chau K et al., 2014</td>
<td>75.74</td>
<td>37.38</td>
<td>24</td>
<td>108.11, 47.51</td>
<td>-32.37 [-55.46, -9.28]</td>
</tr>
<tr>
<td>Sevila F., et al., 2010</td>
<td>66.1</td>
<td>20.51</td>
<td>25</td>
<td>157.75, 42.3</td>
<td>-91.65 [-111.37, -71.93]</td>
</tr>
<tr>
<td>Sung V., et al., 2014</td>
<td>256</td>
<td>137</td>
<td>65</td>
<td>238, 113</td>
<td>10.99 [18.00, 26.38]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>154</td>
<td>144</td>
<td>75.7%</td>
<td>-43.76 [-72.47, -11.09]</td>
<td></td>
</tr>
<tr>
<td>High Risk of Bias</td>
<td></td>
<td></td>
<td></td>
<td>IV, Random, 95% CI</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>174</td>
<td>163</td>
<td>100.0%</td>
<td>-47.38 [-66.54, -28.22]</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 802.55; Chi² = 107.75, df = 4 (P < 0.000001); I² = 96%
Test for overall effect: Z = 2.54 (P = 0.01)
Test for subgroup differences: Chi² = 0.49, df = 1 (P = 0.50); I² = 0%
Risk of bias legend:
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Binding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias
Figure 4.16: Day 21 subgroup analysis stratified by low versus high risk of bias

Figure 4.17: Day 28 subgroup analysis stratified by low versus high risk of bias

4.3.7 GRADE Assessment of the Overall Quality of Evidence

The GRADE assessment of average daily crying/fussing times revealed low quality of evidence for this outcome on all days of treatment (Table 4.8). The quality of evidence was most affected by the inconsistency (heterogeneity) and imprecision (limited number of patients) of the RCTs. The GRADE assessment of the outcome related to the number of treatment responders was rated low quality for day 7 and 21 and very low quality for days 14 and 28 (Table 4.8). The overall quality of evidence for the outcome related to treatment responders was deemed inconsistent due to the presence of substantial heterogeneity and highly imprecise due to the small number of events.
Table 4.9: GRADE Summary of Findings (SoF) profile outlining the quality of evidence of the effect of \textit{L. reuteri} DSM 17938 versus placebo for the treatment of infantile colic

**Author(s):** Chau K, Smy L, Kennedy DA, Nickel C, Johnston BC, Ito S  
**Question:** Is Lactobacillus reuteri DSM 17938 more effective than placebo for the treatment of infantile colic?

<table>
<thead>
<tr>
<th>Quality assessment</th>
<th>No of patients</th>
<th>Effect</th>
<th>Quality</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weekly Crying/Fussing Times - Day 7</strong></td>
<td><strong>Crying/Fussing Time</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of studies</td>
<td>Study design</td>
<td>Risk of bias</td>
<td>Inconsistency</td>
<td>Indirectness</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>5</td>
<td>RCT</td>
<td>Not serious</td>
<td>Serious</td>
<td>Not serious</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>RCT</td>
<td>Not serious</td>
<td>Serious</td>
<td>Not serious</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>RCT</td>
<td>Not serious</td>
<td>Serious</td>
<td>Not serious</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>RCT</td>
<td>Not serious</td>
<td>Serious</td>
<td>Not serious</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Number of Responders to Treatment - Day 7 Responders</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>RCT</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Not serious</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Number of Responders to Treatment - Day 14 Responders</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>RCT</td>
<td>Not serious</td>
<td>Serious</td>
<td>Not serious</td>
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</tbody>
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112
<table>
<thead>
<tr>
<th>Number of Responders to Treatment - Day 21 Responders</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RCT</strong></td>
</tr>
<tr>
<td>4</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Responders to Treatment - Day 28 Responders</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RCT</strong></td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

| **CI:** Confidence interval; **MD:** Mean difference; **RR:** Risk ratio |

1. High statistical heterogeneity ($I^2 = 96\%$; $P < 0.00001$) was detected that was unable to be explained by subgroup analysis.
2. The sample size was 350 on day 7 of treatment and, therefore, is less than the threshold rule-of-thumb optimal information size (OIS) value of 400 (assuming $\alpha$ of 0.05, and $\beta$ of 0.2, which represents a small effect). As such, the quality of evidence was deemed imprecise and thus, was downgraded.
3. On one hand, the quality of evidence was rated up for large magnitude of effect, as the effect of L. reuteri was rapid, showing significant decrease in crying/fussing time as early as 7 days. On the other hand, this increase in quality of evidence was downgraded as a cautionary action due to the outcome of crying/fussing times being subjective.
4. There was high statistical heterogeneity ($I^2 = 89\%$; $P = 0.00001$) that cannot be explained by subgroup analysis.
5. The sample size was 337 on Day 14 of treatment and therefore, is less than the threshold rule-of-thumb value of 400 (assuming $\alpha$ of 0.05, and $\beta$ of 0.2, which represents a small effect). As such, the quality of evidence was deemed imprecise and thus, was downgraded.
6. There was moderate statistical heterogeneity ($I^2 = 66\%$; $P = 0.02$) that cannot be explained by subgroup analysis.
7. The sample size is 335 on Day 21 of treatment and therefore, the total population size is less than 400 (a threshold rule-of-thumb value; assuming $\alpha$ of 0.05, and $\beta$ of 0.2, which represents a small effect). As such, the quality of evidence was deemed imprecise and thus, was downgraded.
8. There was high statistical heterogeneity ($I^2 = 96\%$; $P = 0.00004$) that cannot be explained by subgroup analysis.
9. The sample size was 246 on Day 28 of treatment and therefore, is less than the threshold rule-of-thumb optimal information size (OIS) value of 400 (assuming $\alpha$ of 0.05, and $\beta$ of 0.2, which represents a small effect). As such, the quality of evidence was deemed imprecise and thus, was downgraded.
10. Imprecision was rated down 2 levels, as the total number of events remains small (e.g. <100 events). Therefore, the threshold rule-of-thumb of control event rate 0.2 and RRR 25% is not met and as such, fails to meet OIS criteria.
11. The effect size was considered large, as RR is greater than the threshold rule-of-thumb, RR >2; therefore, the quality of evidence was upgraded.
12. There was moderate statistical heterogeneity ($I^2 = 72\%; P = 0.01$) that cannot be explained by subgroup analysis.
13. Using the threshold rule-of-thumb of control event rate 0.2 and RRR 25%, approximately 325 events were required to meet OIS criteria; therefore, imprecision was rated down 2 levels, as the total number of events remain small (e.g. <100).
14. High statistical heterogeneity ($I^2 = 80\%, P=0.002$) was detected that was unable to be explained by subgroup analysis.
15. Using the threshold rule-of-thumb of control event rate 0.2 and RRR 25%, approximately 325 events were required to meet OIS criteria; therefore, imprecision was rated down 2 levels, as the total number of events remain small (e.g. <100).

16. High statistical heterogeneity ($I^2 = 87\%$; $P=0.00004$) was detected that was unable to be explained by subgroup analysis.

17. The total number of events remain small (e.g. <100) and the 95% CI overlaps no effect (i.e. CI includes RR of 1.0); therefore, using the threshold rule-of-thumb of control event rate 0.2 and RRR 25%, the OIS criteria were not met. Thus, the quality of evidence was rated down 2 levels for imprecision.

### 4.3.7 Safety and Tolerability

A definition of what constitutes an adverse event was not stated in any of the 5 RCTs; however, adverse events were monitored and information regarding treatment-related adverse events was reported in all of the studies (Chau et al., 2015; Mi et al., 2015; Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a). All RCTs reported no adverse events, with the exception of one (Savino et al., 2010). In Savino et al., the adverse events reported included rhinitis ($L. reuteri$ group, $n = 1$), eczema (placebo group, $n = 1$), otalgia (ear pain) (placebo group, $n = 1$), and gastroesophageal reflux (placebo group, $n = 1$) (Savino et al., 2010).
CHAPTER 5
Discussion

5.1 Clinical Efficacy of Probiotics in Pediatrics

Contents in this section contains current work that is in preparation for publication and has been registered on PROSPERO:


[KC designed the protocol, co-searched the databases, screened and selected eligible reviews, extracted the data, performed the analysis, conducted the GRADE analysis, and prepared the manuscript for submission].

In the recent years, a marked increase in the use of probiotics in pediatrics has been demonstrated, particularly with the emergence of research demonstrating its efficacy in the treatment of gastrointestinal disorders, such as infantile colic, infectious and non-infectious diarrheal conditions, IBS and FGIDs. As such, probiotics represent a potential therapeutic advance in pediatrics due to its good safety profile in otherwise healthy children and in subgroups of children with chronic disease. To explore the role that probiotics may play, large head-to-head trials and high-quality systematic reviews that use advanced methods, such as network meta-analyses and individual patient data meta-analyses, are needed. It can only be expected that, as more data is accumulated and more probiotic organisms are discovered or engineered, probiotics may be used therapeutically to prevent, manage and treat various acute and chronic diseases. Of importance is the fact that strain specificity exists, such that the individual efficacy of each specific organism is specific a distinct clinical effect. Therefore, the success of one strain of a particular species of probiotic to produce a beneficial clinical effect for a certain GI condition does not imply that all related strains of the same species will produce a comparable clinical response.
5.1.1 Functional Gastrointestinal Disorders and Irritable Bowel Syndrome

Functional gastrointestinal disorders are very common conditions that affect all ages of people; it is estimated that 40% of the population suffers from FGIDs. Furthermore, as GI symptoms commonly overlap, it is highly probable that one person could be affected by more than one FGID simultaneously (Talley, 2008). Therefore, FGIDs are categorized by type of chronic and recurrent symptoms of functional origin that cannot be explained in terms of structural or biochemical abnormalities according to the Rome III consensus process 2006 (Drossman, 2006). For the pediatric population, FGID is classified into 3 separate categories: (1) abdominal pain- and/or discomfort-related FGID, (2) defecation-related FGID associated with a change in bowel habits, and (3) vomiting and aerophagia. There is no curative treatment for FGIDs; however, there are agents, such as probiotics, that provide symptom relief and periods of remission. Probiotics have been shown to reduce the manifestation of FGID symptoms related to abdominal pain and frequency of defecation (Korterink et al., 2014). Efficacy has been shown to reduce abdominal pain-related FGID with the use of LGG, *L. reuteri* DSM 17938 and VSL#3, which contains a mixture of eight strains of probiotics.

Based on the findings of the included systematic review, it appears that probiotics have an overall trend of a therapeutic effect in improving IBS symptoms (Moayyedi et al., 2010). Due to substantial unexplained heterogeneity, the authors concluded that, based on the dichotomous data, high dose multi-strain probiotics were more effective than a single strain, specifically *Lactobacillus rhamnosus* GG. Furthermore, treatment with *Bifidobacterium spp.* tended to show a trend toward improving IBS symptoms, but did not reach statistical significance. Use of VSL#3 was also demonstrated to be effective at decreasing the frequency of abdominal pain, but not the intensity and severity of pain. As it appears, the evidence demonstrates a trend favouring probiotics for the improvement of FGIDs and IBS symptoms; however, our GRADE assessment of the quality of evidence for overall change in FGIDs and IBS symptoms (e.g., reduction in abdominal pain, bloating and/or flatulence), both deemed the most patient-important outcomes of benefit, revealed low to very low quality of evidence. This suggests that little confidence is placed on the overall pooled estimate of effect for the selected outcomes of benefit, as the true effect is likely to be substantially different. Based on our GRADE assessment, the quality of evidence for use of probiotics to improve global FGIDs and IBS symptoms suggests more research may likely have an important impact on our confidence.
in the effect estimate, which may also change the estimate. Despite the low quality of evidence, we still stand to advocate the consideration of adopting probiotics in routine practice, particularly for the improvement of FGIDs (at dose range of 100 million to 10 billion CFU/day) and IBS (at a dose range of 10 million to 3 billion CFU daily, up to 100 billion CFU four times a day) symptoms relating to reduction of abdominal pain and bloating, as limited adverse events were associated with its use. However, it is acknowledged that more research may be needed to determine which strain and dose of probiotics are most efficacious, as the current evidence varies greatly in dose.

5.1.2 Diarrheal Diseases

Acute Infectious Diarrhea (AID)

Probiotics have been suggested to prevent the progression of AID through their action of inhibiting pathogenic bacterial adhesion and colonization by competitively occupying binding sites, lowering the luminal pH, through production of bacteriocins, and increased mucin production (Isolauri, 2003). The available evidence indicates that LGG is efficacious at reducing the duration of diarrhea by close to 10% compared to placebo, and reducing the number of days of hospital stays (Ahmadi et al., 2015). Similarly, a dose-dependent reduction in the duration of diarrhea was observed with the use of low doses ($10^7$ CFU/g per day for 5 days) and high doses ($10^{10}$ CFU/g per day for 5 days) of *L. reuteri* when compared to placebo (1.9 days and 1.5 days versus 2.5 days, respectively) (Shornikova, Casas, Isolauri, Mykkänen, & Vesikari, 1997). Other probiotics, including *L. acidophilus* LB, *B. lactis*, *S. thermophilus* and *S. boulardii*, have all shown to be efficacious at reducing the duration of diarrhea compared to placebo, and as a result, the length of hospitalization was also reduced by approximately 21 to 24 hours, compared to infants receiving oral rehydration therapy alone or placebo. Overall, the findings from the included review provide significant evidence for a positive effect of probiotics in the reduction of duration of diarrhea (MD 0.41; 95% CI: -0.56 to 0.25; $P<0.01$). However, heterogeneity was detected ($I^2 = 39\%$, $P=0.046$) that could not be explained by subgroup analysis (Ahmadi et al., 2015).

Following our GRADE assessment of the main efficacy outcome relating to the duration of diarrhea, the quality of evidence was categorized as moderate, suggesting that the
true effect is likely to be close to the estimate of effect, but a possibility that it may be different. The quality of evidence was downgraded due to the lack of a RoB assessment for the included RCTs in the review. For the outcome of harm relating to the incidence of adverse events, the quality of evidence was rated as low due to the possibility of selective reporting bias. The low quality of evidence suggests that our confidence in the effect estimate is limited due to the possibility that the true effect may be substantially different from the presented estimate of effect for the incidence of adverse events when given probiotics for AID. However, probiotics were generally well tolerated, as no serious adverse events attributable to the use of probiotics were reported. As such, based on our GRADE assessment of moderate quality of evidence, we strongly advocate consideration of moving probiotics towards routine use as a primary treatment option for the reduction of the duration of diarrhea in AID patients at a dosage range of 10 million to 50 billion CFU/day. Furthermore, with the good safety profile of probiotics, despite the low quality of evidence, the use of probiotics in this subset of patients appear to be more beneficial than harmful.

**Antibiotic-Associated Diarrhea**

The composition and diversity of the intestinal microbiota is altered by exposure to antibiotics, which is known to produce various clinical symptoms, one of which is acute diarrhea. This is particularly prevalent in children under the age of 2 years on antibiotic therapy, as approximately 18% in first-line treatment experience episodes of antibiotic-associated diarrhea irrespective of the reason for the antibiotic use, notably more common in those children treated with antibiotics or antibiotic combinations associated with amoxicillin-clavulanic acid (23%). Despite the frequency of AAD, the large majority of cases were classified as mild to moderate in severity and rarely required hospitalization (Turck et al., 2003). Based on the evidence presented in the Cochrane systematic review included this overview conducted by Goldenberg *et al.* (2015), moderate quality of evidence indicated a positive protective effect of probiotics in preventing AAD, as the pooled estimates of effect suggest a precise probiotic effect with a number-needed-to-treat of 10. Specifically, the most effective probiotics strains that produced the most beneficial effect were *Lactobacillus rhamnosus* GG or *Saccharomyces boulardii* given at a dose ranging from 5 to 40 billion CFU per day. Importantly, even at the highest dose administered, the likelihood of adverse events in
otherwise healthy children was very rare, whereas, immunocompromised or severely debilitating children with underlying risk factors were at higher risk of serious adverse effects (Goldenberg et al., 2015). Our GRADE assessment downgraded the quality of evidence to moderate with respect to the main patient-important outcome of duration of diarrhea, as this outcome was most affected by unexplained substantial heterogeneity following subgroup analyses. This suggests that more studies may have an important impact on our confidence in the estimate of effect and while more studies are needed to examine the small differences that may exist between strains, and to examine the duration of probiotic treatment, LGG and \textit{S. boulardii} at 5 to 40 billion CFU per day may potentially be considered for routine use among otherwise healthy children.

\textbf{\textit{Clostridium difficile}-Associated Diarrhea (CDAD)}

\textit{Clostridium difficile} is a pathobiont that may colonize the gut due to an imbalanced or disrupted intestinal microbiota, which may lead to asymptomatic infections, diarrhea, colitis, and pseudo-membranous colitis, and sometimes, death. Therefore, restoring an imbalanced or disrupted intestinal microbiota with probiotics was shown to inhibit and reduce the risk of \textit{C. difficile} adherence and colonization; ultimately, reducing the risk of CDAD by 64% compared to no treatment or placebo. The use of \textit{S. boulardii} was shown to be most effective at preventing CDAD, while other strains, such as LGG, \textit{B. lactis} and \textit{S. thermophilus} showed a moderate positive trend towards a beneficial effect in preventing CDAD. There was no statistical significant difference in adverse events between the probiotic and placebo group and in both treatment and control group; the most commonly reported adverse effects included abdominal pain and cramping, nausea, fever, soft stool, flatulence, and taste disturbances. Of importance, our GRADE assessment of the quality of evidence categorized the patient-important outcome relating to the incidence of CDAD as moderate quality, as the quality was downgraded due to substantial heterogeneity that could not be explained despite various subgroup analyses. Although further research may impact our confidence in the effect estimate and may even change the estimate, based on the moderate quality of evidence, we can advocate that adopting probiotics in routine clinical practice for the treatment of CDAD may potentially improve the duration of diarrhea. As such, it appears that the short-term use of probiotics, specifically, \textit{S. boulardii} at a dose range of 960 billion to 100 trillion CFU per day, may potentially be
effective at reducing the duration of CDAD, particularly when administered concomitantly with antibiotics. However, the optimal patient population, dose and the length of probiotic treatment remain to be determined (Goldenberg et al., 2013).

5.1.3 Acute Gastroenteritis

Acute gastroenteritis (AGE) is defined as the inflammation of the mucus membranes of the GIT with viruses (e.g., rotavirus or norovirus) being the most important etiology. It is a common childhood disease, particularly in developing countries, with a high risk of morbidity and mortality. Children in developed countries are also commonly affected; however, in this setting, it seldom causes death (King, Glass, Bresee, Duggan, & Centers, 2003). AGE is characterized by dehydration, a decrease in the consistency of stools (loose or liquid) and/or an increase in the frequency of bowel movements (≥3 within 24 hours), which may or may not be accompanied by vomiting and/or fever. Typically, diarrhea lasts up to 7 days, but can potentially last up to 14 days; beyond that it is referred to as persistent diarrhea (Guarino et al., 2008). Profuse watery diarrhea and/or frequent vomiting require immediate management of AGE by rehydration. Although drugs may have an impact on the duration and symptoms of AGE, they are sometimes ineffective, particularly in the case of antibiotics, as it has limited utility against viral infections (Britton & Versalovic, 2008). Hence, probiotics have gained favour over the use of drugs for the treatment of AGE, as it has been shown to be effective at restoring the microbial community to a healthy state. Overall, several meta-analyses of numerous RCTs have documented probiotics, as a group including various strains and combinations of strains, to decrease the duration of AGE in pediatric patients by approximately 1 day (35 RCTs, n = 4555, MD: 25 hrs; 95% CI: 16 to 34).

Based on the findings of the included meta-analyses (Szajewska et al., 2013b; Szajewska et al., 2014a; Szajewska et al., 2014b), LGG (10^{10} CFU/d) was shown to be most effective producing the most significant reduction in the duration of diarrhea (Szajewska et al., 2013b) and to a lesser extent, L. reuteri (ATCC 55730 and DSM 17938) and L. acidophilus LB. Despite the fact that Szajewska and colleagues reported significant methodological limitations of the included RCTs, they concluded that the evidence their reviews provided warrants a strong recommendation for the use of LGG in the management of AGE (Szajewska et al., 2013b).
Our GRADE assessment of the overall quality of evidence for the use of probiotics as a treatment for AGE and AGE-related symptoms revealed an overall very low to low quality of evidence for the most patient-important outcomes relating to duration of diarrhea and adverse events, due to possible selective reporting bias, substantial heterogeneity and imprecision of results. This suggests that very little confidence is placed on the estimate of effect, as the true effect of probiotics to reduce the duration of diarrhea in AGE patients and the incidence of AEs relating to probiotic use are likely to be substantially different from the presented estimate of effect. Although we acknowledge that further research may very likely have an important impact on our confidence in the effect of probiotics to reduce the duration of diarrhea, which may possibly change the direction of the effect estimate, the reported incidence of AEs suggests that probiotics are generally well tolerated, as no serious adverse events attributable to probiotics were reported (Szajewska et al., 2013b; Szajewska et al., 2014a; Szajewska et al., 2014b).

Overall, specific strains of probiotics have been shown to provide an important strategy for the treatment of AGE possibly through its mechanism of action of inhibiting pathogen proliferation and function, resulting in diminished severity or duration of diarrhea associated with AGE. While it may be premature to draw definitive conclusions about the efficacy and safety of other probiotic agents for pediatric AGE, the evidence advocates that consideration to move towards using probiotics in general practice for AGE and AGE-related symptoms.

5.1.4 Necrotizing Enterocolitis

Necrotizing enterocolitis is a condition that affects the GIT and is more prevalent in preterm infants; however, term infants are also affected. It is characterized by intestinal wall necrosis, of various lengths and depth, where approximately one third of infants affected suffer from complete perforation of the bowel (Kafetzis, Skevaki, & Costalos, 2003). Unfortunately, it is a major cause for morbidity and mortality, particularly in those with very low birth weight (VLBW) <1500 g. Although the etiology of NEC remains uncertain, NEC is proposed to likely represent complex interactions of factors that cause mucosal injury (Neu, 1996). The prominent theory is that NEC occurs with the presence of two of the three pathologic events: (1) intestinal ischemia, or (2) colonization of the intestine by pathogenic bacteria, and (3) the presence of antigenic material and relative hypoxia in the intestinal lumen (Kosloske, 1984b).
As bacterial colonization was deemed necessary for the development of NEC, this theory has led to the notion that probiotic supplementation may potentially prevent the incidence or progression of NEC severity (Millar, Wilks, & Costeloe, 2003). Probiotics may protect the intestinal barrier through its effect of inducing mucin production thereby, inhibiting the migration of pathogenic microbes and their products through the mucosa, competitively exclude the pathogens, modify the host’s response to microbial products, and possibly improve enteral nutrition (Kafetzis et al., 2003).

The results from the meta-analysis included in our OoSR, conducted by Alfaleh and colleagues, revealed that preterm infants administered prophylactic probiotics showed significant reduction in the incidence of severe stage II to III NEC (RR: 0.43; 95% CI: 0.33 to 0.56; \( P < 0.00001; \) NNT=30; in 20 trials) and significantly lowered mortality rate (RR: 0.65; 95% CI: 0.52 to 0.81; \( P = 0.00017; \) NNT=41; in 17 trials) compared to preterm infants in the control group (placebo or no treatment). Although a positive trend toward the probiotics was observed, the probiotics did not show a significant difference in the incidence of cultured-proven sepsis between the preterm infants given the probiotics compared to the control group (RR: 0.91; 95% CI: 0.80 to 1.03; \( P = 0.16; \) in 19 trials). As such, based on the reported results, Alfaleh and colleagues concluded that prophylactic use of probiotics in preterm infants (<37 weeks gestation and birth weight of <2500 g) significantly reduced the severity progression of NEC to stage II or more and mortality rate compared to preterm infants not administered probiotics.

Following our GRADE assessment of the quality of evidence of probiotic use for the prevention of NEC in preterm infants, although we acknowledged that more precise data is needed to determine the most effective preparation and dosing of probiotics to use, the quality of evidence was rated as moderate. This suggests that we are moderately confident in the effect estimate in that the true effect of probiotics for preventing the progression of NEC severity to stage II or stage III is likely to be close to the estimate of effect, but there is a slight possibility that the true effect may be different. Further research may likely to have an impact on our confidence in the effect estimate and may or may not change the estimate; however, the fact is that there still exists convincing evidence of probiotic safety and efficacy compared to other available interventions for NEC and with the overall moderate quality of evidence, a change in practice and the adoption of probiotics prophylaxis in the management of NEC in preterm infants may be warranted above the use of other interventions. However, caution should be
taken regarding the dosage and specific strain of probiotics used in the special high-risk population of preterm infants.

### 5.1.5 Infantile Colic

Infantile colic is a common symptom of unknown condition that affects approximately 5 to 40% of infants (Lucassen et al., 2001). It consists of repeated episodes of inconsolable crying and irritability that lasts for 3 or more hours per day, for 3 or more days per week and, oftentimes, more than 3 weeks with no discernible cause and produces significant distress in parents and caregivers (Brazelton, 1962; Wessel et al., 1954). A systematic review and meta-analysis of RCTs (Sung et al., 2013) demonstrated that supplementing breastfed colicky infants with *L. reuteri* significantly improved colic symptoms compared to infants receiving simethicone, an anti-foaming agent thought to reduce colic crying caused by excessive gas, and placebo.

Since the publication of this review, additional RCTs have been conducted (Chau et al., 2015; Mi et al., 2015; Sung et al., 2014) and the findings have been inconsistent (Sung et al., 2014). Sung and colleagues conducted the largest, well-designed RCT investigating *L. reuteri* for the treatment of colic involving both breast- and formula-fed infants. Overall, 167 breastfed infants were randomized to receive the probiotic or placebo and with multiple follow-up periods, the authors concluded that no significant reduction in crying and/or fussing time was observed between the probiotic and placebo group. We undertook the task of updating the evidence by conducting a systematic review and meta-analysis to examine the efficacy of *L. reuteri*. Overall, the pooled estimates for the 5 included RCTs demonstrated that infants supplemented with *L. reuteri* strain DSM 17938 showed a significant improvement in crying/fussing time compared to infants receiving placebo. Further, a higher proportion of infants in the probiotic group successfully responded to treatment compared to the placebo group. The quality of evidence was evaluated using the GRADE approach and the results revealed that the quality of evidence was low relating to the number of treatment responders. The quality of evidence was downgraded due to substantial heterogeneity, which could not be explained by subgroup analyses, and imprecision, a result of the small sample size. The overall low quality of evidence for this patient-important outcome suggests that our confidence in the effect estimate for probiotics in the treatment of infantile colic is limited, as the true effect may
be substantially different from the effect estimate that was presented. Although further research may likely have an important impact on our confidence, or that it may change the estimate, due to the general safety profile of probiotics and the lack of an effective treatment for colic, we advocate strong consideration of adopting probiotics, specifically containing *L. reuteri* DSM 17938 at a dose of 100 million CFU per day, in routine clinical practice for the treatment of infantile colic.

### 5.1.6 Safety and Tolerability of Probiotics

The most commonly used probiotic strains are considered *generally recognized as safe* (GRAS), primarily due to the fact that many probiotics naturally reside in the human GIT. However, there still remains certain safety concerns surrounding their use. Various commonly used species and strains of probiotics have been shown to become opportunistic and translocate through the GI epithelial barrier and have potential adverse immunologic effects. For example, *L. rhamnosus, L. acidophilus, L. plantarum, L. casei, L. paracasei, L. salivarius, L. plantarum,* and *L. lactis,* have been isolated from bloodstream infections and various other types of infective lesions (Saarela, Matto, & Mattila-Sandholm, 2002). Some studies have identified various strains of lactobacilli to be causative factors in endocarditis; however, in these cases, indigenous bacteria, not the microbes that were introduced as a probiotic, were revealed to be the causative agents (Axelrod, Keusch, Bottone, Cohen, & Hirschman, 1973; Bayer, Chow, Betts, & Guze, 1978). A Finnish study conducted to screen a diverse population (*e.g.*, preterm infants, healthy children and adults) consistently taking LGG, ranging from low to high dose and for short and long periods, failed to show a single case of infection associated with its use (Saxelin et al., 1996). Furthermore, there have been other studies that have investigated the use of various strains of probiotics in children between the age of 0 to 18 years (van den Nieuwboer, Claassen, Morelli, Guarner, & Brummer, 2014) and even in immuno-compromised children (Hempel et al., 2011), which have concluded that absolutely no adverse events were reported to be associated with probiotic use and therefore, probiotics containing *Lactobacillus* and *Bifidobacterium* species appear to be safe.

Consistent with the other available studies on probiotic safety, the safety statements from all 11 systematic reviews included in the OoSR pertaining to the included RCTs in the respective reviews suggest that no serious adverse effects are associated with the use of
probiotics in pediatrics. Safety statements included, “no AEs were reported during the treatment period and the study product was well tolerated” (Ahmadi et al., 2015), “no significant different in AEs were reported between treatment and placebo groups” (Goldenberg et al., 2013; Goldenberg et al., 2015; Korterink et al., 2014; Szajewska et al., 2013b; Szajewska et al., 2014a; Szajewska et al., 2014b), and “no AEs related to the study product were reported” (Moayyedi et al., 2010; Tiequn et al., 2015). Additionally, in one review, the authors listed the AEs that were reported, but made a general statement that the “reported AEs were considered minor and/or rare and did not appear to lead to further discomfort” (Ahmadi et al., 2015; Korterink et al., 2014; Moayyedi et al., 2010; Tiequn et al., 2015).

Of importance, our GRADE assessment of the main patient-important outcome of harm relating to adverse events for all reviews included in our OoSR categorized the overall quality of evidence as low to very low. The overall quality of evidence was most affected by inconsistency of the measured adverse events and imprecision of results, as many of the studies only reported a limited number events (e.g. <400 events). Many reviews were also deemed at high risk for potential selective reporting bias due to the lack of reporting any adverse events, which resulted in downgrading the quality of evidence. Although the low to very low quality of evidence suggests that further research may likely have an important impact on our confidence in the estimate of effect of the incidence of adverse events, and may even change the estimate, the reported safety data from the included reviews align with previous analyses that probiotic use in pediatrics is safe, with limited adverse effects (van den Nieuwboer et al., 2014).

5.1.7 Strengths and Limitations

With the inclusion of 11 systematic reviews in this overview, the overall number of RCTs and participants included allows for reasonably robust conclusions regarding the efficacy of various probiotics as an intervention for GI-related condition in the pediatric population. As such, there are several strengths of this OoSR, one being that the evidence presented centers around evidence-based medicine, which provides up-to-date evidence through the use of systematic reviews that utilized Cochrane methods to search, identify, assemble, critically appraise, and integrate the relevant evidence (e.g., formatted review using PICO, multiple database searches, multiple reviewers to assemble and analyze findings from primary studies). This is a major advantage, as the evidence reported in systematic reviews of RCTs provides
unbiased evidence for clinical practice, and importantly, guideline development. Also, the increase in the number of RCTs and systematic reviews has led to initiatives like the National Institutes of Health’s (NIH) ClinicalTrials.gov, Cochrane Collaboration and PROSPERO (an international prospective register of systematic reviews) to encourage registration of RCTs and systematic reviews, respectively, and in turn, this has made clinical practice more transparent and consistent (Evidence-Based, 1992; Sackett, Rosenberg, Gray, Haynes, & Richardson, 1996). The aim of these initiatives is to promote transparent reporting of research, and therefore, prompts the conduct of better quality trials, which ultimately leads to an increase in the quality of research evidence available for guideline development and clinical decision-making (Schünemann, Fretheim, & Oxman, 2006). With the rise in the amount of research being conducted, this is particularly important, as publication of research findings in medicine is not always merely driven by altruism, but more often than not, can be motivated by an researcher’s desire for recognition and/or financial gain (Woodcock & Luger, 2015). Evidence of this poor publication practice is demonstrated by the ten-fold increase of scientific research that was retracted since 1975 due to fraud and misconduct by researchers (Fang, Steen, & Casadevall, 2012). Accordingly, in line with encouraging transparency of research conduct and publication, the Good Publication Practice (GPP) guidelines were implemented in the publication of intervention studies, as these types of manuscripts frequently contain recommendations for treatment, as well as, information that directly affects a patient’s health and well-being (Woodcock & Luger, 2015). Thus, the GPP guideline was implemented to ensure complete transparency and to ensure that principles of evidence-based medicine have been applied. Therefore, the evidence presented in this OoSR adds another layer to GPP, as the evidence presented is obtained by searching, identifying, assembling and critically appraising findings from systematic reviews of RCTs. Furthermore, since the quality of both the primary studies and the systematic reviews has undergone quality assessments prior to selection, it can be certain that the findings arose from the inclusion of evidence from high quality trials and reviews, as we sought to include only moderate and high quality evidence based on the AMSTAR assessment, which allowed for the exclusion of reviews that utilized methods associated with high RoB.

This overview is also constrained by limitations in the included systematic reviews. The majority of the included systematic reviews investigated an array of different probiotics strains
of either *Lactobacillus* or *Bifidobacterium* species, at varying ranges of dosages (e.g., $10^5$ to $10^{12}$ CFU per dose, at multiple doses per day); therefore, this variability makes it extremely difficult to draw any overarching generalizable conclusions regarding the specific effects of probiotics. Additionally, results from the included reviews may not be applicable to vulnerable pediatric populations, such as immunocompromised children, due to the potential of probiotics to result in bacteremia in this subgroup (Saarela et al., 2002; Salminen et al., 2002).

Additionally, given the substantial heterogeneity detected in 7 of the included reviews (Ahmadi et al., 2015; Goldenberg et al., 2015; Korterink et al., 2014; Moayyedi et al., 2010; Szajewska et al., 2013b; Tiequn et al., 2015), it cannot be concluded that the observed positive effect reflects a true and consistent finding or whether it is an overrepresentation of a few trials. On the contrary, however, evaluating and pooling all RCTs of various probiotics in this overview allows the identification of trends that would not be apparent if trials examined individual strains. For example, the finding gleaned from dichotomous data suggest that all probiotics have an overall general beneficial efficacy on GI-related conditions when administered at high doses. The continuous data revealed that single species/strains of probiotics might have limited impact on symptoms; while high doses of multi-strain probiotics are more like to improve symptoms.

Also, there were limitations that occurred at the level of individual studies, as a few of the RCTs in the included systematic reviews showed high or unclear quality in terms of randomization, allocation concealment, blinding and high rate of loss to follow-up. In fact, there were a few included systematic reviews that did not draw any firm conclusions because of potential methodological flaws of the included trials (Ahmadi et al., 2015; Goldenberg et al., 2015; Korterink et al., 2014; Moayyedi et al., 2010; Szajewska et al., 2014b; Tiequn et al., 2015). Additionally, two systematic reviews themselves failed to explicitly pre-specify the inclusion and exclusion criteria according to the PICO (Moayyedi et al., 2010; Tiequn et al., 2015), which may have led to the inclusion of irrelevant RCTs. As a result, it may explain the substantial heterogeneity observed in the dataset. Notably, one aspect of the observed heterogeneity may be due to the various types of probiotic species studied across all RCTs in the included reviews.

Furthermore, the overall limitation of this OoSR is that because of the general inherent variability of probiotic research due to the dynamic nature of the intestinal microbiota during infancy, the variability in the type of probiotics studies across included reviews, and the wide
range of dosages administered, presents a hindrance for successful knowledge translation of evidence to practice. Thus, this highlights the need for more specificity and consistency in terms of the specific clinical effects of specific strains, as well as, specific outcome measures, in probiotic research, particularly in the pediatric population.

5.1.8 Implications for Clinical Practice and Research

This overview identified a considerable number of systematic reviews investigating probiotics for the treatment of GI-related conditions in the pediatric population and thus, provided a comprehensive view of controlled assessments of probiotics as a potential therapeutic agent.

Although the currently reviewed evidence for probiotics use for GI-related conditions in the pediatric population appears to favour its use, the estimated size of effect for the studied conditions varies across the included primary RCTs. Thus, the evidence still remains insufficient to explicitly confirm with confidence the use of probiotics at a particular dose and for what duration as a primary treatment option. However, probiotics have been shown to successfully reduced hospital stay by approximately 1 to 4 days when used for the treatment of diarrheal conditions, irritable bowel syndrome and AGE and for the prevention of antibiotic-associated diarrhea. Furthermore, the evidence is strong in support of probiotics for the management and treatment of infantile colic crying. It is noteworthy to mention that most of the evidence suggests that *Lactobacillus* spp. may be considered the best studied and perhaps most effective probiotics compared to other species; however, the optimal effective dose and duration of treatment remain to be explored and explicitly defined.

5.1.9 Conclusions

Overall, with the direction of probiotic research, it is undeniable that probiotics in the near future may have a definite role in several GI-related conditions, in both pediatric and adult populations. Although the currently available evidence is quite encouraging, the primary limitations for the recommendation are the methodological issues with the currently available RCTs that limit the quality of the evidence and the heterogeneity of treatments (probiotic strain and dose, mode, dose and duration of supplementation, primary outcomes, etc). Some specific strains are promising, for example, *L. reuteri* DSM 17938 for the treatment of infantile colic
and LGG for gastroenteritis. At present, there is no indication for the use of a particular strain of probiotics for the treatment of NEC, IBS and FGIDs, as more studies are needed to confirm the existing evidence. Future studies should place more emphasis on explicitly identifying the optimal dose and duration of treatment of probiotics, especially since the evidence in favour of the beneficial effects of probiotics with limited adverse effects is promising. Furthermore, research should also focus on the development of more precise tools for assessing important clinical outcomes. Lastly, while there exists some promising evidence for certain strains of probiotics, these findings must be interpreted with caution, as the data cannot not be regarded as generalizable to all conditions. Therefore, it can be stated that the evidence on the use of probiotics to treat common pediatric GI-related conditions as incomplete, as no firm conclusions can be made without further research on strain specificity of clinical effects, optimal effective dosages, mode of administration to achieve optimal bacterial viability, and duration of treatment to achieve the desired clinical outcome.

Despite the limitations mentioned, this overview identified a substantial number of convincing evidence regarding probiotics safety and efficacy. Additionally, with the assessment of the quality of evidence, we can advocate the move towards potentially including the use of probiotics as a therapeutic option for many GI-related conditions, particularly in the pediatric population, in routine clinical practice. However, ultimately, a general practice guideline or a widespread recommendation or even a blanket statement regarding probiotic use in pediatrics for all GI-related conditions cannot yet be explicitly defined.

### 5.2 Efficacy of *Lactobacillus reuteri* DSM 17938 for the Treatment of Infantile Colic

Contents in this section have been published in the Journal of Pediatrics:


[KC amended the protocol for SickKids REB and Health Canada approval, recruited the participants, conducted the trial, collected the data, performed the analysis, and prepared the manuscript for submission].
In the present Probiotics for Infantile Colic study, it was successfully demonstrated that administration of *Lactobacillus reuteri* DSM 17938 to exclusively breastfed Canadian infants diagnosed with infantile colic is more effective than placebo at reducing colic crying and fussing symptoms. In fact, colicky infants treated with *L.* *reuteri* showed a significant improvement in daily crying and fussing time in as early as 7 days after initiation of probiotic therapy. Furthermore, an analysis of the number of responders to treatment (defined as the number of participants demonstrating a ≥50% reduction in crying and/or fussing time from baseline) revealed a similar trend was observed with improvements of colic symptom in the *L.* *reuteri* group beginning on day 14; however, significance was not reached until day 21 of treatment.

A few minor differences notwithstanding, our results are consistent with other previous reported studies in Italy and Poland, which used similar definitions of infantile colic and primary outcome measures (Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a). In the trial by Savino and colleagues (Savino et al., 2010), breastfeeding mothers were instructed to refrain from consuming dairy containing cow’s milk protein; however, similar to Szajewska and colleagues’ trial (Szajewska et al., 2013a), we did not request breastfeeding mothers to adhere to a cow’s milk protein elimination diet; despite this, we observed comparable improvements in daily crying and fussing times in the *L.* *reuteri*-treated group. This demonstrates that merely eliminating cow’s milk protein is not effective in reducing daily crying times of colicky infants, as was previously suggested (Barr et al., 1988; Hall et al., 2012; Iacovou, Ralston, Muir, Walker, & Truby, 2012; Lucassen & Assendelft, 2001) and as such, avoiding cow’s milk protein dairy does not appear to significantly impact the efficacy of *L.* *reuteri* observed in Savino and colleagues’ trial.

As with previous studies (Savino et al., 2010; Szajewska et al., 2013a), infants administered the probiotic, *L.* *reuteri* DSM 17938, demonstrated a significant decrease in daily crying and fussing times when compared to infants who received the placebo. The need for a placebo group is essential in this type of self-limiting condition, which tends to improve over time. In our study, 6 of 28 infants (6/28; 21%) administered placebo showed a significant reduction in crying and fussing times from baseline (day 0) to end of study (day 21) compared to 15 of 24 infants in the *L.* *reuteri* group (15/24; 63%). A placebo effect was also observed in Savino’s, Szajewska’s and Sung’s trials (Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a); however, the effect was far greater in those studies (71%, 37.5%, and not reported,
respectively). In contrast to the previously mentioned studies (Savino et al., 2010; Szajewska et al., 2013a) and our own trial, Sung and colleagues (Sung et al., 2014) reported that infants in the probiotic group did not improve compared to the placebo group. In fact, among their formula-fed subgroup, the placebo group performed significantly better than the probiotic. Because this observation does not have any biological plausibility, it suggests that some uncontrolled variables may have affected this trial (Sung et al., 2014). The lack of significant difference between the two groups in Sung's study may be explained by their recruitment of infants contemporaneously treated with drugs (such as proton pump inhibitors) and dietary approaches (such as formulas containing probiotics or hypoallergenic formula). These confounding factors needed to be evaluated individually in order to perform an appropriate multivariate analysis and to distinguish the effects of the tested probiotic from the placebo. The apparent greater response to placebo in previous studies may be partially attributed to the average age of entry of infants in both trials; 28.5 days in Savino et al. trial (Savino et al., 2010), 38.1±11.7 days in Szajewska et al. trial (Szajewska et al., 2013a), and 48.4±17.5 days (6.9±2.5 weeks) in Sung et al. trial (Sung et al., 2014) compared to 41.1±9.4 days in our trial. Thus, the lack of response in Sung’s trial may be due to the natural course of the condition and the self-limiting nature of colic symptoms, as the average infants’ age at entry was older (Barr, Rotman, Yaremko, Leduc, & Francoeur, 1992).

An infant’s intestinal microbiota is highly dynamic and is more variable in its composition until approximately two years of age (Rautava et al., 2012). Previous studies have shown that the acquisition and development of the intestinal microbiota during this period is a complex process that is influenced by various factors, including mode of delivery, feeding type (Adlerberth, 2008; Gronlund et al., 1999; Rautava et al., 2012), and geographical regions (Fallani et al., 2010; Suzuki & Worobey, 2014; Yatsunenko et al., 2012). Furthermore, de Weerth and colleagues (2013) reported that colicky infants displayed lower microbiota diversity and stability compared to non-colicky infants (de Weerth et al., 2013a; de Weerth et al., 2013b). Accordingly, the marked differences observed in the effect of L. reuteri in the Sung et al. trial compared to the results in our trial and those of Savino and Szajewska may be attributed to differences in colonization patterns and stability of the intestinal microbiota of infants from Australia, Italy, Poland and Canada. Hence, it is of great importance to continue to investigate the efficacy of L. reuteri for the treatment infant colic across different geographical
regions to strengthen the evidence for this intervention, as infants from one area may be more susceptible to the effects of probiotics than other regions.

5.2.1 Strengths and Limitations of the Probiotics for Infantile Colic Trial

An important strength of this study is that, to date, this is the first North American randomized, double-blind, placebo-controlled trial to investigate the effectiveness of probiotic therapy for the treatment of infantile colic. Furthermore, with the recent evidence of a negative effect of *L. reuteri* DSM 17938 to improve colic symptoms, our results precisely define the efficacy of *L. reuteri* DSM 17938 in treating infant colic in colicky Canadian infants. As well, our diagnostic criteria of infantile colic and outcome measures were similar to the other trials (Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a). This is of great importance as this allows us to make comparisons between studies investigating the same intervention for the same condition.

A potential limitation of this study, similar to previous studies, is that the measure to assess the duration of crying and fussing times in colicky infants solely relied on the mother’s report of the duration of crying and fussing episodes in the maternal diaries, which poses well-known problems, such as poor adherence and accuracy of recordings due to the tendency to retrospectively record. However, this potential shortcoming could not be expected to introduce bias in any particular arm of this blinded study. Another limitation is that objective measures of compliance with the medication were not assessed. A potential method of assessing compliance is by weighing the study bottles pre- and post-dispensing; however, this method was reported to produce highly variable results (Vitolins, Rand, Rapp, Ribisl, & Sevick, 2000). Lastly, collection of fecal samples to determine the colonization pattern of colicky infants and whether a difference is observed following probiotic supplementation was not included in this present study. Therefore, we were not able to precisely confirm whether probiotic supplementation with *L. reuteri* DSM 17938 improves its profile in the infant intestinal microbiota.

As such, our conclusions based on the findings of the Probiotics for Infantile Colic trial support the beneficial effects of administering *L. reuteri* DSM 17938 to treat infantile colic in breastfed, colicky Canadian infants as previously reported in other geographical regions. Of particular importance, our study provides, for the first time, evidence from North America that supplementation of probiotics in early infancy is effective in managing colic symptoms.
5.3 Systematic Review and Meta-Analysis of *Lactobacillus reuteri* DSM 17938 for the Treatment of Infantile Colic

Contents in this section contains current work that is in preparation for publication and has been registered on PROSPERO:


[KC designed the protocol, co-searched the databases, screened and selected eligible RCTs, extracted the data, performed the meta-analyses and GRADE assessments, and prepared the manuscript for submission].

The results from the meta-analysis of the evidence demonstrated that the administration of *Lactobacillus reuteri* DSM 17938 was associated with reduction of average daily crying/fussing in the population of colicky infants as early as day 7 of treatment, showing continued reduction on days 14, 21 and 28 from baseline when compared to infants treated with a placebo. Further, there were significantly more infants who responded to treatment in the *L. reuteri* group compared to infants receiving the placebo on days 7, 14 and 21; however, this effect was not observed on day 28. Importantly, these results are limited due to the significant heterogeneity observed across all treatment days for daily average crying/fussing time and on days 14, 21 and 28 of treatment for the number of treatment responders. The observed heterogeneity remained unexplained after performing a subgroup analysis categorizing trials by treatment duration (e.g., 21- versus 28-day).

5.3.1 The GRADE Approach to Assessing Quality of Evidence of *Lactobacillus reuteri* DSM 17938 to Treat Infantile Colic

The GRADE assessment revealed low quality of evidence for the average daily crying/fussing times outcome for all weekly endpoints and very low to low quality of evidence relating to treatment responder outcome (Table 4.9). The very low quality of evidence for treatment responders on days 14 and 28 can be explained by serious inconsistencies, due the presence of substantial heterogeneity, and very serious imprecision due to the few crying/fussing events (*i.e.*, <100 events). Similar to the favourable effect of *L. reuteri* DSM 17938 based on average daily crying/fussing times outcome, the number of treatment responders also favoured *L. reuteri* DSM 17938, as significantly more infants in the probiotic group responded to treatment with a decrease in crying/fussing of ≥50% by day 7 and the
number continued increasing for days 14 and 21. However, this effect was not significant by day 28 of treatment (87/127 responders versus 57/119 responders, respectively). A possible explanation for this result may be the natural course of colic. As demonstrated in previous studies, colic can manifest as early as 2 weeks of age with peak crying times at 6 weeks of age and a spontaneous but gradual remission of crying to baseline levels by approximately 12 weeks of age, which represents the same crying curve of non-colicky infants (Brazelton, 1962). With the combined total of mean age of entry being comparable in the *L. reuteri* DSM 17938 and placebo groups (38.2 ± 9.2 days and 36.9 ± 8.5 days, respectively), it is possible that the apparent lack of treatment responders on day 28 may be explained by the natural, spontaneous remission of colic symptoms in both treatment and placebo group. Generally, colic symptoms resolve in 60% of colicky infants by 3 months of age and in 80 to 90% of infants by the age of 4 months (St James-Roberts & Halil, 1991). The results of this meta-analysis suggest that treatment with *L. reuteri* reduces colic symptoms sooner than spontaneous resolution of the condition.

The inconsistencies between studies observed in this meta-analysis, which resulted in downgrading of the quality of evidence, refers to the substantial heterogeneity among the included RCTs (Table 4.9). However, despite performing a subgroup analysis stratified by duration of treatment (e.g. 21- versus 28-day), the heterogeneity remained unexplained, as there was no significant difference between the subgroup of 21-day treatment versus 28-day treatment (*P*=0.80) (Figure 4.16 and 4.17). This may be the result of *L. reuteri* showing an improvement in colic symptoms earlier than 21 or 28 days, as confirmed by the significantly higher number of treatment responders as early as day 7 in the *L. reuteri* group compared to the placebo group. Other possible explanations for the heterogeneity may include the inter-individual variability of the intestinal microbiota between infants of different geographical regions (Fallani et al., 2010; Suzuki & Worobey, 2014; Yatsunenko et al., 2012) and the inherent effect of probiotics on the dynamic nature of the intestinal microbiota during early infancy (Rautava et al., 2012; Sommer & Bäckhed, 2013). Previous studies have shown that the intestinal microbiota is more unstable and less diverse in colicky infants than that of non-colicky infants and its development and colonization pattern during early infancy is strongly impacted by extrinsic factors, such as mode of delivery, method of feeding (e.g., breast- or formula-fed) and geographical region (Adlerberth, 2008; Bezirtzoglou, 1997; Blaut & Clavel, 2007; Yatsunenko et al., 2012). For example, in a study comparing the fecal microbiota of non-
Western children with Western (American) children 0–3 years of age, significant differences in the phylogenetic composition of the microbiota were found (Yatsunenko et al., 2012). Additionally, even infants residing in the same geographical region, but with different dietary habits (Marques et al., 2010; Xu & Knight, 2015) and different cultural traditions (e.g., perspectives and attitudes of keeping companion animals, since close physical contact with animals affects the acquisition and exchange of microbes) (Serpell JA & P, 2011), will have a varied composition of the gut microbiota. Therefore, it is probable that the variation among the included RCTs may be attributed to the different geographical locations of the infant populations (Australia (Sung et al., 2014), Canada (Chau et al., 2015), China (Mi et al., 2015), Italy (Savino et al., 2010) and Poland (Szajewska et al., 2013a) and the differences in cultural traditions and dietary habits across those geographical regions lend to significant inter-individual variability of the intestinal microbiota. Furthermore, due to the fact that an infant’s intestinal microbiota is less stable (Faith et al., 2013; Rajilic-Stojanovic, Heilig, Tims, Zoetendal, & de Vos, 2012), some studies have shown that it is possible for their intestinal microbiota to return to baseline levels of bacterial composition similar to the composition before the administration of probiotics (Goldin et al., 1992). This effect is termed colonization resistance, referring to the inability of exogenously administered probiotics to permanently adhere and persist in fecal samples for more than a week after discontinuation of probiotic administration (Alander et al., 1999). Moreover, it is only at approximately 3 years of age that the intestinal microbiota converges toward an adult-like microbiota and becomes more stable (Faith et al., 2013). Therefore, it is possible that less inter-individual variability may be observed in adult intestinal microbiota with respect to the effect of probiotics, compared to the high inter-individual variability expected in an infant’s response to probiotics.

An additional explanation for the observed contradictory results from Sung et al. trial is that in the Savino et al. (2010) trial, breastfeeding mothers were requested to adopt a diet that was free from cow’s milk protein for the duration of the 21-day study period (Savino et al., 2010; Sung et al., 2014), whereas mothers in the Sung et al. trial did not implement any dietary restrictions. Rather, in the Sung et al. trial, infants diagnosed with cow’s milk protein allergy were excluded, but infants treated with proton pump inhibitors (PPI) were eligible and included in the patient population, as 21 versus 24 colicky infants on PPI therapy were randomized to the probiotic and placebo group, respectively (Sung et al., 2014). Therefore, it is highly probably that these infants were misdiagnosed with infantile colic and rather, were suffering
from gastro-esophageal reflux, which could explain the lack of significant difference between the probiotic- and placebo-treated groups. Importantly, the inclusion of infants receiving concomitant treatment, such as PPIs, or differences in dietary approaches, such as the inclusion of formula-fed infants on hypoallergenic formulas, introduces additional confounding factors, which need to be explored separately in order to determine the true effect of the probiotics being investigated.

Accordingly, due to the complexity of the intestinal microbiota, it is undeniable that studies that examine the role and effect of the intestinal microbiota are difficult to control, as there are a multitude of factors that are involved in determining its composition and activity within its host. Therefore, it is generally accepted that variability of results between similar studies will inevitably exist, despite attempts to control factors that may be the cause for the inconsistencies (Fuller, 2006).

With respect to tolerability and safety of *L. reuteri* DSM 17938, it is encouraging that none of the included RCTs reported any incidents of serious adverse events and no differences were observed in the incidence of any adverse events between treatment and control group.

5.3.2 Possible Mechanisms of Action of *Lactobacillus reuteri* DSM 17938

It is well established that the colonization process of an infant intestinal microbiota proceeds in a systematic manner from birth and during early infancy (Rautava et al., 2012). This process is considerably influenced by various factors, such as mode of delivery, maternal diet, probiotic or antibiotic use during pregnancy and after delivery, type of feeding and environmental factors, which include the hospital setting as well as the geographical location (Adlerberth, 2008; Bezirtzoglou, 1997; Blaut & Clavel, 2007; Fallani et al., 2010; Yatsunenko et al., 2012). Therefore, conceivably, alterations of any of these factors may lead to disruption of the development of a balanced intestinal microbiota composition during infancy, which has become the primary theory of the underlying cause of infantile colic. Consequently, this has led to the idea that supplementation with probiotics to restore the imbalanced microbiota composition may be an effective treatment option to alleviate excessive crying in colicky infants.
Current evidence indicates that different probiotic strains exert their beneficial effects through different mechanisms of action; moreover, some probiotic agents mediate their effects on the host through multiple actions (Wang et al., 2010). Fundamentally, probiotics act by transiently colonizing the gastrointestinal tract to create a favourable balance of beneficial microbes and thereby stabilizing the gut microbiota to benefit the host (Boirivant & Strober, 2007; Marco et al., 2006; Sherman et al., 2009; Wallace, 2009). Although the exact mechanism by which *L. reuteri* exert its effects is still uncertain, a purported mechanism is its direct action on pain perception (Indrio et al., 2008) and improved gut motility and function (Indrio et al., 2008), which may result in a calming effect to ease infant gut discomfort and, in turn, reduce crying and fussing in colicky infants (Savino et al., 2007). Moreover, a recent study by de Weerth et al. (2013) demonstrated that colicky infants have lower microbiota diversity and reduced specific microbiotic signatures, specifically with respect to lactobacilli, which implicates the role of an underdeveloped microbiota in the onset of colic (de Weerth et al., 2013a; de Weerth et al., 2013b; Savino et al., 2007). Furthermore, since colicky infants have increased colonization by coliforms (e.g., *Escherichia coli*) and decreased colonization of lactobacilli (Savino et al., 2004a; Savino et al., 2005a; Savino et al., 2009), recent evidence suggests supplementing with *L. reuteri* can improve colic symptoms by diversifying the microbiota through displacement of colic-associated bacteria (Liu et al., 2014). There is also evidence that excessive crying and/or fussing may be due to increased inflammation, as shown by higher levels of fecal calprotectin, and thus, resulting from increased level of pathogens colonizing the guts of colicky infants, (Liu, Fatheree, Mangalat, & Rhoads, 2010; Rhoads et al., 2009). As it has been shown that *L. reuteri* also exerts an anti-microbial effect against pathogens (Liu et al., 2010), it is likely that administration of *L. reuteri* may indirectly induce an anti-inflammatory response through its anti-microbial action of inhibiting the growth and virulence of intestinal bacterial pathogens (Liu et al., 2010; Wang et al., 2010). Although there is evidence confirming the efficacy of supplementing with *L. reuteri* to reduce colic crying (Chau et al., 2015; Mi et al., 2015; Savino et al., 2007; Savino et al., 2010; Savino et al., 2015; Sung et al., 2014; Szajewska et al., 2013a), the exact mechanisms of action by which *L. reuteri* is able to bring forth this effect still requires further study.
5.3.3 Study Limitations

While the majority of included RCTs are well-designed with low RoB (Chau et al., 2015; Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a), there are some limitations for this meta-analysis that should be considered.

There is evidence of substantial heterogeneity among the studies included in this meta-analysis. As such, due to the possible uncertain etiology of infantile colic, the inter-individual variability in infant intestinal microbiota and the variable effect of *L. reuteri* DSM17938 on the intestinal microbiota of colicky infants of different etiology, a random effects model for the analysis of the average daily crying/fussing times and treatment responders are justified. However, it is important to note that the variation among the RCTs occurs more in the magnitude of effect rather than the direction of effect.

Additionally, since 2 of 5 RCTs lacked a post-treatment follow-up period (Chau et al., 2015; Savino et al., 2010) and 2 of 5 only had a short-term post-treatment follow-up (e.g., only 1-week) (Mi et al., 2015; Szajewska et al., 2013a), it was not possible to assess a post-treatment outcome as part of this meta-analysis. Since infantile colic is a condition that improves spontaneously over time (Barr, 1990; Barr et al., 1992; Brazelton, 1962; Wessel et al., 1954), it is difficult to ascertain whether improvement in colic symptoms is solely due to the administration of *L. reuteri* DSM 17938 or the natural course of the condition. Therefore, with the short study duration of all included RCTs, the inclusion of an appropriate follow-up period post-treatment administration may have helped to definitively determine whether the improvements of colic symptoms are attributable to *L. reuteri* DSM 17938.

Another limitation of this review is that the primary focus was solely to investigate *Lactobacillus reuteri* DSM 17938 for the treatment of colic. Therefore, the results cannot be extrapolated to other probiotic strains, or for treatment of any other pediatric conditions.

Lastly, and not surprisingly, various biases are common in randomized controlled trials and may be a potential source of systematic error (Jüni, Witschi, Bloch, & Egger, 1999). Therefore, a critical appraisal of trial quality and an assessment of the RoB are widely recommended (Higgins & Green, 2011). However, based on the results of the RoB subgroup analysis, there remained a statistically significant reduction in crying/fussing time in the infants administered *L. reuteri* compared infants receiving placebo. This suggests that there may not be a significant association between the methodological quality of a particular trial and the overall effect of *L. reuteri* on reducing crying/fussing times. Additionally, we were unable to
properly assess publication bias from the funnel plot or based on other more advanced tests due to the inadequate number of included trials. In spite of that, it is unlikely that such a significant publication bias occurred that would substantially impact the magnitude and direction of effect of \textit{L. reuteri} DSM 17938 found with this meta-analysis.

5.3.4 Evidence on the Efficacy of \textit{Lactobacillus reuteri} DSM 17938 for the Treatment of Infantile Colic

Overall, with the emergence of RCTs investigating treatment options for infantile colic, including our Probiotics for Infantile Colic trial, probiotics—specifically \textit{L. reuteri} DSM 17938—appears to be the most promising therapeutic option to reducing colic crying and fussing. Consistent with other RCTs from various different geographical regions (Mi et al., 2015; Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a), the findings from the Probiotics for Infantile Colic study support the beneficial effects of administering \textit{L. reuteri} DSM 17938 to treat infantile colic in breastfed, colicky Canadian infants. Infantile colic is a symptom of a self-limiting condition; however, persistent infant crying and irritability has a substantial impact on parents’ well-being and ability to provide adequate care. Despite conflicting results from one RCT (Sung et al., 2014), the promising results observed in our study, along with previous studies (Mi et al., 2015; Savino et al., 2010; Szajewska et al., 2013a), suggest that the use of \textit{L. reuteri} DSM 17938 for the treatment of infantile colic is more effective than placebo and potentially, other currently available options. Of particular importance, the Probiotics for Infantile Colic study provides, for the first time, evidence from North America that supplementation of \textit{L. reuteri} in early infancy is effective in managing colic symptoms. However, it is important for repeat studies in different geographical regions to further strengthen the current evidence for \textit{L. reuteri} DSM 17938.

The data compiled in the meta-analysis presents the current literature and reported important findings on the efficacy of \textit{L. reuteri} DSM 17938 for the treatment infantile colic. The findings reveal that \textit{L. reuteri} DSM 17938 may be efficacious for the treatment of infantile colic demonstrated by the fact that supplementation led to a significant reduction of colic symptoms. Moreover, as observed in all included RCTs (Chau et al., 2015; Mi et al., 2015; Savino et al., 2010; Sung et al., 2014; Szajewska et al., 2013a), a clinically significant reduction in crying/fussing time occurred within 7 days and, with continued treatment, there
was a progressive increase in the difference between the *L. reuteri* DSM 17938 and placebo groups, most notably by day 21. Although this meta-analysis does not evaluate whether *L. reuteri* DSM 17938 may prevent the development of infantile colic, it is reasonable to suggest that *L. reuteri* DSM 17938 may potentially be used prophylactically by administration up to 7 days prior to the usual time for onset of symptoms and it will likely not result in any significant adverse effects. Of note is that the low quality of evidence following the GRADE assessment for daily crying/fussing outcomes across all treatment days and for the number of treatment responders on day 28 suggests that more high quality RCTs with longer post-treatment follow-up periods would be beneficial in confirming the true therapeutic effect of *L. reuteri* and to make definitive recommendations for the use of *L. reuteri* DSM 17938 for the treatment of infantile colic.

Taken together, the results from the Probiotics for Infantile Colic RCT and the overall findings from the available evidence presented in the meta-analysis suggest that administration of *L. reuteri* DSM 17938 may potentially mitigate the severity of colic symptoms by reducing the duration of crying/fussing times sooner than by spontaneous resolution of the condition. Additionally, a significant number of infants treated with *L. reuteri* DSM 17938 responded to treatment throughout the duration of the study compared to colicky infants treated with placebo. Of importance is that the majority of studies reported positive outcomes and good tolerability, as no significant adverse events were observed in any colicky infants included in the RCTs.
6.1 Summary of Evidence: Is there a role of Probiotics as a Therapeutic Agent in Pediatrics?

Interestingly, the onset of various pediatric GI-related conditions, such as infantile colic, certain diarrheal and other GI-related diseases, coincides appropriately with the maturation process of the intestinal microbiota, oftentimes affecting the compositional development and diversity of the microbiota. Processes such as immunological maturation and the colonization of specific microbes, may occur in response to antigen challenges by the present of cow’s milk protein and microbial adherence and colonization (Garofalo & Goldman, 1999; Koleva et al., 2015; Marques et al., 2010; Round & Mazmanian, 2009). Accordingly, the simultaneous occurrence of the development of the microbiota and the onset of various pediatric conditions provide further evidence to the notion that an orderly colonization pattern of the intestinal microbiota may be a contributing factor to the cause or be a consequence of certain conditions, such as infantile colic. However, despite the growing knowledge of the composition of the gut microbiota during infancy, the relationship between the microbiota and host, and consequently between the microbiota and the onset of certain pediatric conditions, such as infantile colic, still largely remains unknown, which may be a reflection of contradictory results observed between similar well-designed trials. This necessitates the need to continue the investigation of the exact role of the intestinal microbiota on the development of various diseases, particularly in the pediatric population, before the use of probiotic interventions can be deemed effective.

In spite of this, the series of results presented in this thesis reinforce the existing evidence that an aberrant or alterations in the intestinal microbiota diversity and composition may potentially play a significant role in the development of various pediatric GI-related conditions, including infantile colic. Although the exact mechanisms of action of probiotics is specific to the nature of the conditions or disease state, it is widely accepted that probiotics generally act through direct and indirect effects on the immune system and enhancement of mucosal barrier function. Although some inconsistencies and limitations of the current evidence exist surrounding the efficacy of probiotics as a treatment option in pediatrics and the
uncertainty of possible mechanisms of action, the overall evidence presented in this thesis is of significant importance in the context of probiotic use in pediatrics globally, as it provides a critical analysis of the role of probiotics for the treatment of pediatric conditions, with an emphasis on infantile colic.

6.2 Future Directions

Firstly, probiotics are considered ‘generally recognized as safe’ (GRAS) and with a good tolerability profile among otherwise healthy children, with the most commonly reported adverse effects being mild bloating and flatulence (Boirivant & Strober, 2007; Gareau et al., 2010; Marco et al., 2006; Sherman et al., 2009). These effects typically subside with continued use of probiotics and therefore, oftentimes are not even reported as an ‘adverse’ effect. However, despite the fact that many probiotic strains are indigenous to the human GIT and although the occurrence is rare (e.g., approximately 0.3 cases/100,000 inhabitants/year during 1995 to 2000 in Finland) (Salminen et al., 2002), the use of certain probiotic strains has been associated with the potential to cause systemic bacteremia and fungemia, particularly among children with serious illnesses (Kunz, Noel, & Fairchok, 2004; Snydman, 2008). For example, *Lactobacillus rhamnosus GG* is the most studied probiotic strain for the treatment of many pediatric conditions; in the particular cases of bacteremia associated with its use, it was reported that these adverse effects might be attributed to the increase in incorrect use of the appropriate probiotics to treat or prevent the specific condition (Salminen et al., 2002). Therefore, it is of great importance to focus future research in identifying the exact mechanisms of action of specific species and strains of probiotics and their actual clinical effects on specific disease states, in order to prevent the misuse of probiotics, which may in rare cases lead to serious adverse events. In particular, due to the strain specificity of probiotics, only clinically proven probiotics should be considered and recommended for use in vulnerable populations, such as children with NEC, Crohn’s disease or ulcerative colitis.

Secondly, it is important to note that a major challenge of probiotic efficacy studies is the lack of a standard definition of what constitutes ‘normal’ intestinal microbiota, particularly in infants, due to the dynamic nature of their microbiota (Adlerberth, 2008; Grönlund et al., 2011; Koleva et al., 2015; Marques et al., 2010; Penders et al., 2006; McFarland, 2014; Sanders, 2011; Savino et al., 2004b). Therefore, there is difficulty proving that a causal
relationship exists between the use of probiotics leading to colonization of the bacteria in the intestinal microbiota and subsequent improvement of symptoms of the condition. More studies are needed to fully understand what constitutes the baseline state for a healthy infant microbiota in order to definitively conclude the efficacy of probiotics in specific pediatric conditions.

In the context of infantile colic research, an important area for future work is modernizing and improving the current and arbitrary definition of infantile colic, as several definitions currently exist, some of which conflict with others. There is no consensus regarding the definition of excessive crying, as the currently available definitions mostly concerns the duration of infant crying or its effect on the parents or caregivers (Reijneveld, Brugman, & Hirasing, 2001). The commonly used and most often-cited definition of colic based on duration of crying is that coined by Wessel et al. (1954), which describes a colicky infant as “one who, otherwise healthy and well-fed, had paroxysm of irritability, fussing or crying lasting for a total of more than three hours a day and occurring on more than three days in any one week” (Wessel et al., 1954). Although this definition is the one most commonly used in clinical trials (Chau et al., 2015; Mi et al., 2015; Savino et al., 2007; Savino et al., 2010; Savino et al., 2015; Sung et al., 2014; Szajewska et al., 2013a), it is regularly modified, which, as might be expected, can lead to the inclusion of heterogeneous populations of crying infants classified as ‘colicky’ (Barr et al., 1992; Reijneveld et al., 2001). Ultimately, it may also result in different study findings such as in the case of the trials investigating L. reuteri for the treatment of infantile colic. As such, for an accurate interpretation of the research findings regarding the incidence, etiology and more importantly, effective treatments for infantile colic, a set of standard criteria must be established in order to define to what extent the variation in colic definition would include the same population of infants. To date, there exists only one known study that assessed the impact of various definitions of excessive crying on the estimate of prevalence of colic and to what extent the various definitions comprised the same group in crying infants (Reijneveld et al., 2001).

In addition to a lack of a standard definition of infantile colic, there is a lack of an objective method for recording crying/fussing times. The various methods used to report the duration of crying and fussing episodes include infant cry diaries, parental recall interviews, questionnaires, and follow-up well-baby visits. All of these methods call to question the accuracy and precision of self-reported data. Despite the fact that there is a validated data
acquisition method for crying times called The Baby Day Diary (Barr et al., 1988), the data recorded remains subjective in nature and, like the definition of colic, is oftentimes used with modifications. Inconsolable crying is understandably exhausting and engenders feeling of frustration in parents of colicky infants (Akman et al., 2006; Barr, 1998; Stifter & Bono, 1998); therefore, although the crying/fussing time data is typically collected prospectively, it is not inconceivable that recall bias may still exist, which can result in unintentional omission of crying/fussing episodes or recalling more frequent or longer duration of crying/fussing episodes than actually occurred, a phenomenon called telescoping (Bruijnzeels, van der Wouden, Foets, Prins, & van den Heuvel, 1998). Moreover, the fatigue experienced by parents of colicky infants may reduce the willingness to accurately recall the colic episode and complete the diary in the same detail over periods of days to weeks (Bruijnzeels et al., 1998). Therefore, to accurately determine the duration of colicky infant crying, which may lead to the development of a systematic diagnosis definition of colic, there must also be a focus on devising an objective measure of cry times before more studies are conducted to determine potential management or treatment options.

Finally, an interesting area for future studies investigating possible interventions for the treatment of colic is establishing potential intestinal markers to objectively measure the composition of the intestinal microbiota before and after the colic period, which may include from birth up to later childhood and even into adulthood. This area of study would bring forth more insight into the etiology of condition(s) resulting in colic crying colic, as well as, possible long-term consequences of colic, which are important areas of focus since the cause and long-term effects still remain unknown despite decades of research. As previously discussed, infantile colic can manifest as early as 2 weeks of age, peaking at 6 weeks of age, at which point, may spontaneously resolve at 3 months of age (Brazelton, 1962). However, there are colicky infants whose excessive crying extend beyond 3 months and continue up to 5 to 6 months of age and it is possible that this group of colicky infant might be of distinct etiology (St. James-Roberts, 2012). Accordingly, since the development of colic coincides chronologically with the development of the intestinal microbiota composition, it is possible that there exists a relationship between colic and the intestinal microbiota. Thus, it would be of great interest to explore in greater detail the causal relationship between the impact of colic on the colonization pattern of the intestinal microbiota or whether it is the variation in the composition of the gut microbiota that results in the onset of colic. Currently, most studies
extrapolate the composition of the intestinal microbiota from fecal samples; although it is the least invasive method, fecal samples may not accurately reflect specific features of the intestinal microbiota in the upper GIT (Mentula, Tuure, Koskenala, Korpela, & Kononen, 2008). Therefore, identifying a possible objective method to evaluate the intestinal microbiota would allow further exploration into factors (e.g., maternal microbiotic health, diet, and maternal probiotic and antibiotic use in breastfed infants) that may influence the colonization process of the intestinal microbiota in colicky infants, which have not been widely studied. Taken together, research in this area may help elucidate the exact etiology of colic and with a clearer understanding of the cause, a better idea of possible treatment options and the mechanisms of action of therapeutic agents, such as probiotics, at reducing colic crying may be clarified.


Supplementation of infant formula with probiotics and/or prebiotics: a systematic review and comment by the ESPGHAN committee on nutrition. *J Pediatr Gastroenterol Nutr*, 52(2), 238-250.


reviews. *Hum Reprod Update, 11*(2), 103-104.


Medicina Biologica.
Lin, P. W., Nasr, T. R., Berardinelli, A. J., Kumar, A., & Neish, A. S. (2008b). The probiotic Lactobacillus GG may augment intestinal host defense by regulating apoptosis and


Persaud, N. N., Azad, M. B., Chari, R. S., Sears, M. R., Becker, A. B., & Kozyrskyj, A. L.


Saarela, M., Matto, J., & Mattila-Sandholm, T. (2002). Safety aspects of Lactobacillus and Bifidobacterium species originating from human oro-gastrointestinal tract or from


Savino, F., Pelle, E., Palumeri, E., Oggero, R., & Miniero, R. (2007). Lactobacillus reuteri (American Type Culture Collection Strain 55730) versus simethicone in the treatment of


Lactobacillus reuteri ingestion and IK(Ca) channel blockade have similar effects on rat colon motility and myenteric neurones. Neurogastroenterol Motil, 22(1), 98-107, e33.
## Appendix A: AMSTAR Checklist

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<th>Question</th>
<th>Yes</th>
<th>No</th>
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<td>1. Was an 'a priori' design provided?</td>
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<td>The research question and inclusion criteria should be established before the conduct of the review.</td>
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<td>2. Was there duplicate study selection and data extraction?</td>
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<td>There should be at least two independent data extractors and a consensus procedure for disagreements should be in place.</td>
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<td>3. Was a comprehensive literature search performed?</td>
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<td>At least two electronic sources should be searched. The report must include years and databases used (e.g., Central, EMBASE, and MEDLINE). Key words and/or MESH terms must be stated and where feasible the search strategy should be provided. All searches should be supplemented by consulting current contents, reviews, textbooks, specialized registers, or experts in the particular field of study, and by reviewing the references in the studies found.</td>
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<td>The authors should state that they searched for reports regardless of their publication type. The authors should state whether or not they excluded any reports (from the systematic review), based on their publication status, language etc.</td>
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<td>In an aggregated form such as a table, data from the original studies should be provided on the participants, interventions and outcomes. The ranges of characteristics in all the studies analyzed e.g., age, race, sex, relevant socioeconomic data, disease status, duration, severity, or other diseases should be reported.</td>
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<td>'A priori' methods of assessment should be provided (e.g., for effectiveness studies if the author(s) chose to include only randomized, double-blind, placebo controlled studies, or allocation concealment as inclusion criteria); for other types of studies alternative items will be relevant.</td>
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<td>For the pooled results, a test should be done to ensure the studies were combinable, to assess their homogeneity (i.e. Chi-squared test for homogeneity, P). If heterogeneity exists a random effects model should be used and/or the clinical appropriateness of combining should be taken into consideration (i.e. is it sensible to combine?).</td>
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<td>10. Was the likelihood of publication bias assessed?</td>
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<td>An assessment of publication bias should include a combination of graphical aids (e.g., funnel plot, other available tests) and/or statistical tests (e.g., Egger regression test).</td>
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<td>11. Was the conflict of interest stated?</td>
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<td>Potential sources of support should be clearly acknowledged in both the systematic review and the included studies.</td>
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Appendix B: Probiotic for Infantile Colic
Informed Consent Form

SUBJECT INFORMATION AND CONSENT FORM

Title: *Lactobacillus reuteri* DSM 17938 versus placebo in the treatment of infantile colic: A randomized double-blind controlled trial

Principal Investigator: Dr. Gideon Koren
Co-Investigators: Dr. Saul Greenberg, Dr. Sheila Jacobson, Dr. Eddy Lau
Study Research Associate: Dr. Parvaneh Yazdani-Brojeni
Research Study Coordinator & PhD Candidate: Kim Chau
Research Site Telephone: 416-813-7283 or 1-800-436-8477

Please read this document carefully before you sign it!

Your baby’s participation is voluntary.
Your baby should take part in the study only if you want your baby to do so.
You will be asked to sign this consent form.
You may refuse to allow your baby to take part or can withdraw your baby from this study at any time without penalty or loss of benefits to which your baby is otherwise entitled.
Your baby’s treatment will not be affected should you decide not to take part in this study.
The purpose of this form is to give you information about the research study and, if signed, to show your decision to allow your baby to take part in the study. This form describes the purpose, procedures, benefits, risks, discomforts and precautions of the research study.
The following information will describe the study and your role in it.
Please read this Subject Information and Consent Form and ask as many questions as needed. This Consent Form may contain words you do not understand. If you do not understand some terms and words, please ask the study team to explain any part that is unclear.
A copy of this form will be given to you to review at your leisure; please feel free to ask for advice from others before signing.
Background

Infantile colic is a condition, when otherwise healthy baby, has prolonged, unsoothable crying without obvious cause. It is one of the most common problems within the first 3 months of life, affecting up to 28% of infants. This condition causes distress for both parents and paediatricians and despite 40 years of research, the reason for infantile colic is still unclear. Some researchers relate this condition to infants’ difficult temperament, difficulties in mother-baby bonding, abnormal gut function, allergy problems, smoking at home. Because of these multiple independent causes and generally healthy condition of baby, there are different approaches to treat infantile colic.

Recent studies have been shown that changes of gut bacteria (or intestinal microflora) of newborns, during the first months of life, may play an important role in colicky infants. *Lactobacillus reuteri* is one of the few natural lactobacterial species in the human gastrointestinal tract. *Lactobacillus reuteri* DSM 17938 is a bacterium that is a member of the broad class of lactic acid bacteria. *Lactobacillus* species are found in the gastrointestinal tract of healthy humans of all ages, where they are among the “normal” bacteria. *Lactobacillus* is a non-pathogenic lactic acid bacterium, which means that they cannot cause disease in the human gut. It has been used safely for many years as a probiotic dietary supplement in adults, and recent data demonstrated safety after long-term dietary supplementation for newborn infants. *Lactobacillus reuteri* DSM 17938 is also known as *L. reuteri* Protectis, which is very closely related to the particular family of probiotics used in foods worldwide. Recent studies have shown that *Lactobacillus reuteri* DSM 17938 is well tolerated and improves the symptoms of infantile colic without any bad side effects related to the probiotic. Therefore, the probiotic, BioGaia, containing natural bacteria *Lactobacillus reuteri* DSM 17938, was suggested in this study to improve infantile colic.

Purpose of the Study

Your baby is being invited to participate in this study because he/she has been diagnosed with infantile colic, which is characterized by excessive crying or is gassy-fussy or experiences symptoms of acid reflux (also know as gastroesophageal reflux). We do not know whether a probiotic *Lactobacillus reuteri* DSM 17938 is effective in improving the symptoms of infantile colic or not. Therefore, our study will try to find differences in the condition of colicky infant treated with a probiotic *Lactobacillus reuteri* DSM 17938 or with placebo (no treatment).

Overall, we expect this study to take a little over a year to complete; however, your participation in the study will only be 3 weeks.

Description of the Research

Study Product

In our study, we are going to use a probiotic *Lactobacillus reuteri* (DSM 17938) in a commercially available form supplied by BioGaia (BioGaia AB, Stockholm, Sweden). BioGaia Probiotic drops have been approved by Health Canada as a Natural Health Product; however, it has not yet been approved for infants in this age group and is considered an investigational Natural Health Product in this study. This oil suspension is stable for 21 months at 2⁰C to 8⁰C and should be kept in refrigerator when it is not in use.

Study Product Administration

Consent form
Version date: April 17th, 2014
Page 2 of 7
If you agree to be in the study, you are asked to give your baby 5 drops of the probiotic by mouth, once a day (preferably at the same time), 30 minutes after breastfeeding for 3 weeks (21 days) of the study.

For the control group, we will use a placebo (drops without probiotic _L. reuteri DSM 17938_) in a liquid that looks the same as the probiotic BioGaia, and we ask that it is given to your baby in the same way, 5 drops by mouth once a day, 30 minutes after breastfeeding for 3 weeks (21 days) of the study.

If your baby spit out/vomit a study product within 15 minutes after receiving it, you will be instructed to repeat a dose (5 drops). Only one attempt to repeat a dose can be made daily (i.e. maximum of 10 drops per day). You will be asked to record this event in a diary.

You will receive BioGaia or the placebo free of charge.

No invasive procedures will be required during the study.

All participants will be randomized (like flipping a coin) to one of the two groups: treatment group (receiving the probiotic BioGaia) and control group (receiving placebo, drops without probiotic _L. reuteri DSM 17938_). We expect that a total of 100 babies will be involved in our study at several paediatricians’ offices in Toronto. Your baby has an equal chance of being in the treatment group or placebo group.

Our study is a double blind randomised control study; that means that neither you nor your paediatrician or the study team members will know what product, probiotic or placebo, your baby will receive during the study.

During the 21-day study, we ask that you do not introduce any new foods or food supplements to your baby until your baby has completed the study because it may interfere with the effect of the study product.

**Study Procedures**

If you agree to allow your baby participate in the study, you will receive a maternal diary to fill out, which will help us to assess your baby’s colic signs. **You will be asked to record in a diary the daily crying time, the number of colic episodes, their duration, stool consistency and frequency, and any observed adverse events (e.g., constipation or vomiting) starting from the day of recruitment.**

Your baby will undergo a medical examination by a pediatrician, and background information about type of delivery, birth weight, gestational age, and family history of gastrointestinal problems, allergies and if you have any dietary restrictions will be collected.

**You will be contacted by telephone by a study team member on days 7, 14 and 21 for follow up. The study team member will be asking you questions about your baby’s progress on the study and if there has been any change in your baby’s colic symptoms or if your baby has experienced any adverse side effects. These calls are part of the study only and are not part of routine care for your baby. Each follow up call should last approximately 15-20 minutes.**

One of the researchers will be available by phone to answer your questions and to help you to maintain correct documentation and confirm that the study products are administered correctly. Please inform the study team when any new medication is taken by your baby during this clinical trial. This includes prescription and non-prescription medications, natural health products, probiotics, prebiotics etc. If your baby needs any
antibiotic therapy or other probiotic treatment during the study they will be removed from the trial.

Possible Hazards, Risks and Discomforts of Participating in the Study

Safety studies have been conducted on healthy newborns (including premature babies), small children, adults, elderly and immuno-deficient adults (HIV positive). In all studies, *L. reuteri* DSM 17938 has been free from side effects, even when administered in doses exceeding the levels normally contained in BioGaia Probiotic products. A few individuals have experienced temporary mild gas passing. There are no known side effects related to the study placebo.

Caution and Contraindications of BioGaia Probiotic Product

Discontinue use and consult a health care practitioner if symptoms of digestive upset (e.g. diarrhea) occur, worsen, or persist beyond 3 days. Do not use if your baby experiences nausea, fever, bloody diarrhea or severe abdominal pain. Do not use if your baby has an immune compromised condition (e.g. AIDS, lymphoma, patients undergoing long-term corticosteroid treatment).

We know of no harm that taking part in this study could cause your baby, but there may be harms that we do not know about. We do not expect any serious adverse effects with this product. We will monitor all changes in the health of your baby during the study period.

Potential Benefits

To Participant

Infantile colic is an unpleasant condition for a baby and a stressful situation for parents. Therefore, while participating in our study, your baby and you may benefit from the probiotic supplementation with BioGaia product, and it may result in some improvement in your baby’s colic condition. Your baby, irrespective of treatment with probiotic or placebo, will be initially examined by a certified paediatrician and followed up 3 times throughout the study by a study team member to make sure your baby is healthy and is not experiencing any negative side effects.

You will be advised how to manage a crying baby.

At the end of the study, a copy of the final published results will be mailed to you.

To Society

The results of this study may help us develop a new approach to the treating of infantile colic by simple and safe way using probiotic BioGaia, *Lactobacillus reuteri* DSM 17938.

Alternatives to participation

If you decide that your baby should not take part in this study, it will not affect the medical care that he or she will receive.
Confidentiality

We will respect you and your baby’s privacy. No information about who your baby is will be given to anyone or be published without your permission, unless the law requires us to do so. For example, the law requires us to give information about your baby if a baby has been abused, if your baby has an illness that could spread to others, if someone talks about suicide (killing themselves), or if the court orders us to give them the study papers.

Sick Kids Clinical Research Monitors or the regulator of the study may see your baby’s health record to check on the study. For example, people from Health Canada Health Products and Food Branch, if necessary, may look at your records. By signing this consent form, you agree to let these people look at your baby’s records. The data produced from this study will be stored in a secure, locked location. Only members of the research team (and those individuals described above) will have access to the data. Following completion of the research study, the data will be kept confidential and stored for 25 years, in accordance with Section 76 of the Natural Health Products Regulations and then destroyed as required by the Hospital for Sick Children policy. Published study results will not reveal your baby’s identity.

Once this Informed Consent Form is signed by you, we will put a copy of this consent form in your baby’s research file. We will give you a copy for you to refer to, as needed, throughout the study.

Reimbursement

No reimbursement will be provided for participation in this study.

Participation

It is your choice to allow your baby to take part in this study. Your baby’s participation is voluntary. You can stop your baby’s participation in this study at any time. The care your baby receives will not be affected in any way whether your baby takes part or not in this study. If you decide to withdraw your baby from the study, you should call the study doctor immediately. Your baby’s treatment and the attitude of your doctor towards you and your baby will not be affected should you decide not to allow your baby to take part in this study. Refusal to participate will not affect any benefits to which your baby is otherwise entitled.

If you decide to allow your baby to participate, you will need to sign this consent. Your baby’s participation in the trial will be 21 days.

If we get new information while conducting this study that may affect your decision to allow your baby to take part in this study, we will tell you about the new information as it becomes available and we will ask you again if you still want your baby to be in the study. In addition, your baby’s participation may be stopped, by the study doctor or your healthcare provider, without your consent, even if you and your baby is benefiting from the study.

Although we may make money from these findings, we cannot give you (or your baby) any of this money now or in the future because you (or your baby) took part in this study.

In the event that your baby experiences any adverse reaction (whether or not you consider it to be related to the study drug) during the course of this study, you should immediately contact your study doctor (for name and telephone number see “Contacts” below).

If your baby becomes ill or is harmed during study participation, the treatment will be covered by OHIP. For the duration of the study, you must notify the study doctor and/or study

Consent form

Version date: April 17th, 2014

Page 5 of 7
team member of any other medical treatments that may be necessary for your baby to undergo. Signing this consent form does not interfere with you or your baby’s legal rights in any way.

**Sponsorship:**
The study is sponsored by Dr. Gideon Koren and the Hospital for Sick Children. The study product and placebo will be provided by BioGaia Company (Ferring Canada, Inc.) for free.

**Contacts**
You will be given a copy of this informed consent form for your records. If you have any questions or require any additional information, you can contact one of the study doctors or study team member.

**Contact phone:** 416-813-7283.
**Research Site Telephone:** 1-800-436-8477.
If you have questions about your rights as a subject in a study or for information on whom to contact in the event of injuries during a study, please call the **Research Ethics Manager** at 416-813-5718. A Research Ethic Board (REB) is a group of scientific and non-scientific individuals, who perform the initial and ongoing ethical review of the research study with the study subject's safety and welfare in mind.

**Conflict of Interest**
The Principal Investigator, Dr. Gideon Koren, and the other research team members have no conflicts of interest to declare.
Parent’s Statement of Consent
I understand that my baby has met the criteria for the study, and I would like to take part in this research study.
I understand that my baby’s participation in this research study is voluntary.
I may decide not to have my baby take part or to withdraw from the study at any time without penalty or loss of benefits or treatment to which my baby is entitled.
I also understand that the study may be stopped, without my consent, by the study doctor conducting the study.

By signing this form, I agree that:
1. The study has been explained to me. All my questions were answered to my satisfaction.
2. The possible benefits and harms (if any) of this study have been explained to me and I understand them.
3. I understand that I have the right to refuse to let my baby take part in this study and the right to take my baby out of the study at any time.
4. The decision about my baby taking part in this study will not affect my baby’s health care.
5. I am free now, and in the future, to ask any questions about the study.
6. I have been told that my baby’s medical records will be kept confidential, except as described to me.
7. I understand that no information that would identify my baby and/or I will be released without asking me first.
8. I have read and understood pages 1 to 7 of this consent form. I agree, or consent, that my baby may take part in this study.
9. I understand that I do not give up my legal rights by signing this form.
10. I understand that I should sign both copies of the consent form provided. I understand that I may keep one copy of the consent form.
11. I voluntarily agree to take part in this research study.
12. I have voluntarily signed this Informed Consent Form prior to any study related procedures being performed.

Print Name of Mother of Participant

Date

Time

Mother of Participant’s Signature

Print Name of Investigator/Designee

Date

Time

Investigator/Designee’s Signature

Consent form
Version date: April 17th, 2014
Page 7 of 7
### MATERNAL MEDICAL HISTORY

[ ] Check (✓) if no significant Medical History

<table>
<thead>
<tr>
<th>Diagnosis / Symptoms</th>
<th>*Medication at Start of Study to Treat Condition? Yes No</th>
<th>Start Date Resolution Date</th>
<th>Response</th>
</tr>
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<tbody>
<tr>
<td>1. HEART</td>
<td>☐ Yes ☐ No</td>
<td></td>
<td></td>
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<tr>
<td>2. HYPERTENSION</td>
<td>☐ Yes ☐ No</td>
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<tr>
<td>3. LUNGS/ASTHMA</td>
<td>☐ Yes ☐ No</td>
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<tr>
<td>4. GI DISORDERS</td>
<td>☐ Yes ☐ No</td>
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<tr>
<td>5. DIABETES</td>
<td>☐ Yes ☐ No</td>
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<td>6. ALLERGIES</td>
<td>☐ Yes ☐ No</td>
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<td>7. KIDNEY</td>
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<td>8. PSYCHIATRIC</td>
<td>☐ Yes ☐ No</td>
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Signature_________________________  Date_________________________
INFANT DATA
Date of Birth: MMM YYY
Sex: ☐ Male ☐ Female
Birth Weight: _________(lb/ounces) _________(kg/g)
Apgar Score: _________ (1 min) _________ (5 min)
Length of stay in Hospital: _________(days), reason: ________________________________
Complication after delivery: ☐ NO ☐ YES, specify:__________________________
Breastfeeding started on: DD MMM YYY
Exclusively Breastfed: ☐ NO ☐ YES
Problems with Breastfeeding: ☐ NO ☐ YES

INFANT COLIC HISTORY
Meet criteria for Infantile Colic or related symptoms: ☐ NO ☐ YES
☐ Excessive crying ≥ 3 days/week ☐ Gassy-fussy symptoms
Weight at time of enrollment: _________, _________(kg) _________(lbs)
Length: _________, _________(cm) _________(in)
Is the infant teething? ☐ NO ☐ YES
Comments: ___________________________________________________________

PARENTAL QUALITY OF LIFE
Please check the number that best corresponds to your well-being and stress level:

0 (Perfect Well-Being/Not Stressed) 1 2 3 4 5 6 7 8 9 10 (Worst/Very Stressed)

Signature_________________________________________ Date_________________________
**Inclusion Criteria** (Check [✓] Yes or No to confirm eligibility)

1. Healthy term infant 21-180 days (3wks to 6 mths) of age
   - Yes [ ] No [ ] N/A
2. Birth weight > 2500 g
   - Yes [ ] No [ ] N/A
3. Exclusively breastfed
   - Yes [ ] No [ ] N/A
4. Colic symptoms (excessive crying for >3d/wk, gassy-fussy)
   - Yes [ ] No [ ] N/A
5. Gestational age between 37 to 42 weeks
   - Yes [ ] No [ ] N/A
6. Apgar score higher than 7 at 5 minutes
   - Yes [ ] No [ ] N/A

*If any Inclusion Criteria response is NO, this subject must NOT enter this study.*

**Exclusion Criteria** (Check [✓] Yes or No to confirm eligibility)

1. Any chronic illnesses or gastrointestinal disorders
   - Yes [ ] No [ ]
2. Any history of antibiotic treatment or probiotic supplementation
   - Yes [ ] No [ ]
3. Infants with acute illnesses
   - Yes [ ] No [ ]
4. Allergies to any ingredients in the study product or placebo
   - Yes [ ] No [ ]
5. Participation in other clinical trials
   - Yes [ ] No [ ]

**Eligibility Criteria:** Has the subject met all Inclusion / Exclusion Criteria?

- Yes [ ] No [ ]

**Randomization**

Has the subject been randomized? [ ] No [ ] Yes; If Yes, Date: __________

Copy of consent form given to parents/guardian? [ ] Yes [ ] No

Drops dispensed to parents/guardian? [ ] Yes [ ] No

Patient Information Leaflet given to parents? [ ] Yes [ ] No

Maternal Diary provided? [ ] Yes [ ] No

Signature________________________________________ Date________________________

Page 1 of 4
Version Date: April 17th, 2014
21-Day Maternal Diary

Probiotic for Infantile Colic Study:  
A Randomized, Double-Blind, Placebo-Controlled Trial Investigating *Lactobacillus reuteri* DSM 17938

Subject ID:  
Randomization Date:  

INSTRUCTIONS  
- Please complete all fields  
- Fill in the diary every day using a blue or black pen  
- Tick (✓) inside the proper box  
- Record the number of drops administered  
- See "Sample - How to complete diary" for instructions

Please give five (5) drops of the study product 30 minutes *after* breastfeeding ONCE per day. Please select a time of day that is most convenient for you; we request that the drops are given at approximately the same time each day.

****Remember to bring your diary and any unused/empty study product to your last visit.****
# Daily Maternal Diary

**Probiotics for infantile Colic Study**

**Subject ID:**

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<tr>
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<th>Date:</th>
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### Daily Crying and Fussy/Karzy Time

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<th>Start Time (HH:MM)</th>
<th>Stop Time (HH:MM)</th>
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<tbody>
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### BREAST FEEDING TIME (HH:MM)

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<th>Hard</th>
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### Bowel Movements

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<th>Consistency</th>
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### Study Product Administered?

- **YES (complete during time and number of doses)**
- **NO - provide comment**

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<th>Dosing Times</th>
<th>Number of doses</th>
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**COMMENTS:**

Protocol Number: 1000018594
Version Date: April 24, 2014
Appendix E: Follow Up Visit Intake Form

SickKids
THE HOSPITAL FOR SICK CHILDREN

Lactobacillus reuteri DSM 17938 versus placebo in the treatment of infantile colic: A randomized double-blind controlled trial

Site/Subject Number

Follow Up Visit: □ Day 7 □ Day 14 □ Day 21

Visit Date: ____________

DD MMM YYYY

Maternal Medical History
Exclusively Breastfeeding? □ NO □ YES
Problems with Breastfeeding? □ NO □ YES

Parental Quality of Life
Please check the number that best corresponds to your well-being and stress level:
0 (Perfect Well-Being/Not Stressed) 1 2 3 4 5 6 7 8 9 10 (Worst/Very Stressed)

Comments:

Infant Data
Infant Age: ____________ (months/weeks) Birth Weight: ______ (lb/ounces) ______ (kg/g)

Weight gained from previous visit: ____________ (g/lb/ounce)

Colic symptoms (compare symptoms from previous week):
□ No change □ Mild improvement □ Significant improvement □ Worsened

Have you used other products/methods other than the study drops to console your baby during an episode?
□ NO □ YES, specify:
□ Maternal cow’s milk restriction □ Herbal tea □ Gripe Water □ Increased carrying
□ Decrease infant stimulation □ Other: __________________________

Adverse Events: □ NO □ YES, specify: __________________________

Is the infant teething? □ NO □ YES

Comments: __________________________

Signature: __________________________ Date: __________________________
Appendix F: The Principle of Equipoise in Pediatric Drug Trials

Contents of this Appendix have been published in Pediatric Drugs:


[KC collected the data and prepared the manuscript for submission].
While this principle is straightforward and sounds to be the logical approach to guide clinical research, it has not yet been officially endorsed by all regulatory authorities; for example, The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is mute about equipoise [2], whereas the Canadian Tri-Council Policy endorses it [3].

Maybe the most cited exchange regarding the definitions and limits of equipoise emerged in the context of drug therapy for the human immunodeficiency virus (HIV), where long after randomized placebo-controlled trials (RCTs) proved the superiority of azidothymidine (AZT) over placebo, American-based studies conducted in Africa continued to randomize patients to placebo with full knowledge that AZT is superior. A dramatic example was the continuation of administration of placebo to pregnant women, when it was already proven that AZT successfully reduces fetal-neonatal exposure to the virus by 70%. The researchers claimed that AZT was not available in Africa at that time, and therefore, giving placebo did not practically change the natural course of these pregnancies. Furthermore they pointed out that they preferred to perform a study relevant for Uganda, rather than conducting an “irrelevant” study for the African context. For example, according to them the Ugandan health care system could not have the necessary level of prenatal care required for an American pregnancy AZT regimen, including intravenous infusions and expenses. Lastly, they reasoned that Ugandan scientists were active team members who agreed with all aspects of the study. These views, however, were strongly contested and rejected by many members of the medical community. As an example, Dr. Marcia Angell, the executive editor of the New England Journal of Medicine at that time responded to these arguments, saying [4]:

“The drug AZT is known to cut dramatically the transmission of AIDS from pregnant women to newborns. In choosing to deny any form of this treatment to some of the women in their trials, the researchers knowingly conspire many newborns to HIV.”

The Helsinki declaration states, “in any medical study, every patient, including those of a control group, if any—should be assured of the best proven diagnostic and therapeutic method.” It also states that “in research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.”

Similar violations of the equipoise principle occurred in other therapeutic HIV trials in Africa [5], leading to the emergence of the view that one cannot use different standards in different regions: if a drug was shown to be superior to placebo or to standard therapy in North America, for example, one cannot expose vulnerable and unprivileged persons to placebo using the argument that they would not be able to obtain the new medication anyway. As discussed below, there are other situations where adherence to the principle of equipoise is more difficult.

2 How is Equipoise Decided?

According to van der Graaf and van Delden [6], “in a randomized controlled trial, a control group shall not be denied a superior medically established procedure that has net clinical relevance for a specific condition that is under study for the population that the control group represents.” The term “medically established procedure” sets a standard of evidence for the treatment of the control group. However, this definition does not deal with the acceptable level of evidence. London [7] tried to address the issue of generalizability of “standard of care” when doing an RCT by stating, “If we assume that some intervention (I) has been shown to be effective in treating patients with some condition (C) in one treatment setting (S), then in order to infer that no doubts exist about the benefits of I for treating patients with C in some other treatment setting (S*), we must be confident that S and S* are sufficiently similar that causal relationships that exist in the former will obtain in the latter.” Can a drug or vaccine proven effective in North America (S) be assumed to be equally efficacious in Africa (S*)?

In reality, the understanding that equipoise is a fundamental prerequisite of clinical trials has led to increasing scholarly discussions among specialists in different groups, arguing whether certain modalities are still at an equipoise state [8]. It is conceivable that the most effective way to decide whether equipoise exists at a certain time and place should be based on systematic review of the literature comparing the two (or more) arms of the study, e.g., new agent versus placebo or versus standard therapy. Often times, there are practice guidelines by professional societies, which aim to define the state of the art in comparing drug A to drug B (or placebo).

3 Pediatric Aspects

Focusing on pediatric trials, there have been only a few attempts to engage practitioners in defining the state of the art and the existing equipoise. For example, Hirshberg et al. [9] surveyed North American pediatric intensivists to find out their willingness to conduct a pediatric trial of blood glucose control and to determine whether self-reported practices were influenced by adult-specific data. The authors conducted a survey of North American pediatric intensive care units (PICUs) in 96 institutions.
While this principle is straightforward and sounds to be the logical approach to guide clinical research, it has not yet been officially endorse by all regulatory authorities; for example, The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is mute about equipoise [2], whereas the Canadian Tri-Council Policy endorses it [3].

Maybe the most cited exchange regarding the definitions and limits of equipoise emerged in the context of drug therapy for the human immunodeficiency virus (HIV), where long after randomized placebo-controlled trials (RCTs) proved the superiority of azidothymidine (AZT) over placebo, American-based studies conducted in Africa continued to randomize patients to placebo with full knowledge that AZT is superior. A dramatic example was the continuation of administration of placebo to pregnant women, when it was already proven that AZT successfully reduces fetal-neonatal exposure to the virus by 70%. The researchers claimed that AZT was not available in Africa at that time, and therefore, giving placebo did not practically change the natural course of these pregnancies. Furthermore they pointed out that they preferred to perform a study relevant for Uganda, rather than conducting an “irrelevant” study for the African context. For example, according to them the Ugandan health care system could not have the necessary level of prenatal care required for an American pregnancy AZT regimen, including intravenous infusions and expenses. Lastly, they reasoned that Uganda scientists were active team members who agreed with all aspects of the study. These views, however, were strongly contested and rejected by many members of the medical community. As an example, Dr. Marcia Angell, the executive editor of the New England Journal of Medicine at that time responded to these arguments, saying [4]:

“The drug AZT is known to cut dramatically the transmission of HIV from pregnant women to newborns. In choosing to deny any form of this treatment to some of the women in their trials, the researchers knowingly consign many newborns to HIV.”

The Helsinki declaration states, “in any medical study, every patient, including those of a control group, if any—should be assured of the best proven diagnostic and therapeutic method.” It also states that “in research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.”

Similar violations of the equipoise principle occurred in other therapeutic HIV trials in Africa [5], leading to the emergence of the view that one cannot use different standards in different regions: if a drug was shown to be superior to placebo or to standard therapy in North America, for example, one cannot expose vulnerable and unprivileged persons to placebo using the argument that they would not be able to obtain the new medication anyway. As discussed below, there are other situations where adherence to the principle of equipoise is more difficult.

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△ Alisa
covering 37 states/provinces. While the median definitions of hyperglycemia (150 mg/dL) and hypoglycemia (≤60 mg/dL) looked reasonable, practice patterns were variable. Although the majority of respondents denied a change in clinical practice based on the published literature, the preferred blood glucose target range increased from 80–110 mg/dL in 2005 to 90–140 mg/dL in 2009. The proportion of intensivists who preferred a blood glucose target of 80–110 mg/dL decreased from 43 to 6 % (p < 0.001). Almost half of respondents indicated that the acceptable severe hypoglycemia rate for a protocol should not exceed 2.5 %. The majority (93 %) indicated they would be willing to enroll patients in a pediatric trial of blood glucose control.

This study highlights the difficulties in achieving a genuine equipoise against which new therapies should be tested. The participants reported that they control blood glucose with insulin in critically ill children and do not necessarily adopt adult-specific data or a single uniform blood glucose target. These responses have led the authors to conclude that persistent variation in practice begs a multicenter clinical trial of blood glucose control in critically ill children. This paper may serve as an example of how the discussion about equipoise (or lack of) should be conducted in the pediatric context.

In a similar manner, Harrison et al. [10] reviewed published studies of analgesic effects of sweet solutions in neonates to ascertain areas with sufficient evidence of effectiveness and areas of uncertainty, ultimately, to define the current state of equipoise. After analyzing 298 publications, the authors concluded that a state of clinical equipoise regarding analgesic effects of small volumes of sweet-tasting solutions no longer exists. Therefore, there is no justification for conducting additional RCTs with placebo or no-treatment groups for infants in medically stable condition. Another valuable outcome of such data synthesis procedures is identifying where uncertainty still exists and thus where future clinical research is still warranted. In this context, they identified uncertainties related to outcomes after prolonged use of sweet solutions, concomitant use of other analgesics, and effectiveness beyond the newborn period. In this case, the lack of clear equipoise makes it very challenging to define ethical boundaries of interventions.

In another example, Vohra and colleagues [11, 12] investigated whether or not equipoise existed with respect to wrapping premature newborns immediately after delivery to reduce hypothermia. The authors point out that, based on their systematic review, they were reasonably confident that wrapping reduces heat loss in this population [12]. In contrast, equipoise exists with regard to the long-term outcome of wrapping premature newborns, and they believe that this practice should not be included as part of the standard of care until more evidence is presented [11].

It is critical to create a new climate where such analyses will be expected as the new norm against which new studies should be designed and executed.

There are immense ethical and practical difficulties in conducting drug trials in children, and these are coupled with the reality that the majority of medications are not labeled for use in children [13, 14]. These hurdles have led to wide areas of poor evidence on effectiveness and safety, and consequently blurred boundaries as to what has been sufficient evidence to show the superiority of a particular therapeutic modality.

Some examples of pediatric practices not anchored in strong evidence include the use of decongestants for upper respiratory infections [15], the use of histamine 2 receptor (H2) blockers for infantile colic [16] and the need to treat febrile seizures [17]. Under these circumstances, when the relative effectiveness of different drugs is not clear, it is difficult, if not impossible, to define an equipoise. The climate change of the last 2 decades, with the emergence of systematic reviews and meta-analysis in pediatric therapeutics, should help close this gap.

4 The Role of Data Synthesis in Defining Equipoise

As stated earlier, well designed data synthesis should be expected as the new norm against which new studies should be designed and executed. Without such investigations, studies may continue to be performed unsystematically long after cumulative experience has shown that one treatment is better than another, or superior to placebo. Perhaps the most known example of such a need is the cumulative meta-analysis conducted by Lau et al. [18], which showed that studies on the effectiveness of the enzyme streptokinase in improving the outcome of myocardial infarction, which started in 1959, reached robust statistical significance in 1973 after eight trials involving 2,432 patients. Yet, similar studies continued to randomize patients to placebo in 23 separate trials enrolling an additional 35,000 patients with no change in effect size [18]. This is a powerful example, albeit in adults, why the equipoise principle must be endorsed and practiced.

A recent study surveyed the perceptions of institutional review board (IRB) members to determine how they would decide equipoise and revealed that there was no clear consensus as to the threshold of allowing a randomized study to start in terms of equipoise [19]. Fifty percent of the IRB members surveyed would approve an RCT addressing the efficacy of two drugs for the management of headache even if 80 % of experts favor one treatment over another. Similarly, half of participating IRB members would approve the study when the median distribution of equipoise among experts was 70 % (70 in favor of treatment A.
vs. 30 in favor of treatment B) for treatment of leukemia, 60 % for treatment of geriatric patients and 70 % for treatment of newborns [19]. This study indicates, on the basis of the views of those making decisions in ethics committees, that the boundaries of equipoise are quite flexible, fluid and, in more than one way, a moving target. They are context related and the different make-up of different ethics committees may lead to different equipoise. This dimension of equipoise has rarely been discussed before, as most authorities relate to it as a firm definition, based on evidence of what has been proven to be superior and what has not.

5 Change in Equipoise During the Conduct of a Study

Quite often researchers start a study when a genuine equipoise exists, not knowing whether the experimental drug is better or safer than standard therapy. However, if during the conduct of the trial, another study (or studies) with similar design have been completed elsewhere and may have even been published, should the ongoing study be stopped if the published data show the experimental drug to be inferior or less safe?

Typically, the ultimate decision of continuing an active study under these circumstances resides with the local research ethics board (REB), often informed by its data safety monitoring board (DSMB), which is required to be apprised of new emerging data that may change the status of the trial. It is critical to remember that quite often a seemingly first decisive study is followed by studies with opposing results. Memorable examples of this situation include the claimed effectiveness of antioxidant vitamins in preeclampsia that started in a fanfare and ended with a whisper [20], or the claimed effectiveness of antioxidant vitamins in decreasing cardiovascular risks [21]. Hence, the need for additional studies may be critical and may affect the decision of REBs to allow continuation of a study despite new emerging data that seem to disrupt a given equipoise.

5.1 Interim Analysis Leading to Change in Equipoise

Quite often, clinical trials build into their designs an interim analysis [22], typically for one of two reasons:

a) To examine, in the case of a potentially important new drug, whether it has reached a preset level of effectiveness superiority over standard therapy or placebo, in a manner that does not justify avoiding it in the control group. A dramatic example was the interim analysis of the AZT effectiveness study for HIV/AIDS over placebo, which led to discontinuation of the study after the interim analysis and all patients being offered AZT from that point on [23].

b) The investigational arm is anticipated to potentially cause serious or life threatening adverse effects, which may preclude continuing randomization of patients to that arm.

We have recently conducted a pre-planned interim analysis of a study, which compared oral morphine to ibuprofen for post-tonsillectomy analgesia for children with obstructive sleep apnea. The interim analysis showed that children in the morphine arm had significantly more sleep apnea and hypoxemia after surgery than those in the ibuprofen arm, including a case of life threatening adverse central nervous system depression (Kelly L, personal communication). The DSMB of the study ruled that the study could not continue because morphine exposure constituted an unsafe practice.

Presently, the extent of issues and violations of equipoise has not been reported. Similarly, no consensus policies have been developed to guide DSMBs regarding their role in relation to the principle of equipoise, and there is no published evidence that DSMBs have taken a consistent approach when applying the principle of equipoise to pediatric clinical trials [2, 3].

6 Conclusions

In summary, the basic principles underlying equipoise are anchored in justice and fairness and the concern of ensuring that subjects are not unfairly exposed to an inferior therapy. Yet, the route to define equipoise for a particular mode of therapy in a particular situation is often convoluted, challenging and not well described in any road map. It is the researcher and his/her community of other clinicians and researchers who need to step up to the plate and ensure ethical definition and interpretation of equipoise.

Presently, the concept of equipoise is not widely incorporated in pediatric research, and a major change in climate will be needed to ensure that children are not exposed to the risks of exposure to placebo or an inferior option of therapy when a better option has been proven.

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References

2. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use.


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