Meta-analyses

Prebiotic supplementation in preterm neonates: Updated systematic review and meta-analysis of randomised controlled trials

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A R T I C L E   I N F O

Article history:
Received 11 September 2012
Accepted 14 May 2013

Keywords:
Prebiotic oligosaccharides
Bifidobacteria
Lactobacillus
Necrotising enterocolitis
Enteral feeds
Sepsis

SUMMARY

Background & aims: Regular administration of prebiotic oligosaccharides promote beneficial gut flora in infants. We aimed to systematically review randomized controlled trials evaluating the safety and efficacy of prebiotic oligosaccharide supplementation in preterm infants ≤37 weeks of gestation.

Methods: Available studies from Medline, Embase, comparing formula milk supplemented with or without prebiotics, reporting on safety and the incidence of necrotising enterocolitis (NEC), late onset sepsis, feed tolerance, physical growth and various stool characteristics were eligible.

Results: 7 trials (n = 417) were included. Five trials (n = 345) reported on the incidence of NEC. 3 trials (n = 295) reported on the incidence of late onset sepsis. Meta-analysis revealed a pooled RR (95% CI) of 1.24 (0.56–2.72) for NEC, 0.81 (0.57–1.15), p = 0.23 for the risk of late onset sepsis. 3 individual trials (n = 295) did not observe any improvement in time to enteral feeds post intervention. Meta-analysis indicated a statistically significant difference in the growth of bifidobacteria in the oligosaccharide group with a weighted mean difference of 0.53 (95% CI: 0.33, 0.73) *10⁶ colonies/g, p < 0.00001. A reduction in stool viscosity and pH was also observed. None of the trials reported life threatening adverse effects.

Conclusions: Supplementation with prebiotic oligosaccharides was safe and did not result in decreased incidence of NEC, late onset sepsis and time to full enteral feeds but resulted in a significantly higher growth of beneficial microbes.

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1. Introduction

Significance of gut flora in the gastrointestinal tracts of newborn infants is well understood.²,³ Prebiotic oligosaccharides (prebiotic OS) are non-digestible food ingredients that selectively stimulate the growth of probiotic bacterial species in the colon, such as bifidobacteria and lactobacilli, which have the potential to improve host health. Human breast milk is the natural source of prebiotic OS. Various synthetic prebiotic OS such as short chain galacto oligosaccharides (GOS), long chain fructooligosaccharides (FOS), inulin, lactulose are available that mimic natural pre OS. Different prebiotic OS tend to offer different advantages. A combination of short chain and long chain pre OS has been thought to mimic natural human milk oligosaccharides the best. Regular administration of prebiotic OS has been shown to improve the gut flora and minimise the growth of pathogenic bacteria in preterm neonates.⁴ Although prebiotic OS supplementation is considered safe, its clinical benefits in preterm infants (e.g. improving feed tolerance, preventing necrotising enterocolitis (NEC)) have not been evaluated properly.⁴ The prebiotic summit in 2008 called for well designed clinical trials to advance further knowledge in this field.⁶ Since the publication of our systematic review in 2009, more trials have been reported that assessed the clinical benefits of prebiotic OS in preterm infants.⁷,⁸ These trials have demonstrated trend towards fewer episodes of late onset sepsis and a possible benefit towards promoting enteral feed tolerance in preterm infants.⁷,⁸ A recent systematic review did not find any evidence that prebiotic supplementation improved weight, length, and head circumference in preterm infants.⁹ Given the clinical importance of such findings, we aimed to update our previously published systematic review.⁵
1. Primary objective

To systematically review randomised controlled trials assessing the safety and efficacy of prebiotic OS supplementation in reducing the risk of NEC and late onset sepsis, and facilitating feed tolerance and physical growth in preterm infants born less than 37 weeks of gestation.

1.2. Secondary objectives

Evaluate the effect of prebiotic OS supplementation on gut colonisation, physical characteristics of stool and gastrointestinal transit time in preterm infants.

2. Materials and methods

Guidelines from the Cochrane Neonatal Review group, PRISMA statement and the Centre for Reviews and Dissemination group were followed for conducting and reporting this systematic review and meta-analysis.\textsuperscript{10,11} In order to be included in this review, the trials had to meet the following criteria:

2.1. Study design

Only randomised controlled trials and quasi-randomised trials published in any language were eligible for inclusion. Case series, retrospective studies, cross over trials, and uncontrolled trials were not eligible.

2.2. Participants

Trials in the preterm infants with gestation < 37 weeks at birth were eligible for inclusion. Trials were excluded if the post-conceptual age at randomisation was >40 weeks.

2.3. Interventions

Trials comparing formula milk supplemented with prebiotic OS vs placebo or unsupplemented formula milk were eligible for inclusion. Trials that supplemented breastfed infants with prebiotic OS were also eligible for inclusion. The prebiotic OS could be GOS, FOS, acidic oligosaccharide (AOS), inulin or lactulose. The supplementation should have continued for at least two weeks. Trials comparing combination of pre and probiotics vs controls were excluded. Trials in which composition of the intervention formula was different from that of controls (e.g., high quantity of beta palmitic acid, use of hydrolysed formula, etc.) were not eligible for inclusion.

2.4. Outcomes measures

Trials with at least one of the following outcome measures were included: incidence of NEC according to Bell stage, blood culture positive late onset sepsis, enteral feed tolerance, symptoms of intolerance to OS supplementation such as vomiting, diarrhoea, regurgitation, irritability and crying leading to cessation of supplementation or any other adverse outcome as reported by the authors. Other outcomes included stool colony count of bifidobacteria, lactobacilli and colonisation with enteric pathogenic bacteria, stool characteristics (e.g., pH, consistency, and frequency), age at full feeds, weight gain during hospital stay, death before discharge from the hospital, gastric or gastrointestinal transit time measurement.

2.5. Search strategy

The Cochrane Central Register of Controlled Trials (CENTRAL, the Cochrane library, Issue 2, 2012), Medline (1966 to July 2012), CINAHL (Cumulative Index of Nursing and allied Health Literature) EMBASE databases, and proceedings of the Pediatric Academic Society Meetings (published online from year 2007), and Pediatric Gastroenterology conferences (from year 2007) were searched in February and July 2012. Medline was searched using the following MeSH words: “Oligosaccharides” AND “Infant Formula” AND “Infant” OR “Infant, Very Low Birth Weight” OR “Infant, Low Birth Weight” OR “Infant, Extremely Low Birth Weight” OR “Infant, Premature” OR “Infant, Newborn” OR “Infant, Small for Gestational Age” OR “Infant, Premature” with limits of “Randomised Controlled Trial, Clinical trial”. The search was repeated using the text word “prebiotic” instead of “Oligosaccharides.” Text words, ‘Inulin’ and ‘Lactulose’ were used to identify additional studies. The reference lists of identified articles and key review articles were searched for additional studies. RS and SR searched the literature independently and assessed the eligibility of trials for inclusion in the review. Any differences were resolved by discussion with the third reviewer (SP).

2.6. Assessment of risk of bias

The methodological quality and the risk of bias of the included trials in terms of randomisation, blinding, allocation concealment, bias, internal validity was assessed separately by the reviewers RS and SR using the Cochrane methodology for systematic review of interventional studies.\textsuperscript{10} In the event of disagreement, consensus was reached by discussion with the third reviewer (SP).

2.7. Data extraction

RS and SR independently extracted the data on a custom designed data collection form. Important data items included demographic characteristics, age at starting prebiotic OS, duration of supplementation, predefined outcome measures and adverse effects. Inconsistencies were resolved by discussion between all three reviewers. In the previous review, the authors of identified studies were contacted to improve the methodological quality of reporting and the results.\textsuperscript{5} Since the newly added studies were assessed to be of good quality, a decision was made to use only the published data.

2.8. Statistical analysis

Meta-analysis was done using Review Manager 5.1 software from The Cochrane collaboration.\textsuperscript{12} Weighted mean difference (WMD) and 95% confidence interval (CI) were calculated for continuous outcomes. Risk ratio was used for summary measure. Heterogeneity was estimated by the I squared statistic. A fixed effects model was used. The results were cross checked with the random effects model.

3. Results

3.1. Search results

Medline search using the previously described MeSH words and combinations revealed 180 studies. The study log and study selection process is presented in Fig. 1.

3.2. Methodological quality

The assessment of methodology and the risk of bias were performed using the Cochrane methodology for interventional trials.\textsuperscript{10}
Each response was categorised as low risk, high risk, and can’t tell. The details of the assessment are provided in Table 1. Most trials were of high quality and constituted low risk in their conduct and reporting. Only Boehm et al. had inadequate details in the published reports and further information was not available after 3 attempts to contact the authors as described in our previous systematic review. Further attempts to clarify the methodology were not made this time.

3.3. Trial characteristics

Seven RCTs (n = 417) were included in this updated review, of which three were new. Westerbeek et al. reported various outcomes from the CARROT study. Five publications from the same study were identified as Boehm 2002. Using the Cochrane methodology 6 trials were considered to be of good quality with a low risk of bias (Table 1). The type, concentration, duration and the timing of supplementation of prebiotic OS and the main outcomes of the trials are highlighted in Table 2.

3.4. Primary outcomes

NEC: None of the included trials were primarily designed and powered to assess the effect of prebiotic OS on the incidence of NEC. Data on this outcome was available from Mihatsch et al., Indrio et al., Riskin et al., Modi et al., Westerbeek et al. NEC did not occur in any of their study infants in Mihatsch and Indrio et al. In the OS vs control group, NEC occurred with a RR of 0.43 (95% CI 0.04–4.25) in Riskin et al., 1.74 (95% CI 0.68–4.71) in Westerbeek et al. and 0.55 (95% CI 0.05–5.99) in Modi et al. respectively. Meta analysis revealed that the pooled RR (95% CI) for OS group was 1.24 (0.56–2.72) compared with the control group with no evidence of heterogeneity (I² statistic 0%) (Fig. 2).

3.5. Late onset sepsis

Data from 3 new trials were available for inclusion (Riskin et al., Westerbeek et al., Modi et al.). Late onset sepsis was defined as being blood culture positive sepsis across all the studies with or without information on clinical conditions. In the pre OS vs control group, late onset sepsis occurred with an RR of 0.43 (95% CI 0.09–1.99) in Riskin et al., 0.78 (95% CI 0.53–1.16) in Westerbeek et al., 1.05 (95% CI 0.45–2.44) in Modi et al. respectively. Westerbeek et al. also observed that in the prebiotic OS-supplemented group, 9 of 55 (16%) infants had >1 serious endogenous (defined as non-coagulase negative infection) infection compared with 17 of 58 (29%) in the placebo group (RR: 0.4, 95% CI: 0.17, 1.16, p = 0.10). Meta analysis observed that the pooled RR (95% CI) for the risk of late onset sepsis for the OS group was 0.81 (0.57–1.15), p = 0.23 compared to the controls (I² statistic 0%) (Fig. 3).

3.6. Time to full enteral feeds

Data from three new trials were available for inclusion. Westerbeek et al. defined full enteral feeds as 120 ml/kg/day while Riskin et al. and Modi et al. defined full feeds as 150 ml/kg/day. Time to full enteral feeds (mean ± SD) in the pre OS vs control group was 41.0 ± 32.0 vs 54.2 ± 31.9 days in Riskin et al. Westerbeek observed that the median (range) time to full enteral

![Fig. 1. Study selection log.](image)

**Table 1** Risk of bias in included studies.

<table>
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<tr>
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<th>Mihatsch15</th>
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<th>Riskin7</th>
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<tr>
<td>Random sequence generation (selection bias)</td>
<td>Low risk</td>
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<td>Allocation concealment (selection bias)</td>
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<td>Blinding of participants and personnel (performance bias)</td>
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<td>Selective reporting (reporting bias)</td>
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Table 2
characteristics of included studies.

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<th>Study ID</th>
<th>Study population and intervention</th>
<th>Outcomes</th>
<th>Results</th>
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<tr>
<td>Boehm 02</td>
<td>Preterm &lt;32 weeks. Intervention: scGOS + lcFOS (9:1), 1.0 g/dl (n = 10). Control: Maltodextrin placebo (n = 10). Reference group of breastfed infants (n = 12). Duration of supplementation: 28 days Timing if supplementation- while on full feeds</td>
<td>Stool bacterial flora on days 7, 14 and 28, stool pH, frequency and consistency. Plasma and urine calcium and phosphate and plasma alkaline phosphatase levels, symptoms of intolerance, anthropometry.</td>
<td>The prebiotic group had a higher colony counts of Bifidobacteria on day 28, no difference in the colony counts of lactobacilli or any individual pathogenic bacteria, lower quantities of all the pathogenic bacteria, softer and more frequent stools and higher ca/p ratio in the urine. No difference in weight and length gain, symptoms of intolerance, plasma calcium, phosphorus and alkaline phosphatase was observed between the two groups.</td>
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<td>Mihatsch 06</td>
<td>Preterm &lt;1500 g and 24–31 weeks at birth intervention: prebiotic: scGOS + lcFOS (9:1), 1.0 g/dl (n = 10). Control: Maltodextrin placebo (n = 10). Duration of supplementation: 14 days timing of intervention: on full enteral feeds</td>
<td>Stool viscosity and consistency, gut transit time, stool frequency, stool pH on day 14, weight gain during study period</td>
<td>In the prebiotic group, stools were softer, less viscous and more acidic. No differences in weight gain, stool frequency between the two groups.</td>
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<td>Indrio 07</td>
<td>Preterm infants &lt;35 weeks intervention: scGOS + lcFOS (9:1), 0.8 g/dl (n = 10). Control: Maltodextrin placebo (n = 10). Duration of supplementation 30 days timing of intervention: on full enteral feeds</td>
<td>Gastric electrical activity, gastric emptying time at 15 and 30 days, weight gain during the study period</td>
<td>Trend towards lower gut transit time in the prebiotic group, (p 0.079). Gastric electrical activity was not different between the groups. Gastric emptying time was lesser in the prebiotic group on day 15. No difference in the gastric emptying time on day 30. No difference in weight gain. Prebiotic group had higher stool colony counts of bifidobacteria, softer and more frequent stools, and lower counts of pathogenic bacteria. Mean weight gain and arm circumference were higher in the placebo group.</td>
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<td>Kapiki 07</td>
<td>Preterm &lt;36 weeks intervention: lcFOS 0.4 g/dl; n = 20. Control: Maltodextrin as placebo; n = 20. Duration of supplementation: 14 days</td>
<td>Stool colony counts of bifidobacteria and pathogenic bacteria after 7 days of supplementation, stool characteristics, frequency; anthropometry on day 7 and 14</td>
<td>No significant difference in the time to full enteral feeds, anthropometric measures or NEC. Prebiotic supplementation was well tolerated. Prebiotic group tended to have lower incidence of sepsis and significantly lower gastric residuals. Lactulose group had higher maximum bilirubin levels, but required less phototherapy, were less anaemic and there was no difference in the transfusion requirements.</td>
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<td>Riskin 10</td>
<td>Preterm 23–34 weeks of gestation intervention: 1% Lactulose n = 15 Control-Maltodextrin as placebo n = 13 duration of supplementation-beyond discharge or 40 weeks of corrected age whichever was earlier. Timing of intervention: by day 3</td>
<td>Stool colonisation, late onset-sepsis, NEC, length of hospital stay, weight at discharge, time to full enteral feeds, neonatal jaundice, anaemia of prematurity, adverse effects of intervention.</td>
<td>Lactulose supplementation did not demonstrate significant increase in lactobacilli levels, no difference in time to full enteral feeds, anthropometric measures or NEC. Prebiotic supplementation was well tolerated. Prebiotic group tended to have lower incidence of sepsis and significantly lower gastric residuals. Lactulose group had higher maximum bilirubin levels, but required less phototherapy, were less anaemic and there was no difference in the transfusion requirements.</td>
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<td>Modi 10</td>
<td>Appropriately grown infants &lt;32 + 6 weeks of gestation. Intervention: scGOS + lcFOS 0.8 g/dl (n = 71) control-standard formula n = 79. Duration of supplementation-Until discharge or 40 weeks of corrected age whichever was earlier timing of intervention: by day 2</td>
<td>Time to full enteral feeds (150 ml/kg/day), time spent on full feeds in the first 28 days, weight length, and head circumference gain, stool flora, stool characteristics, fluid balance, NEC, culture positive sepsis, adverse G1 effects of supplementation.</td>
<td>No significant difference in the time to full enteral feeds, no difference in the proportion of days between birth and 28 d or discharge (whichever came first) that a total daily milk intake of at least 150 ml/kg was tolerated, no difference in the fecal flora, lactobacilli, bifidobacter growth, tolerance of formula, no difference in anthropometric measures, no difference in the incidence of NEC, blood culture positive sepsis. Prebiotic supplementation did not significantly reduce the risk of serious infectious morbidity, did not enhance the postnatal decrease in intestinal permeability in the first week of life, did not affect fecal IL-8 and Calprotectin levels but decreased stool viscosity and stool pH with a trend towards increased stool frequency post supplementation.</td>
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<td>Westerbeek 08</td>
<td>&lt;32 weeks completed or &lt;1500 g intervention: acidic and neutral oligosaccharide (scGOS + lcFOS, 80% and 20%), 1.5 g/kg/day n = 55 Control-Maltodextrin placebo n = 58 Duration of supplementation-30 days timing of intervention: by day 3</td>
<td>Infectious morbidity such as neonatal sepsis, NEC, time to full enteral feeds (defined as 120 ml/kg/day), stool pH, stool frequency, stool viscosity, anthropometric parameters, intestinal permeability in the first week of life, fecal interleukin-8 and fecal calprotectin.</td>
<td>All the above results were not statistically significant. Modi et al. and Westerbeek at al reported no significance without providing the actual values. Meta-analysis was not possible due to heterogeneity in reporting the weight gain. Meta-analysis of the</td>
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feeds for the OS vs the control group was of 10 (4–48) vs 11(7–50) days, p 0.47. while Modi et al. observed that the time required was median (IQR) of 6 (5–8) vs 7 (6–9) days, p = 0.10. Meta analysis was not possible due to variations in the data format.

3.7. Physical growth

All included trials reported on weight gain during the study period and none showed significant difference between the two groups. Trials used different unit measures to report this outcome (e.g. g/kg/day or g/day). Mihatsch et al. reported average median weight gain (g/kg/day, range) of 17.6 (8.1–23.4) vs 13 (9.3–21.9) grams. Indrio et al. and Kapiki et al. reported a mean ± SD weight gain of day 34.50 ± 6.90 vs 34.60 ± 9.46 g and 22.8 ± 6.0 g vs 27.4 ± 7.0 g/day respectively, Riskin et al. reported a mean ± SD weight at discharge of 2567 ± 355 vs 2846 ± 667 g during the study period. All the above results were not statistically significant. Modi et al. and Westerbeek at al reported no significance without providing the actual values. Meta-analysis was not possible due to heterogeneity in reporting the weight gain. Meta-analysis of the
data from Boehm et al., Kapiki et al. and Indrio et al. (n = 106) showed no significant difference in the weight gain between the two groups (WMD = 1.60, 95% CI: 3.7–0.57 g/day) as reported in our previous publication.5

3.8. Secondary outcomes

As for stool colonisation with bifidobacteria and/or lactobacilli, data from two new trials (Modi et al. and Riskin et al.) were available for inclusion in addition to the previously described data from Boehm et al. and Kapiki et al. Boehm et al. and Kapiki et al. observed a significantly higher growth of bifidobacteria after 28 days of supplementation. Meta-analysis of the data from these two trials estimated a statistically significant difference in the growth of bifidobacteria in the pre OS group with a WMD 0.53 (95% CI: 0.33, 0.73) × 106 colonies/g, p < 0.00001 (Fig. 4). Riskin et al. reported lactobacilli count was not significantly different in the pre OS vs control group 3.3 × 106 vs 0.1 × 106, but no bifidobacteria were grown. Modi et al. reported that the fecal flora for lactobacilli and bifidobacteria were not statistically significant in the two groups.

3.9. Stool colonisation with potentially pathogenic bacteria

Data from two new trials Modi et al. and Riskin et al. were available for inclusion in addition to Boehm et al. and Kapiki et al. Modi et al. and Riskin et al. reported that infants who received prebiotic supplementation had lower colony counts of pathogenic bacteria like Escherichia coli, enterobacteria, and enterococci. However, both the studies did not observe statistical significance. Recently, as a part of their RCT, Westerbeek et al. observed that enteral supplementation with pre OS mixture did not affect the intestinal colonisation of specific bacterial groups including that of potentially pathogenic bacteria such as E. coli.

3.10. Stool frequency, consistency

Data from three new trials Westerbeek et al. and Riskin et al. and Modi et al. in addition to two studies previously reported in our review (Boehm et al., Kapiki et al., and Indrio et al.) showed that prebiotic supplemented group had more frequent stooling of softer consistency. Modi et al. and Westerbeek et al. did not observe any significant difference in the stool frequency. Riskin et al. observed that OS supplementation increased the frequency of stools compared to placebo with no change in consistency.

3.11. Stool pH

Data from two new trials (Westerbeek et al. and Modi et al.) were available for inclusion in addition to the three trials included in our previous review. Boehm et al. and Kapiki et al. reported that the stool pH was lower in the prebiotic OS group compared with the controls. Mihatsch et al. reported that the stool was more acidic in prebiotic supplemented group compared with the placebo group (Median pH 5.1, range 4.0, 5.8 vs 6.2, range 5.4, 7.7, p < 0.001). Modi et al. reported that the stool pH was significantly lower in the OS group after supplementation owing to a higher quantity of acetic acid in the gut. Similarly, Westerbeek et al. reported that the stool pH at day 30 was lower in the prebiotic OS group (5.9 ± 0.6) compared with the placebo group (6.2 ± 0.3) (p = 0.009, 95% CI 0.08–0.53).

3.12. Stool viscosity

Data from one new trial Westerbeek et al. was available in addition to Mihatsch et al. as previously described in our review. Westerbeek et al. reported that median (range) stool viscosity was lower in the prebiotic OS group (16.8 N, 3.9–67.8) compared with the placebo group (26.3 N, 1.3–148.0, p = 0.03). Mihatsch 2006 reported that stools of prebiotic supplemented group had lower viscosity compared with placebo group: 31.8 (19.9, 67.3) vs 157.5 (24.1, 314.00, p = 0.006). Mihatsch also observed that in the prebiotic group, the stool viscosity reduced from 74.1 N at the time of study entry to 31.8 N after 14 days of supplementation. During the same period, in the placebo group, the stool viscosity increased from 50.1 N to 157.5 N.
3.13. Gastrointestinal transit time (GTT)

No new trials reported this outcome. As reported in our previous review, the two trials Mihatsch et al.\textsuperscript{15} and Indrio et al.\textsuperscript{17} reported shorter GTT and lower T1/2 at day 15 after prebiotic intervention.\textsuperscript{5}

3.14. Symptoms of intolerance

Boehm et al. and Kapiki et al. reported this outcome.\textsuperscript{13,16} Both reported no difference in the incidence of symptoms of intolerance like excessive crying, irritability, vomiting, and diarrhoea.\textsuperscript{5} Data from 3 new trials were available for inclusion.\textsuperscript{7,18}

These trials reported that OS supplementation was well tolerated and safe with no adverse effects such as vomiting, diarrhoea, regurgitation, irritability and crying leading to cessation of supplementation.\textsuperscript{7,18}

3.15. Other outcomes

Westerbeek et al. reported intestinal permeability using lactulose/mannitol ratio in the first week of life.\textsuperscript{18} The authors did not find any difference in the intestinal permeability from the start of OS supplementation to day 7 of supplementation.\textsuperscript{21} No difference in the excretion of IL-8 and Calprotectin in the OS supplemented group was observed.\textsuperscript{20} A statistically significant reduction in the incidence of mild bronchopulmonary dysplasia at 36 weeks of age was observed in the OS group.\textsuperscript{14}

4. Discussion

Our updated systematic review found a small number of RCTs (7 trials, \( n = 417 \)) which addressed the efficacy and safety of prebiotic OS supplementation in preterm infants. The results suggest that prebiotic OS supplementation is well tolerated by preterm infants, decreases stool pH, reduces stool viscosity and increases gastric and gastrointestinal transit time. OS supplementation also results in a significantly higher growth of bifidobacteria. However, it did not result in clinically important outcomes such as decreased incidence of NEC or late onset sepsis, and reduced time to full enteral feeds. Weight gain was also not significantly different between the two groups. Since the combined sample size of the included trials in the review was small we could not derive firm conclusions on the role prebiotic OS in preterm infants in relation to such clinically important outcomes.

Over the recent years, it has been shown that augmentation of healthy gut flora using probiotic bacteria in preterm infants confers protection against NEC and all cause mortality.\textsuperscript{20} Prebiotic OS have the potential to confer similar protection by stimulating the growth of healthy gut flora and reduce the load of potential pathogens\textsuperscript{6,24} without the need for administering probiotics.\textsuperscript{5} Prebiotic OS have the added advantage that they are inert and reach the colon intact. The OS molecules act as ligands and receptors-analogs, inhibiting the adhesion of pathogens on the epithelial surface in the gastrointestinal tract\textsuperscript{23} protecting the gut integrity. OS may also exhibit direct immuno modulatory properties by their action on toll like receptors. Along with their immuno modulatory properties, reduction in stool pH by liberating short chain fatty acids may minimise the growth of pathogenic bacteria further shifting the balance towards healthier gut flora.\textsuperscript{5} Thus prebiotic OS administration may confer protection against neonatal conditions such as late onset sepsis and NEC.

The rationale for the role of prebiotic OS in enteral tolerance stems from faster gastric and gastrointestinal motility.\textsuperscript{15,17} Riskin et al. observed that prebiotic OS supplemented group had significantly lower gastric residuals during and after supplementation.\textsuperscript{7} Similarly, improvement in enteral feed tolerance in a subgroup of infants was observed by Modi et al. in their preterm prebiotic study.\textsuperscript{8} Modi et al. observed from the analysis of covariance (ANCOVA) model that the predicted enteral tolerance with the GOS/FOS formula was 2.9% higher for a baby born at 28 wk gestation and 9.9% higher at 26-wk gestation, but decreased or of no benefit in babies born > 31 wk of gestation. These results suggest a potentially beneficial role of prebiotic OS in extremely preterm infants, who are the highest risk group for feed intolerance, NEC and late onset sepsis.\textsuperscript{8}

The variations in the growth of bifidobacteria in various studies could be partly explained by different combinations and doses of prebiotic OS used in different studies (Table 2). Westerbeek et al. used a combination of short chain GOS and long chain FOS and demonstrated an increase in the growth of Bifidobacteria,\textsuperscript{18} while Riskin et al. used 1% lactulose and showed a preferential growth of Lactobacilli.\textsuperscript{7} Although pre OS promotes the growth of beneficial gut flora, not all pre OS are the same. Different pre OS and their combinations exert varying effects on gut flora. The recent studies that reported the time to enteral feeds, OS supplementation was started within the first 72 h when the bacterial colonisation of the gut may not have been well established.\textsuperscript{7,18,19} Moro et al. have reported that the proliferation of bifidobacteria following prebiotic OS supplementation is dose dependent and the degree of prebiotic response is also dependent on baseline counts of bifidobacteria.\textsuperscript{31}

It is noted that OS was added to breast milk in both, the control as well as the intervention group, which may reduce the effect of OS itself as breast milk also contains human milk OS. However in the study by Westerbeek et al., the effect of OS was not significantly modified by the type of feeding, suggesting that addition of OS also has an effect in breastfed preterm infants.\textsuperscript{8,18}

Administration of prebiotic OS and modification of gut flora in the newborn period may not be devoid of complications.\textsuperscript{12} Animal studies have pointed towards increased bacterial translocation in rat pups fed with GOS and inulin mixture and decreased resistance of newborn pups to salmonella colonisation in the gut after administration of fructose oligosaccharide and inulin.\textsuperscript{33} Increased stool acid content due to fermentation of prebiotic OS may increase d-lactic acid levels in the gut, similar to short gut syndrome causing
serious neurological side effects has been reported in hepatic encephalopathies treated with prebiotic OS.34 However, the included trials documented a sto1 pH in the range of 5–7 which are thought to be not harmful in infants. None of the included trials identified any other adverse events during pre OS supplementation. We did not come across any clinical reports of bacterial translocation following pre OS supplementation. Despite this, further research into the beneficial effects of modification of gut flora and its adverse effects warrants targeted studies with adequate measures to document short and long term adverse outcomes.

Preterm infants are frequently exposed to broad spectrum antibiotics.5,53,35 Antibiotic administration has been known to affect the gut flora in preterm infants, for as long as one month after the administration. The beneficial effects of pre OS are expected to be suboptimal in presence of antibiotic exposure.37 Recently, as a part of the CARROT study, Westerbeek et al. found that the use of broad-spectrum antibiotics in preterm infants decreased the growth of all intestinal microbiota and opine that it may negatively influence the normal microbiota development and interfere with the bacterial–epithelial ‘cross-talk’ necessary for normal gut development.15 This interaction needs to be considered while designing future studies.

The strengths of our updated systematic review are that it has been conducted using robust methodology. Since the trials were small and not powered for clinically important outcomes such as late onset sepsis and NEC, firm conclusions could not be drawn. Further trials powered for clinically important outcomes should be conducted to advance the concept of prebiotics and its efficacy in clinically important outcomes.

Funding sources
None.

Statement of authorship
All the authors contributed towards the design, concept and preparation of the manuscript. RS and SR conducted literature search, included the eligible trials, analysed the data and prepared the initial manuscript.

SP was responsible for the intellectual content, concept of the study and finalised the manuscript.

Conflict of interests
None stated.

Acknowledgements
None stated.

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