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Therapeutic Interventions for Infantile Colic: A Systematic Review

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ABSTRACT

Introduction: The long-standing lack of a consensus regarding the etiology of infantile colic contributes to the largely exclusionary nature of current recommendations for diagnostic processes and treatment courses. In May of 2016, the Rome Foundation published a Rome IV revision of their Rome III diagnostic criteria in order to begin to address these and additional criticisms. However, despite the development of these revised criteria, an emphasis was again made upon the necessity for a true consensus on the etiological, diagnostic, and therapeutic aspects of the infantile colic condition.

Purpose: The primary aim of this thesis is to summarize the efficacy of new therapeutic interventions for infantile colic that have been assessed via randomized controlled trial as of May 2016. This summary will be conducted within the context of proposed etiologies for the disease.

Method: A systematic literature review was conducted with PubMed and the Cumulative Index of Nursing and Allied Health (CINAHL) using search terms related to infantile colic and treatments. Articles published from May 2016 to 2023 that included therapeutic methods targeted toward infants determined to have infantile colic were included. This comprehensive review will provide an updated summary of treatment options for infantile colic with the goal of comparing their efficacy in practice.

Results: This review included a total of 72 articles. Of these articles, 22 were randomized controlled trials which assessed the efficacy of interventions directed toward infants diagnosed with colic. The efficacy of these interventions was then interpreted within the context of proposed etiologies underlying infantile colic.

Conclusion: Although many promising interventions were discussed, there remains a persistent need for the implementation of more robust, large-scale randomized controlled trials in order to validate and evaluate their respective efficacies, as well as their mechanisms of action. This was followed by a discussion of my own work investigating potential factors influencing the development of infantile colic.

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Chapter 1. Introduction

Infantile colic is a relatively common disease, with recent assessments of the prevalence of excessive crying in infants estimated to be approximately 21% worldwide by expert consensus. However, reported prevalence of infantile colic varies greatly depending on which diagnostic criteria are used. Furthermore, research efforts interrogating the etiology of infantile colic and evaluating treatment courses for the disease have remained inconclusive, often utilizing a variety of diagnostic criteria and methodologies in their design. Although the condition is generally considered to be benign, it has a considerable negative impact on caretakers and has been associated with the development of future disease, such as the internalization of behavioral problems upon reaching preschool age.

This review focuses on new interventions for infantile colic published after the most recent consensus on diagnostic criteria, the Rome IV criteria for Functional Gastrointestinal Disorders in May 2016; specifically, interpreting these criteria within the context of investigations into the etiological underpinnings of infantile colic. A description of the progressive development and varied implementation of diagnostic criteria for infantile colic precedes a review of randomized controlled trials which evaluate the efficacy of new therapeutic interventions for the disease; the latter will interrogate these interventions as they relate to prior research regarding hypothesized mechanisms of action. To conclude, a brief discussion emphasizing the necessity for further research, along with a synopsis of my own work in the field, is included.

Diagnostic Criteria: Wessel

The development of diagnostic criteria for infantile colic initially faced multiple challenges. The etiology of the disease was unclear and there was no known "hallmark" or "gold standard" sign that distinguished the condition from other causes of excessive crying in infants. Furthermore, the use of measures which could differentiate colicky infants from healthy infants, such as assessments of symptoms including the number, duration, and frequency of crying episodes, varied in means of administration and evaluation. Each of these factors have contributed to varying reports of both the prevalence and presentation of infantile colic, the lack of consensus on which remains a significant focus of current research.

One of the first attempts at addressing these challenges was made by Wessel et al. in 1954, which defined infantile colic as the condition occurring when "A young infant, otherwise healthy and well-fed, has paroxysms of irritability, fussing or crying for a total of more than 3 hours a day and occurring on more than 3 days in any 1 week and lasting for more than 3 weeks." Participants in this study generally began exhibiting symptoms of infantile colic in their second week of life which self-resolved by the end of the second month, but there were many infants who began and continued to experience symptoms before and after this time period; furthermore, this timeline was highly similar to those exhibited by the healthy infants. Specifically, infant behaviors – time spent awake and quiet, awake and crying, being held or rocked, and sleeping – were recorded as they occurred along a 24-hour timescale for the first week of life; after this point, observations regarding the infant's behaviors were collected via multiple surveys and reports at various time points over the course of the first year of life (Wessel et al., 1954). This method of data collection was supported by the 1988 Barr et al.

publication which compared similarly-written observations of symptoms to audio recordings in order to assess relative accuracy of the former. Ultimately, Barr et al. endorsed these methods of documenting symptom presentation and progression as sufficient approximations of actual behavior, although they did note that there was often confusion regarding the distinction between "fussing" and "crying," which varied broadly depending on the recording individual's personal criteria of what distinguished the two (1988).

Wessel et al. also acknowledged that their allocation of infants into the colic ("paroxysmal fussers") and healthy control ("contented") groups based on this definition did not result in a wholly distinct symptom presentation across the two groups, as the authors had to account for a healthy level of crying in the control group, which was not explicitly defined beyond severity of symptom presentation to a lesser degree than that of the colic group (1954). Furthermore, the range of symptoms including duration and daily pattern of crying episodes varied broadly within the colic group, indicative of a continuum rather than dichotomy of infant crying (Wessel et al., 1954). Nonetheless, Wessel's diagnostic criteria have since been frequently utilized in order to differentiate healthy infants from those experiencing infantile colic, even referenced in a recent 2015 publication by Johnson et al. in which national clinical guidelines for the diagnosis of infantile colic were proposed within the scope of the practice of family medicine in America (Helseth & Begnum, 2002).

Diagnostic Criteria: ROME I, II, III, IV

The first official consensus on diagnostic criteria for infantile colic were based off of criteria initially established for adults developed by the Rome Foundation, a group of international experts who consolidated available scientific knowledge pertaining to prevalent functional gastrointestinal disorders (FGIDs) with unclear etiologies in the 1999 publication by Rasquin-Weber et al. Notably, the authors chose to exclude infantile colic from this publication, as the etiology of the disease was so unclear that no evidence-based methodology had even tangentially attributed the condition to a gastrointestinal origin and thus expert consensus on its status as such a disease could not be reached (Rasquin-Weber et al., 1999). Even as the second revision of the Rome Criteria was published later in 1999 within a broader scope of biopsychological contributing factors and consideration of pediatric conditions, infantile colic remained excluded from the FGID designation (Drossman).

Infantile colic was ultimately included in the Rome III criteria published in 2006, in which the Rome Foundation addressed criticisms of over-emphasis on expert opinion and instead focused on consolidaton of evidence-based findings in order to support their consensus on diagnostic criteria for FGIDs (Drossman). Hyman et al. noted that although their initial stance regarding the lack of conclusive evidence supporting a gastrointestinal origin for infantile colic remained unchanged, the clinical presentation of the disease was commonly associated with gastrointestinal distress and therefore its inclusion was necessitated in order to reflect the commonality of referrals to pediatric gastroenterologists and reduce risk of misdiagnosis (2006). In this publication, the following diagnostic criteria are proposed for infantile colic:

"Must include *all* of the following in infants from birth to 4 months of age:

1 Paroxysms of irritability, fussing, or crying that start and stop without obvious cause

2 Episodes lasting 3 or more hours per day and occurring at least 3 days per week for at least 1 week

3 No failure to thrive"

These criteria heavily reference the Wessel criteria published over 50 years prior, with modifications including a reduction in the duration of abnormal crying as well as an emphasis on the otherwise healthy condition of the infant and ultimately self-limiting, benign nature of the condition. The authors also include a broad timeline of less than 5 months for symptom onset and resolution, as well as a brief overview of available treatments. This discussion of treatments focuses on symptom mitigation and caretaker support, reflecting the lack of effective therapeutics available for the condition (Hyman et al., 2006).

An update on therapeutics available for infantile colic was provided in the Rome IV Criteria, published ten years later in 2016 in order to reflect the progression of research in the field (Drossman). Additionally, significant modifications to the previously established diagnostic criteria were made upon establishment of the Rome IV diagnostic criteria. For a more practical, evidence-based application of diagnostics within a clinical context, the modified Wessel criteria were replaced with a broader definition predicated on caregiver indication of unusual crying habits in the infant. In contrast, the diagnostic criteria for infantile colic within the context of clinical research kept the modified Wessel criteria and included a requirement for "a telephone or face-to-face screening interview with a researcher or clinician" as well as "Total 24-hour crying

plus fussing in the selected group of infants is confirmed to be 3 hours or more when measured by at least one prospectively kept, 24-hour behavior diary" (Drossman, 2016).

As these diagnostic criteria were progressively refined in response to the increasing number of proposed etiologies underlying infantile colic, treatments were developed along with them. At first, such treatments primarily promoted pacifier use and other techniques to soothe the infant, altered infant feeding practices, and pharmacological interventions such as antispasmodics and sedatives (Stewart et al., 1954). Over time, most pharmacological interventions were less commonly recommended due to the occurrence of adverse events and a general lack of demonstrable efficacy in clinical trials; although more interventions began to emphasize the need for parental reassurance and support, these were not wholly sufficient to address the disease outcome (Hall et al., 2011). As gut dysbiosis and intestinal inflammation became more prominent hypotheses for the etiology of infantile colic, the administration of oral lactase, probiotics, hydrolyzed formula, and herbal extracts have become increasingly utilized, albeit to varying degrees of efficacy among breastfed and formula-fed infants as assessed by clinical trials (Sarasu et al., 2018).

Ultimately, there remains a great deal of variation in the diagnostic criteria employed in the identification of infantile colic within a research context, even after the establishment of the Rome IV criteria. As shown in Table 1, randomized control trials investigating the relative efficacies of therapeutics for infantile colic since the 2016 Rome IV criteria utilized a variety of diagnostic measures, including eleven articles with Wessel's original criteria, four with some

modification of Wessel's criteria, five with the 2006 Rome III criteria, two with the Rome IV criteria, and one with a completed diary and parental indication of heightened crying.

Chapter 2. Methods and Data Collection

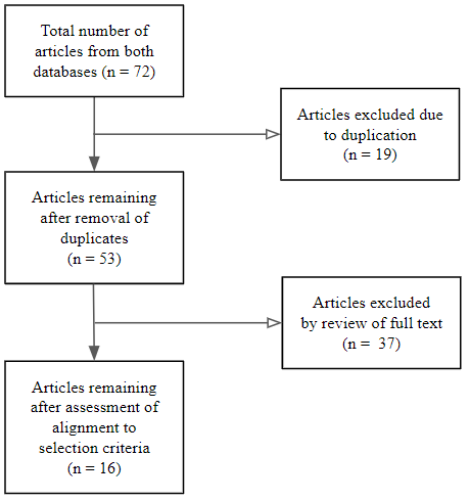
A literature review was conducted in order to evaluate treatments for infantile colic published after the 2016 Rome IV diagnostic criteria were published. Peer-reviewed articles were retrieved from the databases PubMed and the Cumulative Index of Nursing and Allied Health (CINAHL).

The search terms used to conduct this review included terminology associated with infantile colic and treatments. Search terms referring to infantile colic included: “"Colic"[Mesh] or infantile colic or infant colic or excessive crying or inconsolable crying” . Search terms referring to treatments included: “"Therapeutics"[Mesh] or treatment or therapy or mitigate or manage” . Each search included these terms in “All Fields” of all database entries. Searches were also filtered for designation as Randomized Controlled Trial, and articles published after May 2016 were considered.

Use of this search approach resulted in the identification of a total of 72 published articles. Duplicate articles between both databases were removed before the articles were carefully selected for adherence to selection criteria, as determined by a title review followed by a full-text review based on subjective consideration of relevance in accordance with the focus of the thesis. The resulting articles included infants who had been designated as having infantile colic prior to the administration of the intervention. Articles were excluded if they focused on preventive rather than biological therapeutic treatments, and articles were excluded if the effect of the intervention on the disease status or measures of symptom mitigation were not considered in the study outcomes.

The article selection process began with consideration of each title and then each full-text. Before consideration of the full-text of each article, a total of 72 articles were found, and then 19 articles were excluded due to duplication (Figure 1). After reading the full-text of each article, 37 articles were excluded due to subjective determination of lack of adherence to selection criteria (Figure 1). The remaining 16 articles focusing on infant-directed treatments for infantile colic were categorized by nature of intervention: oral lactase, single probiotics, probiotic and extract mixtures, and infant formula. There was overlap of categorization within certain articles (Table 1). Additionally, four articles were used to support the framework of the review.

Figure 1. Flow Diagram of Included and Excluded Articles



Articles were read chronologically by year of publication, and prior review articles were also referenced in order to obtain a deeper understanding of the basis of each focal area.

Chapter 3. Literature Review

The variety of randomized controlled trials assessing the efficacy of therapeutic interventions for infantile colic published since May 2016 reflect the many theories and lack of consensus on the disease's underlying etiology, which is generally assumed to be multifactorial as a consequence (Indrio et al., 2019). The consumption of oral lactase drops is hypothesized to counteract a lactose intolerance which may be attributable to an immature digestive system lacking the enzyme necessary for lactose digestion (Ahmed et al., 2018). Probiotic supplementation is similarly theorized to assist in the development of an immature digestive system which undergoes excessive inflammation and is inappropriately colonized by certain microbes (Pereira et al., 2022). Furthermore, the medication simethicone can be administered to reduce discomfort from flatulence, which can be symptomatic of an imbalanced distribution of intestinal microbiota (Biagioli et al., 2016). In colicky infants who consume formula, different variations of formula, including partially hydrolyzed formulas, may be recommended (Johnson et al., 2015).

Oral Lactase

One of the leading theories regarding the etiology of infantile colic draws upon its namesake reference to gastrointestinal distress, further supported by a common clinical presentation thought to be indicative of such pain: flexion of the legs which appears to protect the abdomen, as well as abdominal distension and excessive flatulence in some cases (Vandenplas et al., 2017). Lactose intolerance is a disease which more commonly arises in late childhood and early adulthood, although it is known to have congenital presentation in rarer cases (De Geyter et al., 2021). Notably, congenital manifestations of the disease generally resolve by three months after birth, overlapping with the time by which symptoms of infant colic self-resolve. The disease manifests due to an insufficiency of lactase activity in the small intestine, resulting in an alternate pathway of lactose metabolism which generates gases including hydrogen and methane which accumulate within the intestines, causing pain and discomfort. The production of these gases can also be measured in exhalations, and a hydrogen breath test is regarded as the best means of determining the presence of this condition, although there is a small but significant proportion of cases for which a measurement of elevated methane provides a more accurate means of diagnosis than hydrogen (Vandenplas, 2017).

However, of the three studies investigating the potential for lactose intolerance as a cause for observed infantile colic, none performed a hydrogen breath test, and one cited historically inconsistent results of the test in the context of infantile colic as justification for its exclusion from the study methodology. Furthermore, an emphasis was made upon the multifactorial hypothesis of the development of infantile colic; lactose intolerance may serve as only one of the

potential causes of infantile colic, rather than being the sole explanation for the disease manifestation (Narang & Shah, 2022).

Nonetheless, the two studies which compared the administration of oral lactase drops to administration of a placebo found significantly decreased duration of crying in the interventional groups when compared to the placebo, indicating that for infants in whom lactose intolerance was the cause of their colicky presentation, consumption of oral lactase was an effective treatment (Ahmed et al., 2018; Narang & Shah, 2022). The study published by Vandenplas et al. did not have a control group and instead compared the relative efficacy of oral lactase supplementation to that of a mixture of probiotics, which resulted in the findings that the probiotic group experienced a significantly shorter duration of crying, on average, in comparison to the lactase-supplemented group (2018).

Ultimately, the administration of oral lactase for the treatment of infantile colic is not uniformly supported. Although the studies investigating its use were adequately powered and found that the consumption of oral lactase resulted in a significant decrease in measured colic symptoms compared to placebos, the studies had methodological limitations including a lack of clear distinction between infantile colic and congenital or transient lactose intolerance (Ahmed et al., 2018; Narang & Shah, 2022). Furthermore, Narang and Shah stated that they were limited in the amount of participants they were able to recruit for their study (2022). However, they had 80 participants in the group which received oral lactase and 82 participants in the group which received a placebo, making their study one of the largest across all studies considered in this

review (Table 1). This highlights the necessity for more large-scale studies in future research regarding interventions for infantile colic.

Supplementation with a Single Probiotic

Probiotics, in particular *Lactobacillus reuteri* DSM 17938, had the strongest body of evidence supporting their supplementation in colicky infants as a means of addressing gut dysbiosis, particularly in breastfeeding infants as opposed to those solely consuming formula (Johnson et al., 2015). Prior studies have implicated abnormal densities of bacterial populations within the gastrointestinal tract in association within infantile colic, specifically identifying significantly lower amounts of *Lactobacillus reuteri* spp. in infants with colic upon comparison to healthy infants (Nation et al., 2017).

In fact, the 2015 national clinical guidelines for diagnosis of infantile colic published by Johnson et al. endorsed the consumption of the probiotic *Lactobacillus reuteri* DSM 17938 to breastfeeding infants presenting with symptoms of infantile colic, while it was contraindicated for consumption by formula-fed infants as it was found to significantly increase rather than decrease duration of crying or fussing in these infants. In contrast, the Rome IV diagnostic criteria published the following year did not support the use of probiotic supplementation due to conflicting findings regarding their efficacy, which varied based on the methodology used to assess it (Benninga et al., 2016). The results of this review reflect this inconsistency; although some trials identify promising findings of efficacious probiotic supplementation, none fully specify a clear mechanism of action, and the validity of their findings are often compromised by an insufficient sample size and conflicting findings despite similar methodologies (Table 1).

For instance, Nation et al. assessed the impact of *Lactobacillus reuteri DSM 17938* supplementation on symptoms of infantile colic and alteration of intestinal colonization with

Lactobacillus reuteri and *Escherichia coli* as indicators of reduced and heightened inflammation, respectively (2017). At the conclusion of the study, median crying time was reduced in both placebo and supplemented groups, and an increase in *Lactobacillus reuteri DSM 17938* was associated with increased rather than decreased crying time, contraindicating its use in therapeutic contexts (Nation et al., 2017).

Similarly, in a series of three publications, Savino et al. aimed to associate markers of the gut microbiome known to be indicative of inflammatory dysbiosis with infant colic status. In their 2017 publication, they measured levels of forkhead box P3 (FOXP3) and retinoid-related orphan receptor-γ (RORγ) messenger RNA, both of which are known to induce colonic T helper and regulatory T cells, which are known to be involved in the development and maintenance of intestinal inflammation; the authors found that a ratio of these two markers was decreased in treated infants with colic in comparison to those who received a placebo, although they were limited by a difficulty in obtaining sufficient samples for analyses from all participants (Savino et al.). Expression of these inflammatory markers had been previously associated with the presentation of infantile colic; as such, they subsequently hypothesized that the administration of the probiotic *Lactobacillus reuteri* DSM 17938 would reduce the expression of such inflammatory markers concurrently with a reduction in symptom severity. Ultimately, the authors corroborated these findings and also demonstrated a significant increase in intestinal colonization of *Lactobacillus* in infants who consumed the probiotic for one month; additionally, there was a relatively significant increase in intestinal colonization of *Escherichia coli* among infants experiencing colic symptoms, as well as heightened fecal calprotectin values, both of which further supported the theory of increased intestinal inflammation associated with the

disease presentation as *Lactobacillus reuteri* DSM 17938 is known to be involved in suppression of the immune response, while *Escherichia coli* is known to be involved in exacerbation of the immune response (Savino et al., 2017).

In their next publication, Savino et al. assessed whether administration of *Lactobacillus reuteri* DSM 17938 would modulate an additional mechanism of intestinal inflammation potentially associated with infantile colic: messenger RNA of the Toll like receptors TLR2 and TLR (2018). TLR2 and TLR4 are known to be implicated in the same inflammatory pathways of T helper and T regulatory cells action which were highlighted in their prior study. Although were able to replicate their findings of reduced symptoms of infantile colic associated with the consumption of *Lactobacillus reuteri* DSM 17938, their findings were inconclusive as TLR2 and TLR4 expression was found to be increased in both treatment and control groups (Savino et al., 2018).

In their 2020 publication, Savino et al. shifted their focus from *Lactobacillus reuteri* DSM 17938 to investigate the influence of another probiotic, *Lactobacillus rhamnosus GG* (ATCC 53103), within the context of infantile colic, also finding a significant reduction in symptom presentation of infantile colic associated with consumption of the probiotic supported by a reduction in levels of an intestinal inflammatory marker, fecal calprotectin. However, across all three publications, Savino et al. noted the lack of double-blindedness as a limitation of their findings, and their sample size was also relatively low, with less than 100 total participants in each study (Table 1).

Chen et al. also interrogated the efficacy of another probiotic, *Bifidobacterium animalis* subsp. *lactis*, BB-12®, in influencing both the severity of colic symptoms and fecal markers of intestinal immunity in a robust, large-scale study (2021). While they found that administration of the probiotic was associated with a significant reduction in crying and fussing duration in primarily breastfeeding infants, although an increase in biomarkers was observed across both groups and to a greater extent in the group supplemented with the probiotic (Chen et al., 2021).

Although promising, the assessment of the efficacy of single probiotics appears to be an area requiring additional research, particularly with more robust, consistent, large-scale and double-blinded study design, in order to reconcile conflicting findings in the literature thus far.

Supplementation with Mixtures

Similarly to evaluations of supplementation with single probiotics, evaluations of multi-probiotics and other mixtures as interventions for infantile colic also vary widely in their implementations and results. For instance, the consumption of multiple probiotics, in live states or dead states which isolate their postbiotic factors, is proposed to address inflammation-inducing imbalances in colonization of the colicky infant gut (Vandenplas et al., 2017). The assessment of the efficacy of each of these mixtures and their components within each trial is referenced against either a placebo or another intervention such as simethicone, making comparisons of relative efficacy both across studies and even within a single study difficult (Table 1). Nonetheless, certain interventions have been found to be relatively effective in their reduction of symptoms associated with infantile colic, and there is a need for further research into what factors determine and mechanisms underly this observed efficacy.

The 2017 publication by Vandenplas et al. investigated the efficacy of oral lactase as an intervention for infantile colic in comparison to AP198K, a mixture containing two heat-killed probiotics and an indigestible carbohydrate which is known to assist in the maintenance of the intestinal barrier. The probiotics included *Lactobacillus reuteri* DSM 17938, which has been shown to have a myriad of effects on the duration of crying and measurements of intestinal inflammation in infants affected by infantile colic. The mixture also included the probiotic *Bifidobacterium brevis* SGB01, which has not been interrogated for efficacy as a treatment for infantile colic prior to this study. The authors found that consumption of this mixture by infants affected by infantile colic could be associated with a significant decrease in the mean duration of crying episodes at each timepoint in comparison to the group which instead consumed an oral

lactase supplement; however, they emphasized the necessity of validation of these findings with a larger-scale study (Vandenplas et al., 2017).

Simethicone is a medication indicated for use in disorders such as infantile colic which are hypothesized to involve some element of gastrointestinal distress due to excessive flatulence and bloating (Ingold & Akhondi, 2023). However, the use of simethicone as a treatment for infantile colic was not endorsed in the 2015 national clinical diagnostic guidelines published by Johnson et al, who cited a recent systematic review finding an overall lack of its efficacy upon comparison to a placebo (Johnson et al., 2015). Nonetheless, simethicone was widely used as an alternate intervention in many of the studies assessing the efficacy of probiotic and other mixtures as treatments for infantile colic.

Most publications investigating the efficacy of probiotic mixtures found them to be more effective in reducing the severity of colic symptoms compared to simethicone or placebos. The 2018 publication by Baldassarre et al. found that consumption of a mixture of eight probiotics was associated with a significantly shorter duration of crying time in comparison to infants who consumed a placebo; similarly, a 2018 publication by Gerasimov et al. assessed the administration of a mixture of two strains of Lactobacilli and also found an association with significantly shorter duration of crying time in comparison to infants who consumed a placebo. The 2020 publication by Maldonado-Lobón et al. investigated the efficacy of a combination of two probiotics, *Bifidobacterium breve* CECT7263 and *Lactobacillus fermentum* CECT5716, in comparison to the efficacy of solely *Bifidobacterium breve* CECT7263 or solely simethicone in relieving symptoms of infantile colic in three groups of affected infants. In the group which only

consumed *Bifidobacterium breve* CECT7263, the total duration of daily crying was significantly shorter compared to that of the group which only consumed simethicone, a finding which occurred at every weekly timepoint. Similarly, the 2021 publication by Piatek et al. investigated a combination of nine probiotics which were found to be significantly more effective in reducing the average number of crying episodes, the number of days crying, and the average duration of evening crying episodes for colicky infants.

In contrast to solely investigating the influence of probiotic consumption on colic severity, the 2017 publication by Martinelli et al. aimed to address the lack of conclusive evidence regarding the efficacies of complementary and alternative medicine-based interventions for infantile colic. In this case, the authors evaluated the efficacy of mixture comprised of *Matricaria recutita* L.*, Foeniculum vulgare* M. var. dulce and *Melissa officinalis* extracts, as well as dead *Lactobacillus acidophilus* (HA122). This efficacy of this mixture in assuaging symptoms of infantile colic was evaluated in comparison to a group which consumed only *Lactobacillus reuteri* DSM 17938 and another group which consumed only simethicone; there was no true placebo group in this study. The authors found that both the group which consumed *Lactobacillus reuteri* DSM 17938 and the group which consumed the mixture resulted in a significantly shorter duration of crying time at 28 days in comparison to the group which consumed simethicone (Martinelli et al., 2017).

These findings are somewhat corroborated by another study investigating the impact of another mixture comprised of extracts, in this case the consumption of commercial combination comprised of *Chamomila, Cina, Colocynthis, Lac defloratum,* and *Magnesium chloratum*

extracts was compared with the consumption of simethicone in the context of mitigating symptoms of infantile colic. To assess the efficacy of both interventions, Raak et al. utilized two quantitative scales which measured the frequency of common caretaker complaints and examination findings associated with the symptom presentation of infantile colic, while the other scale measured the frequency of objective symptoms known to be associated with infantile colic; notably, these scales were not validated, further supporting the necessity for follow-up studies to more accurately determine the efficacy of this intervention. Nonetheless, this study found that this mixture of herbal extracts was significantly more efficacious than simethicone in mitigating symptoms of infantile colic as assessed by both scales. A robust, larger-scale investigation into the mechanisms underlying this observation is warranted (Raak et al., 2019).

In contrast to the prior trials, Khoshnevisasl et al. found that consumption of a symbiotic with both *Lactobacillus reuteri* and simethicone compared to that of a placebo combined with simethicone did not result in a significant difference in symptom severity of infantile colic (2022). Notably, this trial corroborated prior findings conducted by another group with the same symbiotic (Sung et al., 2014).

Infant Formula

The prior studies have largely focused on interventions for primarily or exclusively breastfeeding infants, but infants primarily or exclusively consuming infant formula are also known to develop infantile colic. The 2015 national clinical guidelines published by Johnson et al, as well as the 2016 Rome IV criteria published by Benninga, recommended that formula-feeding infants specifically consume hydrolyzed formulas.

However, Turco et al. found that infants with colic who consumed a partially hydrolyzed formula actually had a significantly higher crying duration on average compared to infants who did not consume the partially hydrolysed formula. Although hydrolysed formula is an intervention that is used to treat cow's milk protein allergy rather than a true underlying cause of infantile colic, it has become a component of the colic treatment course largely due to influence by expert opinion, as well a means of preventing a potential misdiagnosis (Turco et al., 2021).

Chapter 4. Conclusion

This thesis investigated the landscape of research regarding therapies for infantile colic published since the establishment of the Rome IV diagnostic criteria. The range of diagnostic criteria, methods of administration, and resulting efficacies of treatment along with method of evaluation varied broadly even within the same category of intervention, underscoring the still-unclear etiology of the disease, need for consistent implementation of study design, and lack of standardized treatment methods for the disease. Although the etiology of infantile colic remains unclear, the analyses conducted on inflammatory markers and other biological factors of interest in fecal and blood samples have revealed new insights which should be investigated further. Nonetheless, findings must be interpreted with care as studies frequently faced significant limitations, including small sample size and difficulty in obtaining a sufficient quantity of samples for analyses. Studies were also often open-label. Further research with more robust, large-scale randomized controlled trials and long-term follow-up is needed for validation of many of these preliminary findings (Savino, 2017).

While working under the guidance of Dr. Steven Hicks at the Penn State College of Medicine, I also investigated biological factors potentially involved in the etiology and severity of infantile colic through comparative analyses of maternal breastmilk samples in the mothers of infants who had colic versus mothers of healthy controls. Ultimately, our findings corroborated those of an etiology related to abnormal inflammation of the gut by identifying heightened levels of HGF associated with increased severity of colic symptoms; heightened levels of miR-29a-3p and let-7a-5p were associated with increased risk of developing colic; and heightened levels of

commensal *Lactobacillus* appeared to confer a protective effect against the development of colic. Although we could not generalize our findings to a broader context because of the limited sample size and lack of analysis of infant samples, further research investigating these biological factors in a larger-scale study could reveal additional information about mechanisms underlying infantile colic and direct efforts toward developing corresponding therapeutic interventions (Chandran et al., 2023).

Chapter 5. Appendix

Table 1. Summary of articles selected for review of therapeutic interventions for infants diagnosed with infantile colic

Authors, Year	Sample characteristics	Interventional group(s)	Control group	Outcome
Vandenplas et al., 2017	Breastfed and formula-fed infants 0-5 months old with infantile colic determined by Rome-IV criteria, *N* = 46	Oral lactase drops, *n* = 23 APT198K mixture active ingredients: xyloglucan plus heat-killed *Lactobacillus reuteri* SGL01 and *Bifidobacterium brevis* SGB01, *n* = 23		Duration of crying per episode was significantly longer in infants who consumed oral lactase supplement compared to those who consumed the APT198K mixture
Ahmed et al., 2018	Breastfed and formula-fed infants 0-6 months old with infantile colic determined by Wessel criteria, *N* = 104	Oral lactase drops, *n* = 52	Oral placebo formulation, *n* = 52	Duration of crying was significantly shorter in infants who received oral lactase supplement compared to those who received a placebo
Narang et al., 2022	Breastfed and formula-fed infants 0-5 months old with infantile colic determined by Rome-IV criteria, *N* = 162	Oral lactase drops, *n* = 80	Oral placebo formulation, *n* = 82	Duration of crying was significantly shorter in infants who received oral lactase supplement compared to those who received a placebo
Fatheree et al., 2017	Breastfed infants 21-90 days old with infantile colic determined by Barr diaries: at least 2 out of 3 days with 3h daily of nonconsecutive crying and fussing with an affirmation that this amount of crying was typical, *N* = 29	*Lactobacillus reuteri* DSM 17938, *n* = 12	Oral placebo formulation, *n* = 7	Duration of combined crying and fussing time was not significantly different between the two groups of infants
Nation et al., 2017	Breastfed and formula-fed infants 0-3 months old with infantile colic determined by Wessel criteria, *N* = 65	*Lactobacillus reuteri* DSM 17938, *n* = 14	Oral placebo formulation, *n* = 51	No significant difference between median duration of infant crying between infants colonized and infants not colonized by *L. reuteri* by the final timepoint, although there was a significant, positive association between *L. reuteri* colonization density in infants who consumed it and median infant crying time
Savino et al., 2017	Predominantly breastfed infants 0-12 weeks old with infantile colic determined by modified Wessel criteria, *N* = 60	*Lactobacillus reuteri* DSM 17938, *n* = 32	Oral placebo formulation, *n* = 28	Duration of crying and fussing were significantly shorter in infants who consumed the probiotic compared to those who consumed a placebo
Savino et al., 2018	Breastfed and formula-fed infants 0-60 days old with infantile colic determined by Wessel criteria, *N* = 34	*Lactobacillus reuteri* DSM 17938, *n* = 18	Oral placebo formulation, *n* = 16	Duration of crying was significantly shorter in infants who consumed the probiotic compared to those who consumed a placebo
Savino et al., 2020	Breastfed infants 2-10 weeks old with infantile colic determined by modified Wessel criteria, *N* = 47	*Lactobacillus rhamnosus GG (ATCC 53103)*, *n* = 26	Oral placebo formulation, *n* = 21	Average duration of crying was significantly shorter in infants who consumed the probiotic compared to those who consumed a placebo
Gerasimov et al., 2018	Breastfed and formula-fed infants 4-12 weeks old with infantile colic determined by modified Wessel criteria, *N* = 172	*Lactobacillus rhamnosus* 19070-2 and *Lactobacillus reuteri* 12246 *n* = 86	Oral placebo formulation, *n* = 86	Duration of crying was significantly shorter in infants who consumed probiotic compared to those who consumed a placebo
Baldassarre et al., 2018	Breastfed infants 3-6 weeks old with infantile colic determined by Wessel criteria, *N* = 53	Probiotic mixture active ingredients: *Lactobacillus paracasei* DSM 24733, *Lactobacillus plantarum* DSM 24730, *Lactobacillus acidophilus* DSM 24735, *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 24734, *Bifidobacterium longum* DSM 24736, *Bifidobacterium breve* DSM 24732, *Bifidobacterium infantis* DSM 24737, *Streptococcus thermophilus* DSM 24731 *n* = 27	Oral placebo formulation, *n* = 26	Average duration of crying was significantly shorter in infants who consumed the probiotic mixture compared to those who consumed simethicone.

Maldonado-Lobón et al., 2020	Breastfed and formula-fed infants 3-12 weeks old with infantile colic determined by Rome III criteria, *N* = 150	*Bifidobacterium breve* CECT7263 *n* = 49 Probiotic mixture active ingredients: *Bifidobacterium breve* CECT7263 *Lactobacillus fermentum* CECT5716 *n* = 57 Simethicone drops *n* = 44		There was no significant difference between mean duration of crying across all three treatments
Chen et al., 2021	Breastfed and formula-fed infants 3-12 weeks old with infantile colic determined by Rome III criteria, *N* = 192	*Bifidobacterium animalis* subsp. *lactis*, BB-12 *n* = 96	Oral placebo formulation *n* = 96	The mean number of daily crying episodes after the 21-day intervention was significantly reduced in infants who consumed BB-12 compared to those who consumed the placebo. The mean daily sleep duration was markedly increased from baseline to end of intervention in infants who consumed BB-12 compared to infants who consumed the placebo.
Piątek et al., 2021	Breastfed and formula-fed infants 30-90 days old with infantile colic determined by Wessel criteria, *N* = 87	Symbiotic mixture active ingredients: *Lactobacillus acidophilus* LA-14, *Lacticaseibacillus casei* R0215, *Lacticaseibacillus paracasei* Lp-115, *Lacticaseibacillus rhamnosus* GG, *Ligilactobacillus salivarius* Ls-33, *Bifidobacterium lactis* Bl-04, *Bifidobacterium bifidum* R0071, *Bifidobacterium longum* R0175 *n* = 54 Simethicone drops *n* = 33		Infants who consumed the synbiotic had significantly fewer crying episodes on average during the last three weeks of the study, while those who consumed simethicone did not Both infants who consumed the symbiotic mixture and infants who consumed simethicone had a significant decrease in the number of days crying and duration of evening crying during the last three weeks when compared to baseline measurements
Martinelli et al., 2017	Breastfed and formula-fed infants 2 weeks to 4 months old with infantile colic determined by Rome III criteria, *N* = 180	Extract mixture active ingredients: *Matricariae chamomilla* L., *Melissa officinalis* L., tyndallized *Lactobacillus acidophilus* (HA122) *n* = 60 *Lactobacillus reuteri* DSM 17938 *n* = 60 Simethicone drops *n* = 60		Both infants who consumed the mixture and infants who consumed *Lactobacillus reuteri* DSM 17938 had significantly less infant crying time at 28 days when compared with infants who consumed simethicone
Khoshnevisasl. et al., 2022	Breastfed and formula-fed infants 0-3 months old with infantile colic determined by Wessel criteria, *N* = 68	Symbiotic mixture: *Bifidobacterium infantis,* *Lactobacillus rhamnosus,* *Lactobacillus reuteri*, and simethicone *n* = 33 Placebo and simethicone *n* = 35		There was neither a significant difference in crying time, number of crying episodes, nor sleep duration between infants who consumed the symbiotic mixture with simethicone and those who consumed a placebo with simethicone
Raak et al., 2019	Breastfed and formula-fed infants 0-6 months old with infantile colic determined by Rome III criteria, *N* = 125	Homeopathic complex active ingredients: *Chamomilla* D6, *Cina* D6, *Colocynthis* D6, *Lac defloratum* D6, and *Magnesium chloratum* D6 *n* = 74 Simethicone drops *n* = 51		The parents of infants who consumed the homeopathic complex reported significantly fewer symptoms of infantile colic on average than did those of infants who consumed simethicone

Turco et al., 2021	Formula-fed infants 0-4 months old with infantile colic determined by Rome III criteria, *N* = 241	Partially hydrolysed formula: reduced lactose content (40%), added maltodextrins (60%), and *Lactobacillus reuteri* DSM 17938 *n* = 124 Standard formula: intact protein body formula (70% of whey protein, 30% of casein), with 100% of lactose *n* = 117		Mean daily crying time was significantly lower in infants who consumed standard formula compared to infants who consumed the partially hydrolysed formula

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ACADEMIC VITA

DESIRAE CHANDRAN

Education

Bachelor of Science, Biology | *The Pennsylvania State University – Schreyer Honors College* **May 2023**

Work Experience

Part-Time Research Associate, *Penn State College of Medicine* **June 2020 – Present**

- Taught four individuals how to conduct RNA isolations from human breastmilk
- Interpreted findings from RNA and DNA analyses conducted on human saliva and breastmilk, including isolations, polymerase chain reactions, and enzyme-linked immunoassays on saliva
- Developed new organizational measures for thousands of clinical samples utilizing Microsoft Excel
- Utilized the REDCap web application to implement new survey collection measures in multiple research projects

BIOL 155 Teaching Assistant, *The Pennsylvania State University* **August 2022 – December 2022**

- Guided undergraduate student understanding of biological mechanisms underlying human aging
- Provided additional assistance to students as needed during lecture and during review sessions before each examination period

BIOL 437 Teaching Assistant, *The Pennsylvania State University* **January 2022 – April 2022**

- Taught undergraduate students human tissue identification skills in addition to associated physiological functions for the laboratory portion of the course
- Guided students in comprehension and memorization of course material during laboratory periods held biweekly and during review sessions before each examination period

BIOL 110 Tutor, *The Pennsylvania State University* **January 2022 – April 2022**

- Assisted two foreign exchange students from the King Abdullah Undergraduate of Science and Technology, who were participants of the Intensive English Communication Program, in comprehension of course material
- Explained core concepts in introductory biology to students during weekly tutoring sessions

Undergraduate Research Intern, *Penn State College of Medicine* **July 2019 – August 2019**

- Attended and contributed to weekly journal club meetings
- Performed and interpreted RNA isolations on human breastmilk samples
- Developed new sample organization measures for breastmilk samples utilizing Microsoft Excel

Volunteer Experience

Student Red Cross Club, *The Pennsylvania State University* **September 2019 – Present**

Secretary, Website Design Chair, and On-Site Coordinator

- Established the new website in 2019 for the purposes of internal communication, tracking member involvement, and raising awareness about the urgent demand for blood donations at www.psusrcc.com
- Helped oversee blood drives to ensure that the blood donation process runs smoothly

Publications

Chandran D, Confair A, Warren K, Kawasawa YI, Hicks SD. Maternal Variants in the *MFGE8* Gene are Associated with Perceived Breast Milk Supply. *Breastfeed Med*. 2022;17(4):331-340. doi: 10.1089/bfm.2021.0216.

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