Probiotics for preventing and treating gastro-oesophageal reflux/gastro-oesophageal reflux disease in infants: A systematic review and meta-analysis

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Abstract
Background Gastro-oesophageal reflux (GOR) and Gastro-oesophageal reflux disease (GORD) are common during infancy and can cause substantial discomfort in infants, parental distress and financial burden on parents and the health care system. Effective treatment regimens, however, remain elusive. Probiotics given to women during pregnancy and lactation, and babies may have therapeutic effects when it comes to GOR/GORD. The objective of this systematic review and meta-analysis is to evaluate the efficacy of probiotic supplementation for the prevention and treatment of GOR/GORD in infants.

Methods Literature searches were conducted using MEDLINE, EMBASE and the Cochrane Central Register of Controlled trials. Only randomised controlled trials (RCTs) were included. A meta-analysis of included trials was performed using the Cochrane Collaboration methodology where possible.

Results Six RCTs examined the prevention or treatment with probiotics on GOR. There were no studies examining probiotics for GORD. A meta-analysis of 3 studies showed a statistically significant reduction in regurgitation episodes for the probiotic group compared to the placebo group [mean difference -1.44 episodes/day; 95% CI -1.71 to -1.17] but there was high heterogeneity (96%). Meta-analysis of two studies found a statistically significant increased number of stools per day in the probiotic group compared to the placebo group [mean difference 1.26, 95% CI 1.12 to 1.41]. However, there was moderate heterogeneity (69%). Individual studies reported: a decrease in crying time, increased gastric emptying rate, infant length and head circumference, visits to an emergency department or health professional, and loss of parent working days were significantly less with infants receiving probiotic compared to a placebo but more research is needed. Meta-analysis of two studies showed no difference in body weight between the two groups (minimal heterogeneity 23%). None of the studies reported any adverse effects for the women or infants.

Conclusions Probiotic therapy appears promising with some evidence of benefit but most studies are small and there was high heterogeneity between the studies. The use of probiotics could potentially be a non-invasive, cost effective and preventative positive health strategy for both women and their babies. Further well controlled RCTs examining the effect of probiotics for GOR /GORD are warranted.
Background
The North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) in collaboration with the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) define gastro-oesophageal reflux (GOR), also known as ‘functional regurgitation’, as the passage of gastric contents into the oesophagus with or without regurgitation and/or vomiting [1]. Gastro-oesophageal reflux disease (GORD) progresses from GOR when the reflux leads to more serious symptoms or complications such as oesophagitis or stricture [1]. These guidelines have been recently updated following a rigorous review of the literature, but the definitions have been upheld [2].

In clinical practice the terms GOR and GORD have been used interchangeably due to the recognised difficulty in the clinical differentiation, particularly in non-verbal infants. Usually the infant with GOR demonstrate adequate weight gain will have no obvious symptoms other than uncomplicated positing, [3]. However, regurgitation after feeds may occur more than six times per day [4]. In some instances of GOR as well as frequent possetting (spitting up) and vomiting, infants display acute post feed irritability involving back arching and stretching and extended periods of crying which parents find difficult to resolve [1, 2]. These combined behaviours may result in significant interruptions to sleep patterns which in turn contribute to parental distress [1]. As a result most often parental concern regarding their infant’s irritability is the factor driving the push for a diagnosis [2, 4].

In cases where the infant progresses on to develop GORD, clinical symptoms become far more worrying. They include wheezing, apnoea, stridor, recurrent bronchiolitis, aspiration pneumonia, regular bouts of choking and gagging at feeds, disorganised and dysfunctional sucking or swallowing which in turn results in excessive weight loss, feed refusal and delayed feeding development.

Estimation of the true prevalence of GOR versus GORD remains difficult because of the inability to provide specific diagnosis of each. The natural progression of simple GOR is that it is most problematic in infants between birth and four months of age and normally decreases significantly by 12 months of age [4–6]. In Italy, the prevalence of GOR between two to four months of age is approximately 40% of infants [7]; in the USA 40 % [6] and in Australia 41% [8, 9]. Approximately 5–
9% of all infants go on to develop GORD [10–12].

Diagnosis of GOR/GORD is difficult because there is no one investigation for the diagnosis of GOR/GORD in infants and children. Therefore, the infant demonstrating severe symptoms will often undergo a range of diagnostic investigations beginning with a comprehensive physical examination and review of infant - family illness history [4, 13]. While a comprehensive physical examination is essential to rule out the presence of other illnesses, it may not provide a definitive diagnosis as often the severity of reflux may not directly correlate with the severity of symptoms [13]. More intrusive diagnostic investigations may then be indicated. These include gastroesophageal scintigraphy used to explore and describe anatomy, endoscopy and oesophageal biopsy to examine oesophageal mucosa, or oesophageal pH and impedance monitoring to quantify the extent of GOR/GORD [14].

Unfortunately, effective treatment regimens for GORD remain elusive. There remains a lack of evidence to support commonly used pharmacological and non-pharmacological management strategies for GOR and GORD [15, 16]. This creates challenges for clinicians caring for this population [15, 17]. Kirby et al [17] concluded from their national cross-section survey of Australian GPs that often diagnostic and management practices are used despite general practitioners’ concerns regarding the safety and effectiveness. This is especially disturbing as the international rates of diagnosis of GOR/D are reportedly increasing [15] but the rise in the survival of preterm infants, awareness and treatment of GOR/D, and parent support groups may influence this.

Probiotics are a possible therapeutic strategy with minimal side effects that may help to modify GOR/GORD symptoms. Probiotics are defined as live microorganisms that, if taken in adequate amounts, may have a beneficial effect on the host [18]. Mechanisms of action include enhanced epithelial barrier, inhibition of mucosal pathogens and increased adhesion of favourable microorganisms to the intestinal mucosa, and production of antimicrobial substances (bacteriocins, acids etc.) and immune system modulation [19, 20]. All this is done by manipulation of the human microbiome, especially the intestinal microbiota [21]. It is well known that a wide array of illnesses, ranging from inflammatory bowel disease to cancer to major depressive disorder have been linked to changes in the microbiome and their interaction with various bodily systems [21, 22]. Probiotic
microorganisms can alter the immune system at the local and systemic level[20]. At birth the newborn leaves the intrauterine environment and enters a contaminated extrauterine world, which requires potent host defences. Intestinal defences develop through the bacterial colonisation on mode of birth is important [23]. Caesarean births and antibiotic use appear to impact on the infant microbiome. There is increasing evidence that infants born by caesarean section have different gut microbiota to those born by normal vaginal delivery [24, 25]. The intestinal microbiota plays a crucial role in the pathogenesis of gastrointestinal disorders [26–28] and an increasing number of studies are targeting probiotic therapy [29–41] for infants and adults. Therefore, this systematic review sought to determine the effectiveness of probiotics for the prevention and treatment of GOR and GORD in infants.

Methods
The objective of this systematic review is to evaluate the efficacy of probiotic supplementation for preventing and treating GOR and GORD in infants. The systematic review followed the methods described in the Cochrane Handbook for Systematic Reviews of Interventions and by the Cochrane Neonatal Review Group [42].

Search strategy
Eligible studies were identified from the Cochrane Central Register of Controlled Trials (CENTRAL 2019, Issue 4) in the Cochrane Library; MEDLINE via PubMed (1966 to 15 April 2019); Embase (1980 to 15 April 2019); and CINAHL (1982 to 15 April 2019) using the following subject MeSH headings and text word terms: ‘neonate(s)’, ‘newborn(s)’, infant(s)’, AND ‘gastroesophageal reflux’ OR ‘gastrooesophageal reflux’ OR ‘GOR’ OR ‘GORD’ OR ‘infantile reflux’ OR ‘regurgitation’ AND probiotics. Language restrictions were not applied. We searched clinical trials registries for ongoing or recently completed trials (the World Health Organization’s International Trials Registry and Platform www.who.int/ictrp/search/en/; clinicaltrials.gov; ISRCTN Registry https://www.isrctn.com/). All potentially relevant titles and abstracts were identified and retrieved during the search. Independent hand searches were undertaken and the bibliographies of each article were assessed for additional relevant titles.

Study selection
We included all randomised controlled trials that compared probiotics (any dose or composition) to placebo, control or other forms of treatment in mothers during the antenatal period, and infants in the postnatal period (and up to 6 months) for the prevention (mother/infant) and treatment (infant) of GOR/GORD. All definitions of GOR/GORD were accepted and articles in any language we considered if there was an abstract in English.

Data extraction and management
We used the data extraction form available within Review Manager software (RevMan) to extract data on the participants, interventions and control(s), and outcomes of each included trial. Two review authors (JF and KP) screened the title and abstract of all identified studies. We re-assessed the full text of any potentially eligible reports and excluded the studies that did not meet all of the inclusion criteria. Two review authors (JF and KP) independently extracted data from each study without blinding to authorship or journal publication. In case of any disagreement, the two review authors resolved them by discussion until reaching a consensus. One review author (JF) entered data into RevMan (2014), and a second review author (KP) verified them [42].

Methodological quality of the studies
Standard methods of the Cochrane Collaboration as described in The Cochrane Library (www.thecochranelibrary.com) were used to assess the methodological quality of included trial [42]. The methodological details of the studies were extracted from published data. For each trial, information was sought regarding:

Selection bias: Random sequence generation due to inadequate generation of a randomised sequence and inadequate concealment of allocations prior to assignment.
Blinding of participants and personnel: Performance bias due to knowledge of the allocated interventions by participants and personnel during the study.
Blinding of outcome assessment: detection bias due to knowledge of the allocated interventions by outcome assessors.
Incomplete outcome data: attrition bias due to amount, nature or handling of incomplete outcome data.
Selective reporting: reporting bias due to selective outcome reporting. Other sources of bias: bias due to problems not covered elsewhere in the table [42].

Data synthesis
The primary outcome for the infants was effect of probiotics on episodes of GOR/GORD. The secondary outcomes were the effect of probiotics on: crying time (minutes per day), gastric emptying time, number of stools, growth rate (weight, head circumference, length), admissions to hospital
related to infant GOR/GORD, loss of parent working days related to GOR/GORD, number of admissions of mother to hospital due to anxiety/depression, number of visits to any health professional, and adverse events related to probiotic supplementation (mother and infant).

We performed statistical analyses using Cochrane’s Review Manager (RevMan) Version 5.3. We analysed continuous data using mean differences (MDs) and reported the 95% confidence interval (CI) on all estimates and we used the fixed-effect model for all meta-analyses. We assessed the heterogeneity between the included trials, using the I² statistic which describes the percentage of total variation observed across studies due to heterogeneity rather than sampling (random) error. The degree of heterogeneity was graded as non-existent or minimal for an I² value of less than 25%, low for an I² value of 25% to 49%, moderate for an I² value of 50% to 74%, and high for an I² value of 75% to 100%. We planned to assess sources of heterogeneity using sensitivity and subgroup analysis, however, there were insufficient data [42].

Results
A total of 22 potentially relevant citations were obtained through our primary search strategy (Figure 1). Sixteen studies were excluded because they investigated the use of probiotics for infantile colic. Six RCTs met the inclusion criteria [26, 28, 41, 43–45]. No ongoing studies were identified. Overall, the studies had a low risk of bias for random sequence generation, blinding of participants and personnel or low risk/unclear risk for allocation concealment, blinding of outcome assessment and incomplete outcome data (Table 1). All the studies were undertaken in Italy.

A total of 803 infants were enrolled across the six studies. All studies compared probiotics versus placebo for preventing or treating GOR. There were no RCTs examining the effect of probiotics on GORD in infants. Characteristics of the included trials are summarised in Table 2. One study [41] examined the use of four different strains of lactobacilli (L. paracasei DSM 24733, L. plantarum DSM 24730, L. acidophilus DSM 24735, and L. delbrueckii subsp. bulgaricus DSM 24734, three strains of bifidobacteria (B. longum DSM 24736, B. breve DSM 24732, and B. infantis DSM 24737, and one strain of Streptococcus thermophilus DSM 24731. In one study, probiotics were given to mothers four
weeks before the expected delivery date (36th week of pregnancy) until four weeks after delivery [41]. One study [45] administered *L. reuteri* DSM 17938 to healthy breastfed term infants up to 28 days post-delivery, and one study (Indrio 2014) administered *L. reuteri* DSM 17938 to term new born breast fed infants during the first 3 months of life. One study [43] administered *L. reuteri* ATCC 55730 to formula fed preterm infants up to 30 days post-delivery. One study [44] administered *L. reuteri* DSM 17938 for 4 weeks to full-term formula fed infants aged four weeks and five months with GOR diagnosis on the basis of retrospective reports from parents and caregivers and another study [28] administered *L. reuteri* DSM 17938 for 30 days to formula fed healthy infants younger than 4 months of age diagnosed with uncomplicated GOR diagnosed by a paediatrician. In general, included trials had a low risk of bias (Table 2).

**Effect of probiotics on episodes of GOR**

Six trials examined the effect of probiotics on episodes of regurgitation per day at one month of age. Three studies were included in the meta-analysis [26, 43, 44]. Meta-analysis showed a statistically significant reduction in regurgitation in the probiotic group compared to the placebo group (mean difference -1.44 episodes/day; 95% CI -1.71 to -1.17, p < 0.0001) (Figure 2). However, the I² statistic of 96% indicates high heterogeneity. Whilst Indrio (2014) found no difference between the probiotic and placebo groups at one month, a statistically significant reduction in regurgitation episodes per day was reported at three months between the probiotic (Mean 2.9 SD 1.1) and placebo groups (Mean 4.6 SD 3.2, p <.01).

We were unable to include the remaining three studies in the meta-analysis due to the method of reporting. Baldassarre [41] reported that the onset of regurgitation was statistically significantly less frequent in the probiotic group compared to the placebo group X² = 6.944, p = 0.008; Relative Risk = 2.43 (95% C.I 1.14 to 5.62). Logistic regression analysis showed that the only factor with a significant impact on regurgitation was maternal probiotic consumption. Garofoli [46] reported for the group receiving the probiotic, a significant reduction was shown in the average daily number of regurgitations (p = 0.02) from baseline to day 28 of the study period. At the end of 2nd week the difference with the placebo group was significant (p = 0.05) and a trend to a significant result was
reported at the end of the 3rd week of treatment (p = 0.06). Indrio [28] found infants receiving the probiotic had a significant decrease in episodes of regurgitations/day compared to placebo: Median 1.0 (5th percentile = 1.0; 95th percentile = −2.0) vs. Median 4.0 (5th percentile 3.0; 95th percentile = 5.0] calculated over the last 7 days of treatment, (p<0.001).

Crying time
Due to the method of reporting for crying time, we were unable to perform a meta-analysis. Indrio (2008) found a statistically significant decreased crying time in the probiotics group (Mean 32, SD 6) compared to the placebo group (Mean 88, SD 16), p = <0.01 at 30 days. Garofoli [46] reported that a similar pattern in daily average minutes of crying over a 4 week period for the probiotic and placebo groups, however, data was only provided graphically.

Gastric emptying time
Due to the method of reporting for gastric emptying time, we were unable to perform a meta-analysis. Indrio (2008) reported that gastric emptying rate (%) was statistically significantly faster in the newborns receiving probiotics compared with a placebo (p<0.001). Indrio (2011) reported the change in gastric emptying rate (%) before and after the intervention and found a statistically significantly increased gastric emptying rate in infants receiving probiotics compared to placebo (p = 0.01). Indrio (2017) also reported a significantly increased gastric emptying rate percentage change for infants receiving probiotics Median 12.3 (5th percentile = −3.9, 95th percentile = 22.0) compared to placebo Median 9.1 (5th percentile = −27.0; 95th percentile = 25.5); p<0.01.

Number of stools
Meta-analysis of two studies (Indrio 2014; Indrio 2008) found a statistically significant increase in the number per day of stool evacuations in the probiotic group compared to the placebo group at one month [mean difference 1.26, 95% CI 1.12 to 1.41, p = <0.00001]. However, the I² statistic of = 69% indicates moderate heterogeneity (Figure 3). Indrio (2014) also found a significantly increased mean evacuation rate at three months in the probiotic group: Mean 4.2 (SD1.8) compared to the placebo group Mean 3.6 (SD 1.8). However, Garofoli (2014) reported similar pattern for the probiotic and placebo groups in the daily stool frequency and consistency, however, no data was provided.
Baldassarre [41] also reported no significant differences between the probiotic and placebo groups in number of bowel movements (3.7 vs. 4.2, t = 1.17, p = 0.246) and consistency of stools ($\chi^2 = 3.53$, p = 0.317) were found between the two groups.

Growth (body weight; head circumference; length)
Four studies reported on body weight. Two studies were able to be included in the meta-analysis [44, 45]. No statistical difference was found between the probiotic and placebo groups [Mean difference - 79.05, 95% CI 211.77 to 53.67; $I^2 = 23\%$, p = 0.24] (Figure 4). Indrio (2011) reported no difference in body weight between the two groups during, and at the end of the trial but no data was provided. Indrio (2008) reported no difference in weight gain in grams per day over the last 7 days of treatment between the probiotic (Mean 28 grams SD 7.0) and placebo groups (Mean 25 grams SD 8.1). Garofoli (2014) reported no difference in length between the probiotic group (55.1cms, SD 1.94) and control group (56.45, SD 0.65), p = 0.087 at four weeks. Baldassarre [41] reported similar growth patterns between the two groups, according to body mass index at 4 weeks (Time effect: F = 118.95, p < 0.001; treatment effect: F = 0.01, p = 0.92; interaction effect: F = 1.43, p = 0.24). Garofoli [46] reported no difference in cranial circumference between the probiotic (37.33cms, SD 1.21) and placebo groups (38.03, SD 1.47), p = 0.108 at four weeks.

Number of admissions to hospital, loss of parent working days, visits to any health professional related to GOR
Only one study reported on these outcomes. Indrio (2014) found statistically significant less emergency department visits at three months in the probiotic group (Mean 0.52 SD 0.72) compared with the placebo group (Mean 1.78 SD 1.11); p <.05. Indrio (2014) also found statistically significant less paediatric visits due to the presence of symptoms in the probiotic group (Mean 1.3 SD 0.6) compared to the placebo group (Mean 2.3 SD 0.7); p <.05. Indrio (2014) found statistically significant fewer loss of parent working days in the probiotic group (Mean 0.54 SD 0.62) compared to the placebo group (Mean 2.89 SD 1.3); p <.05.

Admissions to hospital due to anxiety/depression
None of the studies reported on admissions to hospital due to maternal anxiety/depression.

Adverse effects
None of the studies reported any adverse effects for the women or infants.

Discussion
To our knowledge, we report the first published systematic review of randomised trials exploring the efficacy of probiotics in infantile GOR/GORD. We found six RCTs on the use of probiotics for the prevention or treatment of GOR for inclusion in the systematic review. However, there was considerable heterogeneity between the studies and the results have to be viewed with caution. There were no studies examining probiotics for GORD.

Meta-analysis of three of the trials showed a statistically significant reduction in GOR in the infants receiving probiotics compared to placebo. The remaining individual studies also reported a statistical reduction in episodes of GOR with the use of probiotics (Garofoli, 2014; Indrio, 2011). Only one study looked at maternal probiotic use in the antenatal and postnatal periods and showed a significant reduction in the onset of infant regurgitation (Baldassarre, 2016). In addition, infants receiving probiotics were found to have an increased gastric emptying rate and an increase in number of stools, and infants receiving probiotics had less emergency department visits. It does not appear that probiotics have a positive or negative effect on infant body weight, head circumference or length.

The intestinal microbiota plays a crucial role in the pathogenesis of gastrointestinal disorders [26–28] and an increasing number of studies are targeting probiotic therapy [29–41] for infants and adults. The pathophysiology of regurgitation seems to be multifactorial and involves oesophageal, gastric and enteric nervous system abnormalities (Indrio et al., 2011). Abnormal gut microbiota colonisation may play a crucial role, therefore, early probiotic supplementation may alter colonisation and represent a new strategy for preventing functional gastrointestinal disorders. It is also noted that probiotics could play a role in controlling intestinal inflammation (Indrio et al. 2014; Indrio et al. 2015). In addition, gastric distension and impaired fundal relaxation due to disturbed gastric motility might be a contributor to GOR (Indrio et al. 2011). Probiotics seem to mediate the activity on colonic sensory neurons, specifically the calcium-dependent potassium ion channel in enteric sensory nerves, resulting in an improvement in gut motility, function and effects on visceral pain [45, 47]. It is also proposed that beneficial bacteria such as probiotics aid in modulating intestinal motility and beyond
the gut, on the central and autonomic nervous system [48]. These actions are particularly important in the developing gastrointestinal tract of infants.

We recently considered the impact of infant immaturity, disturbance of the microbiome through caesarean section and maternal mental health. This model emerged from a mixed methods study we undertook examining >1 million admissions in NSW to hospitals in the first year following birth [16]. We then looked more closely at >11 thousand babies admitted with GOR or GORD. Infants with GOR or GORD admitted to hospitals were also likely to have other disorders such as feeding difficulties, sleep problems, and excessive crying. The mothers of babies admitted with GOR/GORD were more likely to be primiparous, Australian born, give birth in a private hospital and have a psychiatric condition, a preterm or early term infant (37-or–38 weeks), a caesarean section, an admission of the baby to a SCN/NICU and be a male infant [16]. We also randomly examined the records of 300 women and babies admitted to residential parenting services in NSW (RPS) and found 36% of infants admitted to residential parenting centres in NSW had been given a diagnosis of GOR/GORD [49]. Eight focus groups were undertaken with 45 nurses and doctors working in these RPS and the qualitative data revealed two themes: “It is over diagnosed” and “A medical label is a quick fix, but what else could be going on?”[16].

We also found that mothers with a mental health disorder were nearly five times as likely to have a baby admitted with GOR/GORD in the first year after birth. This finding is significant and needs further exploration as to the possible mechanism and possible prevention/treatment. It is possible that inconsistent parenting by inexperienced and anxious mothers may increase infant crying. The fact that primiparous women were more likely to have an infant with GOR/GORD supported this [16]. However, it is possible that maternal mental health has a bidirectional relationship with a disturbed microbiome in the mother and the baby. This is where we propose there may be a role for probiotics in restoring a balance and thereby impacting on severity of GOR/GORD symptoms.

It has been shown that maternal probiotic administration during pregnancy and lactation modulates milk composition, improves gastrointestinal symptoms in the baby and provides transfer of immune benefits to the infant [41]. Probiotic administration antenatally, and for the first few months following
birth, may also have a modifying effect on maternal mental health by modifying the microbiome and thus impacting on the brain/gut axis. This is particularly effective with stress related psychopathologies such as anxiety and depression. There appear to be no safety concerns with the administration of L. reuteri in non-immunocompromised subjects and in preterm infants [50]. Several in vitro studies have proven that L. reuteri is also found to exhibit antimicrobial activity, producing reuterin, a broad-spectrum antibacterial substance [51, 52] and regulate immune responses [53] as well as reduce intestinal inflammation [54]; thus it is possible that L. reuteri strains act through diverse mechanisms.

Limitations of the studies in the systematic review
There are several limitations with this systematic review. Four of the studies were published by one author. Whilst there was an overall low risk of bias in the conduct of the studies, there was substantial heterogeneity between the trials and the results have to be viewed with caution. For example, for the meta-analysis for the outcome of GOR at one month, Indio et al., (2011) used L. reuteri DSM 17938 1x10^8 colony-forming units in 5 drops once a day for 30 days as a treatment for formula fed infants with an existing diagnosis of GOR; Indrio et al., (2014) used L reuteri DSM 17938 in 5 drops once a day for 90 days as a prevention for GOR in breast or bottle fed infants, and Indio et al., (2017) used L. reuteri DSM 17938 2.8 X 10^6 colony-forming units/g powder in a commercially available formula for 4 weeks as a treatment for formula fed infants with an existing diagnosis of GOR. Five of the six trials used a small sample size, two of these studies did not report a sample calculation, and for three studies GOR was reported as a secondary outcome. In only one of the trials the probiotic was given to breastfeeding mothers in the antenatal and postnatal period, and in the remaining trials probiotics were given to infants (bottle or breastfed) solely in the postnatal period. Overall, two of the studies enrolled infants with existing GOR and probiotics were used as a treatment and the other studies used probiotics as a preventative measure. Finally, the effect of probiotics on women’s mental health when anxious during pregnancy and the development of GOR in their babies has not been examined.

Conclusions
There is insufficient evidence to confidently judge the effectiveness of probiotics for the prevention
and treatment of GOR in infants. Data from the individual trials and subset meta-analysis of studies measuring the effect of probiotics are promising. The use of probiotics could potentially be a non-invasive, cost effective and preventative positive health strategy for both women and their babies.

Further well controlled RCTs are warranted.

Abbreviations
GOR: gastro-oesophageal reflux; GORD: gastro-oesophageal reflux disease

Declarations
Ethics approval and consent to participate
Not applicable.
Consent for publication
Not applicable.
Availability of data and materials
All data generated or analysed in this study are included in the article.

Competing interests
The authors declare that they have no competing interests.

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Authors’ contributions
JF: Conceptualized the study design, protocol development, inclusion selection, quality assessment and statistical analysis, and drafted the initial manuscript. HD: Was involved in the study conception and design, oversaw the protocol development and data interpretation and participated in the manuscript preparation. SF: Critically reviewed and amended the manuscript. NB: Critically reviewed and amended the manuscript. VS: Critically reviewed and amended the manuscript CT: Critically reviewed and amended the manuscript. CS: Critically reviewed and amended the manuscript KP: Played a major role in the study conception, design, protocol development, inclusion selection, quality assessment and statistical analysis and worked collaboratively on the draft of the initial manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 The quality and risk of bias in the trials included in the systematic review

| Study/year/ reference | Random sequence generation (selection bias) | Allocation concealment (selection bias) | Blinding of participants and personnel (performance bias) | Blinding of outcome assessment (detection bias) | Incomplete outcome data (attrition bias) |
|-----------------------|--------------------------------------------|----------------------------------------|----------------------------------------------------------|-----------------------------------------------|---------------------------------------|
| Baldassarre/2016 [41]  | Low risk                                   | Low risk                               | Low risk                                                 | Low risk                                      | Low risk                               |
| Garofoli/2014 [45]    | Low risk                                   | Unclear risk                           | Low risk                                                 | Low risk                                      | Low risk                               |
| Indrio/2008 [43]      | Low risk                                   | Unclear risk                           | Low risk                                                 | Unclear risk                                  | Low risk                               |
| Indrio/2011 [28]      | Low risk                                   | Unclear risk                           | Low risk                                                 | Unclear risk                                  | Unclear risk                           |
| Indrio/2014/ [26]     | Low risk                                   | Unclear risk                           | Low risk                                                 | Low risk                                      | Low risk                               |
| Indrio/2017/ [44]     | Low risk                                   | Unclear risk                           | Low risk                                                 | Unclear risk                                  | Low risk                               |

Table 2 Characteristics of included trials

| Study/year/ reference | Description/ study design | Age at enrolment/ Birth weight | Probiotic agent(s) | Dosage |
|-----------------------|---------------------------|--------------------------------|-------------------|-------|

20
| Study | Description | Methods | Outcome | Remarks |
|-------|-------------|---------|---------|---------|
| Baldassarre/2016 [41] | April 2011 – Dec. 2013. 67 healthy pregnant women, aged 18–44 years, admitted with low obstetric risk. 67 infants were breast or bottle fed. Dept. of Biomedical and Human Oncological Science, University of Bari, Italy. Double-blind prospective randomised controlled trial. | Infants 37-41 weeks gestation. Birth weight 2440-4730 g. Four different strains of lactobacilli (L. paracasei DSM 24733, L. plantarum DSM 24730, L. acidophilus DSM 24735, and L. delbrueckii subsp. bulgaricus DSM 24734), three strains of bifidobacteria (B. longum DSM 24736, B. breve DSM 24732, and B. infantis DSM 24737), and one strain of Streptococcus thermophilus DSM 24731. | 900 billion lyophilized packets given to women 1 month before delivery and during lactation. |
| Garofoli/2014 [45] | 40 full term breastfed infants. Neonatal unit, Italy. Double-blind prospective randomised controlled trial. | Infants 38.8-40.1 weeks gestation. Infants enrolled first 3 days of life. Birth weight 3243-3490 g. L. reuteri DSM 17938. | 1 x 10^8 colony-forming units in 5 drops once a day for 28 days. |
| Indrio/2008 [43] | Jan. – Sept. 2006 20 formula fed Preterm infants with normal Apgar scores. Neonatology Section of the Dept. of Paediatrics, University of Bari, Italy. Double-blind prospective randomised controlled trial. | Infants 34 ± 1.1 weeks. Enrolled 3 to 5 days of life. Birth weight 1890 ± 432 g. L. reuteri ATCC 55730. | 1 x 10^8 colony-forming units in 5 drops once a day for 30 days. |
| Indrio/2011 [28] | July 2008 – Jan. 2010 42 formula fed infants < 4 months of age with diagnosis of uncomplicated regurgitation. Gastrointestinal Unit of the Dept. of Pediatrics at the University of Bari, Italy. Double-blind prospective randomised controlled trial. | Age in days at enrolment 31-45 days. Weight at enrolment 4990-5100 g. L. reuteri DSM 17938. | 1 x 10^8 colony-forming units in 5 drops once a day for 30 days. |
| Study          | Time Period          | Details                                                                 | Treatment                                | Basket Value |
|---------------|----------------------|-------------------------------------------------------------------------|------------------------------------------|--------------|
| Indrio/2014   | Sept. 2010 – Oct. 2012 | 554 breast or formula fed term neonates <1 week of age. 9 different neonatal units, Italy. Double-blind prospective randomised controlled trial. | *Lactobacillus reuteri* DSM 17938, 1 × 10^8 colony-forming units in 5 drops once a day for 90 days. | L. reuteri DSM 17938, 1 × 10^8 units in 5 drops once a day for 30 days. |
| Indrio/2017   | Jan. 2014 - Feb. 2015 | 80 exclusively formula fed full-term infants Diagnosed with functional regurgitation. Recruited in the Paediatric Gastroenterology Clinic, Dept. of Paediatrics) of the University of Bari, Italy), and in a Paediatric Primary Care Clinic in Naples, Italy. Double-blind prospective randomised trial. | *Lactobacillus reuteri* DSM 17938, 2.8 × 10^8 colony-forming units/g powder in a commercially available formula for 4 weeks. | L. reuteri DSM 17938, 2.8 × 10^8 units in powder commerical formula for 4 weeks. |
Figures

Figure 1
PRISMA study flow diagram

| Study or Subgroup | Probiotic | Control | Mean Difference |
|-------------------|-----------|---------|----------------|
|                   | Mean     | SD      | Total | Mean | SD | Total | IV, Fixed, 95% CI |
| Indio 2008        | 2.4      | 0.9     | 10    | 4.2  | 1.1 | 10    | 9.2% -2.10 [-2.84, -1.36] |
| Indio 2014        | 2.7      | 1.5     | 238   | 3.3  | 2.3 | 238   | 57.3% -0.60 [-0.95, -0.26] |
| Indio 2017        | 2.8      | 1       | 37    | 5.3  | 1   | 35    | 33.5% -2.70 [-3.18, -2.24] |
| Total (95% CI)    | 285      |         | 275   | 100.0%        | -1.44 [-1.71, -1.17] |

Heterogeneity: Chi² = 52.45, df = 2 (P < 0.00001); I² = 96%
Test for overall effect: Z = 10.58 (P < 0.00001)

Figure 2
Forest plot of probiotic versus placebo - GOR at one month of age
Figure 3

Forest plot of probiotic versus placebo – number of stools/day at one month

Figure 4

Forest plot of probiotic vs. placebo – body weight at one month of age

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

PRISMA 2009 checklist_Probiotic_GOR SR_Final.doc