Probiotics for the prevention of surgical necrotizing enterocolitis: systematic review and meta-analysis

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Keywords: necrotizing enterocolitis; probiotics; surgery; meta-analysis

Aim of the Study

Probiotic administration to preterm infants has the potential to prevent necrotizing enterocolitis (NEC). Data from randomized controlled trials (RCTs) are conflicting but meta-analyses seem to support this intervention. To date, these analyses have not focussed on surgical NEC. We aimed to determine the effect of probiotic administration to preterm infants on prevention of surgical NEC.

Methods

A systematic review of RCTs of probiotic administration to preterm infants was performed. Studies were included if RCT outcomes included any of (i) Bell's Stage 3 NEC; (ii) surgery for NEC; (iii) deaths attributable to NEC. Article selection and data extraction was performed independently by two authors; conflicts were adjudicated by a third author. Data were meta-analysed using Review Manager 5.3. A random effects model was decided on *a priori* because of the heterogeneity of study design; data are risk ratio (RR) with 95% CI.

Main Results

Thirty-eight RCTs reported NEC as an outcome. Data on surgical NEC could be extracted from 19 RCTs, all of which were included. A variety of probiotic products was administered across studies. Description of surgical NEC in most studies was poor. Only 6/19 specifically reported incidence of surgery for NEC, 12/19 Bell's stage 3 and 13/19 NEC-associated mortality. Although there was a trend towards probiotic administration reducing stage 3 NEC, this was not significant (RR 0.74 [0.52-1.05], p=0.09). There was no effect of probiotics on the RR of surgery for NEC (RR 0.84 [0.56-1.25], p=0.38). Probiotics did, however, reduce the risk of NEC-associated mortality (RR 0.56 [0.34-0.93], p=0.03)

Conclusion

Despite 38 RCTs on probiotic prevention of NEC, evidence for prevention of surgical NEC is not strong, partly due to poor reporting. In studies included in this meta-analysis, probiotic administration was associated with a reduction in NEC related mortality.

Key messages

- The evidence that probiotic administration is associated with a decreased incidence of surgical NEC is limited
- This is mainly due to poor reporting of surgical NEC in randomized controlled trials and we urge better reporting of surgical aspects of NEC in future trials

What is known about the subject

In various RCTs and meta-analyses, it has been suggested that probiotic administration is associated with a decrease in incidence of definite NEC.

What this study adds

The reporting of surgical aspects of NEC in RCTs of probiotic administration is poor.

The evidence that probiotic administration is associated with a decrease in incidence of surgical NEC, or surgery for NEC, is limited.

Probiotic administration is associated with a decrease in NEC-associated mortality.

Introduction

Although necrotizing enterocolitis (NEC) is the most common life-threatening surgical emergency affecting neonates, we still do not know how to prevent or medically treat the disease[1]. Many infants with NEC may have surgery with the aim of removal of necrotic intestine. Although the indications for surgery are not well-defined, radiological evidence for intestinal perforation is often regarded as an absolute indication for surgery, and many surgeons would operate for failure to improve, or clinical deterioration, in response to medical management such as cessation of enteral feeds, antibiotic treatment and supportive treatment[2]. In the last few years, there has been a surge of interest in the potential role of probiotics to prevent NEC and this has resulted in the publication of many randomized controlled trials (RCTs), followed by systematic reviews and meta-analyses of these RCTs[3-6]. Some commentators have asserted that it is 'almost unethical' to withhold probiotic administration to all preterm infants in order to prevent NEC[6]. As the type/strain, dose, duration and timing of probiotics is not standardized, others find the evidence less compelling[7]. The American Pediatric Surgical Association Outcomes and Clinical Trials Committee[8] considered the level of evidence for routine probiotic supplementation and concluded that available data supported the routine supplementation of premature infants with probiotics although no conclusions could be drawn for the extremely-low birthweight population (i.e. those with the highest incidence of NEC) due to lack of data. However, most RCTs, and the systematic reviews and meta-analyses that result, focus on the development of confirmed NEC (i.e. Bell's stage 2) and not on the potential effect of probiotic administration on surgical NEC. We focused on surgical NEC since there is general recognition that infants who are treated surgically have more advanced disease than those who are managed medically and importantly are noted to have worse outcomes including higher mortality, more frequent need for further surgery and greater long term neurodevelopmental impairment.The aim of this study was to perform a systematic review and meta-analysis in order to compare the effects of probiotic administration and placebo on surgical NEC in preterm infants.

Methods

A systematic review of available literature (Ovid Medline Jan 1974-Jun 2017) was conducted using the search strategy (probiotic* OR pro-biotic* or probio* OR lactobacill* OR bifidobacter* OR saccharomyces* OR bacillus) AND ((necrotizing enterocolitis or necrotising enterocolitis or necrotizing entero-colitis or necrotising entero-colitis) OR (necrot* and (enterocoli* or entero-coli*)) OR ("necrotizing" or "entero-colitis" or

"enterocolitis") and NEC)) AND publication type randomized controlled trial. A similar search was also conducted in Ovid Embase (Jan 1980-June 2017). Hand searching of the reference lists of published studies, and citation searching using Web of Knowledge (Thomson-Reuter) was also performed in order to identify additional studies. A formal protocol was not prepared for this study.

Inclusion criteria were defined as follows: (i) RCT; (ii) study compared enteral probiotics to placebo or no treatment; (iii) study population defined as premature infants; (iv) explicit data available on incidence of *either* (A) Bell's Stage 3 NEC, (B) surgery for NEC or (C) NEC-associated mortality. Initial screening for inclusion was performed independently by two authors, using the online tool Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia, available at www.covidence.org). Adjudication regarding inclusion/exclusion was performed by the other two authors. The following data were extracted: number of infants treated with probiotic/ placebo, infants with/without Bell's Stage 3 NEC, infants having surgery for NEC (including peritoneal drain), Bell's Stage 2/3 NEC, deaths attributable to NEC. Data on Bell's stage 2/3 NEC, the outcome measure most frequently reported in meta-analyses of probiotics for prevention of NEC, was extracted from included papers (i.e. only those including surgical outcomes) in order to determine whether this subset of papers was representative of the larger group of studies with broader inclusion criteria.

Data were meta-analysed using Review Manager 5.3. A random effects model was decided on *a priori* because of the heterogeneity of study design; data are risk ratio (RR) with 95% CI; heterogeneity was assessed using I^2 and associated Chi-squared test, and Funnel plots prepared for assessment of bias across studies. An additional analysis (not pre-planned) using bacterial products only was also performed. Power calculations were performed using an online tool (Sealed Envelope Ltd. 2012), Power calculator for binary outcome superiority trial. Available from: https://www.sealedenvelope.com/power/binary-superiority/) using an α error of 5% and β of 80%.

Results

The search strategy yielded 169 abstracts, and further searching an additional abstract that was potentially eligible. Full text screening as described in the methods led to the selection of 19 articles for inclusion[9-27] and 24 articles for exclusion[28-51]. A flow chart indicating screening, inclusion and exclusion of studies is shown in Figure 1. Characteristics of the 19 included studies are shown in Table 1. The lack of consistency and clarity regarding

definition of NEC as an RCT outcome, and reporting of surgical NEC (Bell's stage 3, infants having surgery for NEC) was notable. Most excluded papers did not report surgical NEC; two further papers[26 27] were included as it was possible to extract data on surgical outcomes from the papers only because there was a zero incidence of NEC in either arm (and therefore a zero incidence of surgical NEC) rather than explicit reporting of surgical NEC in the RCT outcomes.

Bell's stage 2/3 NEC

Data on Bell's stage 2/3 NEC were obtainable from 19/19 included studies[9-27]; incidence in the placebo group varied between 16% and 0%. Probiotic administration was associated with a significant reduction in the incidence of Bell's stage 2/3 NEC (RR 0.64 [0.48, 0.84], p<0.002). There was a low degree of heterogeneity between studies (I^2=14%, p=0.29).

Bell's stage 3 NEC

Data on Bell's stage 3 as an outcome were available from 14/19 included studies[9-12 14 16-18 21 22 24-27], of which 12/19 explicitly reported reported Bell's stage 3[9-12 14 16-18 21 22 24 25]; incidence in the placebo group varied between 7% and 0%. Probiotic usage was not associated with a significant effect on the incidence of stage 3 NEC, although there was a trend towards a decrease, with a similar risk ratio to that of Bells' stage 2-3 NEC (RR 0.74 [0.51-1.05], p=0.09, Figure 2A). There was no evidence for significant heterogeneity (I^2=0%, p=0.73).

Surgery for NEC

Data on surgery for NEC were available from only 8/19 studies[10 12 13 17 18 22 26 27] of which only 6/19 explicitly reported incidence of surgery for NEC[10 12 13 17 18 22]. There was no effect of probiotics on the RR of surgery for NEC (RR 0.84 [0.56-1.25], p=0.38, Figure 2B). There was no evidence for significant heterogeneity (I^2=0%, p=0.83)

Deaths attributable to NEC

Data on death attributable to NEC were available from 15/19 studies[9 11-15 17 19-23 25-27], of these 13/19 explicitly reported death attributable to NEC[9 11-15 17 19-23 25]. In one study, some deaths were reported as being from Bell's stage 1, however, by consensus these deaths were not included as the diagnosis of NEC had not been confirmed[25]. Probiotic administration was associated with a significant reduction in the risk of NEC-associated mortality (Figure 2C, RR 0.56 [0.34-0.93], p=0.03) with no evidence for significant heterogeneity (I^2=0%, p=0.85).

Analysis by probiotic product

We repeated the analyses, excluding those using only a fungal probiotic product[14 23 26]. Administration of bacterial probiotic products was associated with a significant reduction in the incidence of Bell's stage 2/3 NEC (RR 0.57 [0.41, 0.80], p=0.001), a trend towards a

decrease in Bell's stage 3 NEC (RR 0.73 [0.50-1.05], p=0.09), no effect on the RR of surgery for NEC (RR 0.84 [0.56-1.25], p=0.38, and a significant reduction in the risk of NEC-associated mortality (RR 0.53 [0.31-0.90], p=0.02).

Assessment of bias

The risk of bias in individual studies is shown in Supplementary Table 1. In order to assess evidence for publication bias, funnel plots for each of the outcomes (Bell's stage 2/3, Bell's stage 3, surgery for NEC and mortality attributable to NEC) were generated (Figure 3). For each outcome, the apex of the funnel plot is a RR of <1, providing some limited evidence for bias towards publication of studies favouring probiotic administration, although the ability to detect publication bias is limited by the low number of publications for some of these outcomes.

Discussion

Over the past 10 years, there have been many meta-analyses and Cochrane Reviews evaluating probiotic administration for the prevention of NEC[3-6]. To our knowledge, none to date has specifically focused on whether or not probiotics reduce NEC requiring surgery. In our present systematic review, we found that surgical aspects of NEC were rather poorly reported in RCTs of probiotic administration; of 37 papers reporting any data on NEC as an RCT outcome, only 18 (49%) specifically reported surgical NEC (and in a further two data could be extrapolated due to the zero incidence of any NEC), and in one of these, data could not be used due to unconventional reporting of Bell's staging[51].

The available data from included papers suggests that probiotic administration was not associated with a significant decrease in the risk of developing Bell's stage 3 NEC or having surgery for this condition. Previous meta-analyses of probiotics have shown a significant effect of probiotic administration in decreasing the incidence of Bell's stage 2-3 NEC. In order to determine whether the 16 papers that specifically reported surgical NEC were representative of the larger group of papers that report Bell's stage 2-3 NEC, we also analysed the effect of probiotic administration on Bell's stage 2-3 NEC in the 16 papers reporting surgical NEC. Consistent with the findings of other meta-analyses with less restrictive inclusion criteria [3-5 52], we also demonstrated a significant decrease in the risk of developing Bell's stage 2-3 NEC with probiotic administration. Although the risk of developing Bell's stage 3 NEC was similar to that of developing stage 2-3 NEC, the difference in risk of Bell's stage 3 disease was non-significant. This was due to wider confidence intervals associated with a smaller number of patients.

This review has demonstrated a statistically significant effect of probiotics in reducing mortality attributed to NEC, with a relative risk of 0.56. It is of interest to analyse specifically mortality attributable to NEC as most meta-analyses examine all-cause mortality[3-5 52] and we are not aware of any that have analysed mortality attributable to NEC. It may seem counter-intuitive that probiotics significantly decrease the risk of NEC associated mortality without a significant effect on the risk of surgical NEC. This can be explained firstly because more studies reported deaths than reported either Bell's stage 3 or surgery for NEC. Secondly, up to 20% of infants who have been diagnosed as having definite NEC die without ever having an operation or a post-mortem examination [53] and in addition, many of the studies reporting mortality from NEC did not have mortality as a defined primary or secondary endpoint.

There are a number of potentially confounding factors that should be considered when interpreting these results. None of the RCTs reviewed for this study included a protocol for the decision to proceed to surgery nor precise indications for surgery in infants with NEC. This is an important factor to consider given the decision or indication to perform surgery may differ between surgeons and centre[2]. A further confounding issue is the likely inclusion of infants with spontaneous intestinal perforation (SIP) in reports of infants with NEC. Many surgeons have debated whether SIP and NEC are a similar disease but there is now greater acceptance that they are distinct disease entities. We are not aware of any reports that suggest probiotics influence the risk of developing SIP. Although our present study does not show evidence that probiotics reduce surgical NEC, we acknowledge that in the absence of consistent reporting of both indications for surgery and definitions of NEC/SIP, we should be cautious when generalising our findings. Diagnosis of NEC, staging of the disease according to Bell's criteria, is a problematic area, and both pneumatosis intestinalis (the main criterion used to define Bell's stage 2 NEC) and pneumoperitoneum (the main criterion used to define Bell's stage 3 NEC) have poor inter-observer agreement- even between expert radiologists[54-56]. It is also worth noting that not all probiotic RCTs had independent radiologists.

There are many difficulties in meta-analysing probiotic trials. Cross colonisation of the placebo group is one, with data from one RCT[12] suggesting that up to 37% of placebo allocated participants were colonized with the probiotic intervention after two weeks. Inconsistent and limited data reporting trial outcomes by colonization status precluded such analyses in our present study, though data from one large RCT suggests non-significant trends towards reduced NEC in babies successfully colonised with probiotics[57]. Furthermore,

probiotics work through a diverse range of mechanistic actions and not all probiotics act via the same mechanism[58]. One of the controversies in using probiotics relates to the uncertainty of which probiotic will achieve optimum benefit. In this meta-analysis, a variety of different probiotic products were used. Even if we concluded that probiotics were effective in preventing surgical NEC, we would not be able to recommend a specific product, strain, concentration or even species. Too few studies are available to be able to be meaningfully analyse by the type of probiotic administered.

Given the observed data, in order to detect a significant difference in Bell's stage 3 NEC, a randomised controlled trial would need to recruit 2757 patients in each arm. This is likely prohibitive, so we may never have robust evidence to answer the question of whether probiotic administration prevents surgical NEC. However, recent advances in understanding the microbiological basis for the development of NEC[59] provide some hope that appropriately targeted probiotic therapies could be effective in reducing the devastating effects of this disease. In conducting future randomised controlled trials we recommend that robust reporting of surgical NEC, SIP and any abdominal surgery (e.g. indications for surgery, operation performed, surgical outcomes) will allow us to more clearly assess the benefits of probiotic interventions.

Acknowledgements

SE gratefully acknowledges support from Great Ormond Street Children's Charity and the NIHR Great Ormond Street Biomedical Research Centre. These funders had no input into the systematic review.

Figure legends

Figure 1

Flow chart showing selection of studies for inclusion in the systematic review and meta-analysis.

Figure 2 Meta-analysis of included studies

A: Bell's stage 3 NEC in infants who received probiotic or placebo

B: Surgery for NEC in infants who received probiotic or placebo

C: Mortality attributed to NEC in infants who received probiotic or placebo

Figure 3 Funnel plots of included studies

A: Bell's stage 2-3 NEC in infants who received probiotic or placebo

B: Bell's stage 3 NEC in infants who received probiotic or placebo

C: Surgery for NEC in infants who received probiotic or placebo

D: Mortality attributed to NEC in infants who received probiotic or placebo

Table 1 – Characteristics of included studies

Study	Year	Probiotic used	Placebo	No of patients		Primary outcome defined		Bells stage 3	Surgery for NEC	Death from NEC
				Probiotic	Placebo					
Al-Hosni	2012	Lactobacillus rhamnosus GG + Bifidobacterium infantis in milk	Unsupplemented milk	50	51	Weight	501-1000g	x	x	
Bin-Nun	2005	Lactobacillusbifidus, streptococcus thermophillus, And bifidobactrium infantis in milk	Unsupplemented milk	72	73	NEC	<1500g BW	x		x
Costeloe	2015	Bifidobacterium breve BBG-001 in milk	Unsupplemented formula	654	661	NEC (stage 2 or 3)	23-30 weeks GA	x	x	x
Dani	2002	Lactobacillus GG in milk	Maltodextrins in milk	295	290	Urinary Tract Infection, Bacterial Sepsis and NEC	<33 weeks GA or <1500g BW		x	x
Demirel	2013	Saccharomyces boulardii	No addition	135	136	NEC stage ≥2, death	<32 weeks GA and <1500g BW	x		x
Fernandez-Carrocera	2013	Lactobacillus 4 spp. Bifidobacteruim infantis, Streptococcus thermophillus	No addition	75	75	NEC	<1500g BW	x		x
Jacobs	2013	Bifidobacterium infantis, Streptococcus thermophilus, and Bifidobacterium lactis	Maltodextrin	548	551	late-onset sepsis	<32 weeks GA and BW < 1500 g			x
Lin	2005	Lactobacillus acidophilus and Bifidobacter infantis	No addition	180	187	NEC, death, sepsis	<1500g BW	x	x	
Lin	2008	Lactobacillus acidophilus and Bifidobacter bifidum	No addition	217	217	NEC, death	<1500g	x		x
Manzoni	2006	Lactobacillus casei subspecies rhamnosus	No addition	39	41	enteric fungal colonization	<1500g	x	x	x

Oncel	2013	Lactobacillus reuteri in oil base	Oil base	200	200	Death, NEC	<32 weeks GA and <1500g BW			x
Rougé	2009	Bifidobacterium longum and Lactobacillus rhamnosus	Maltodextrin	45	49	Enteral feeding	<32 weeks GA and <1500g BW			x
Saengtawesin	2014	Lactobacillus acidophilus and Bifidobacterium bifidum	No addition	31	29	NEC, death	<34 weeks GA and <1500g BW	x		x
Sari	2011	Lactobacillus sporogenes	No addition	110	111	NEC, death	<33 weeks GA and <1500g BW	x	x	x
Serce	2013	Saccharomyces boulardii	Distilled water	104	104	NEC, sepsis, death	<34 weeks GA and <1500g BW			x
Tewari	2015	Bacillus clausii	Sterile water	123	121	Sepsis	<34 weeks GA	x		
Totsu*	2014	Bifidobacterium bifidum	Dextrin	153	130	Full enteral feeding	<1500g	x	x	x
Van Nierkerk	2015	Lactobacillus rhamnosus and Bifidobacterium infantis	Medium chain triglyceride oil	91	93	NEC, sepsis	>500 g and <1250 g, breast milk fed	x		x
Xu*	2016	Saccharomyces boulardii	No addition	63	62	Growth	30-37 weeks and 1500-2500g BW	x	x	x

*Totsu et al. and Xu et al. both reported zero cases of NEC so other NEC outcomes are by definition zero.

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Figure 1

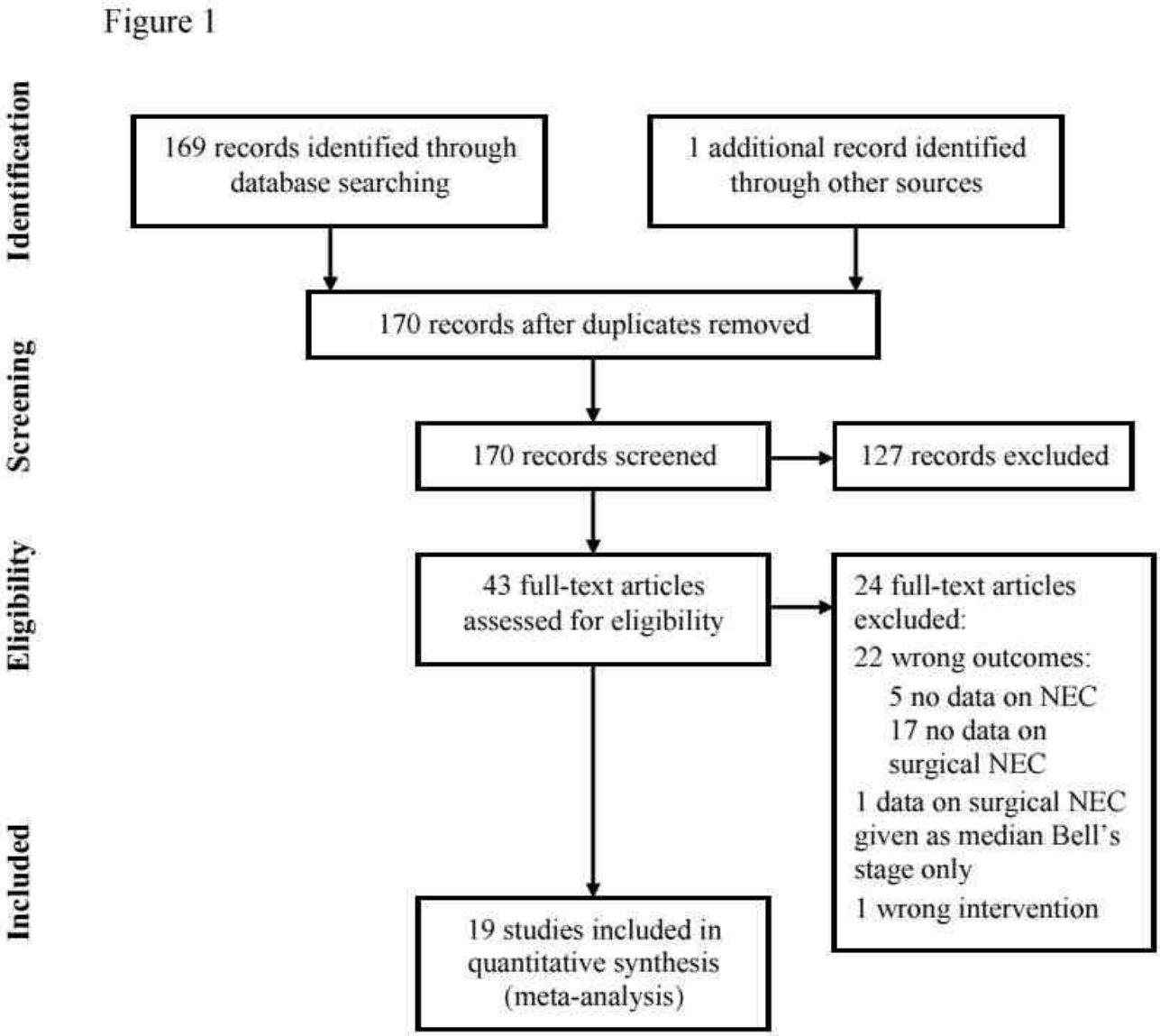


Figure 1
Identification
Screening
Eligibility
Included
169 records identified through database searching
1 additional record identified through other sources
170 records after duplicates removed
170 records screened
127 records excluded
43 full-text articles assessed for eligibility
24 full-text articles excluded:
22 wrong outcomes:
5 no data on NEC
17 no data on surgical NEC
1 data on surgical NEC given as median Bell's stage only
1 wrong intervention
19 studies included in quantitative synthesis (meta-analysis)

Figure 2

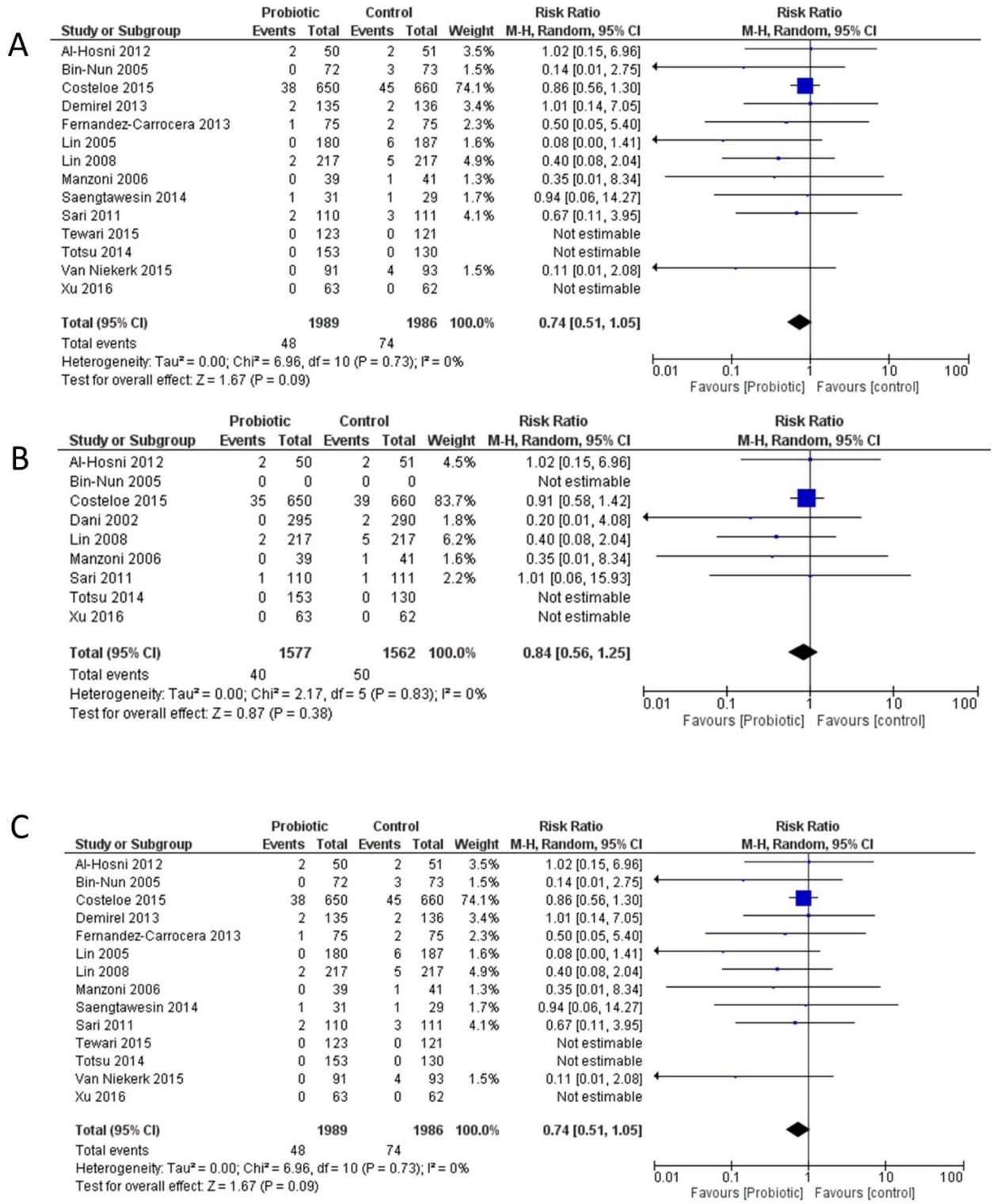
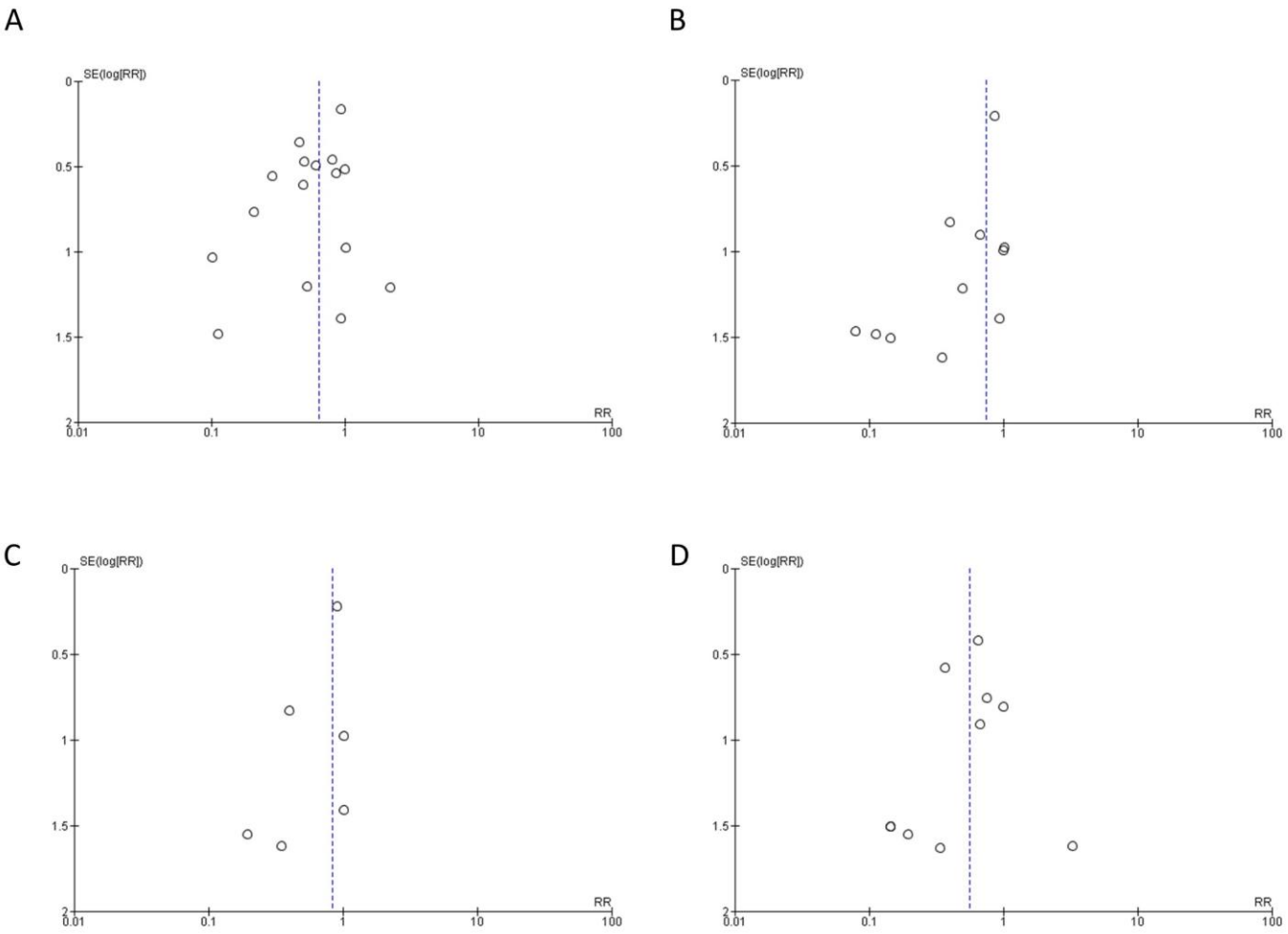

A
Probiotic
Control
Risk Ratio
Risk Ratio
Study or Subgroup
Events Total Events Total Weight M-H, Random, 95% CI
M-H, Random, 95% CI
Al-Hosni 2012 2 50 2 51 3.5% 1.02 [0.15, 6.96]
Bin-Nun 2005 0 72 3 73 1.5% 0.14 [0.01, 2.75]
Costeloe 2015 38 650 45 660 74.1% 0.86 [0.56, 1.30]
Demirel 2013 2 135 2 136 3.4% 1.01 [0.14, 7.05]
Fernandez-Carrocera 2013 1 75 2 75 2.3% 0.50 [0.05, 5.40]
Lin 2005 0 180 6 187 1.6% 0.08 [0.00, 1.41]
Lin 2008 2 217 5 217 4.9% 0.40 [0.08, 2.04]
Manzoni 2006 0 39 1 41 1.3% 0.35 [0.01, 8.34]
Saengtawesin 2014 1 31 1 29 1.7% 0.94 [0.06, 14.27]
Sari 2011 2 110 3 111 4.1% 0.67 [0.11, 3.95]
Tewari 2015 0 123 0 121 Not estimable
Totsu 2014 0 153 0 130 Not estimable
Van Niekerk 2015 0 91 4 93 1.5% 0.11 [0.01, 2.08]
Xu 2016 0 63 0 62 Not estimable
Total (95% CI) 1989 1986 100.0% 0.74 [0.51, 1.05]
Total events 48 74
Heterogeneity: Tau² = 0.00; Chi² = 6.96, df = 10 (P = 0.73); I² = 0%
Test for overall effect: Z = 1.67 (P = 0.09)
0.01 0.1 1 10 100
Favours [Probiotic] Favours [control]
B
Probiotic
Control
Risk Ratio
Risk Ratio
Study or Subgroup
Events Total Events Total Weight M-H, Random, 95% CI
M-H, Random, 95% CI
Al-Hosni 2012 2 50 2 51 4.5% 1.02 [0.15, 6.96]
Bin-Nun 2005 0 0 0 0 Not estimable
Costeloe 2015 35 650 39 660 83.7% 0.91 [0.58, 1.42]
Dani 2002 0 295 2 290 1.8% 0.20 [0.01, 4.08]
Lin 2008 2 217 5 217 6.2% 0.40 [0.08, 2.04]
Manzoni 2006 0 39 1 41 1.6% 0.35 [0.01, 8.34]
Sari 2011 1 110 1 111 2.2% 1.01 [0.06, 15.93]
Totsu 2014 0 153 0 130 Not estimable
Xu 2016 0 63 0 62 Not estimable
Total (95% CI) 1577 1562 100.0% 0.84 [0.56, 1.25]
Total events 40 50
Heterogeneity: Tau² = 0.00; Chi² = 2.17, df = 5 (P = 0.83); I² = 0%
Test for overall effect: Z = 0.87 (P = 0.38)
0.01 0.1 1 10 100
Favours [Probiotic] Favours [control]
C
Probiotic
Control
Risk Ratio
Risk Ratio
Study or Subgroup
Events Total Events Total Weight M-H, Random, 95% CI
M-H, Random, 95% CI
Al-Hosni 2012 2 50 2 51 3.5% 1.02 [0.15, 6.96]
Bin-Nun 2005 0 72 3 73 1.5% 0.14 [0.01, 2.75]
Costeloe 2015 38 650 45 660 74.1% 0.86 [0.56, 1.30]
Demirel 2013 2 135 2 136 3.4% 1.01 [0.14, 7.05]
Fernandez-Carrocera 2013 1 75 2 75 2.3% 0.50 [0.05, 5.40]
Lin 2005 0 180 6 187 1.6% 0.08 [0.00, 1.41]
Lin 2008 2 217 5 217 4.9% 0.40 [0.08, 2.04]
Manzoni 2006 0 39 1 41 1.3% 0.35 [0.01, 8.34]
Saengtawesin 2014 1 31 1 29 1.7% 0.94 [0.06, 14.27]
Sari 2011 2 110 3 111 4.1% 0.67 [0.11, 3.95]
Tewari 2015 0 123 0 121 Not estimable
Totsu 2014 0 153 0 130 Not estimable
Van Niekerk 2015 0 91 4 93 1.5% 0.11 [0.01, 2.08]
Xu 2016 0 63 0 62 Not estimable
Total (95% CI) 1989 1986 100.0% 0.74 [0.51, 1.05]
Total events 48 74
Heterogeneity: Tau² = 0.00; Chi² = 6.96, df = 10 (P = 0.73); I² = 0%
Test for overall effect: Z = 1.67 (P = 0.09)
0.01 0.1 1 10 100
Favours [Probiotic] Favours [control]

Figure 3


A
SE(log[RR])
0
0.5
1
1.5
2
0.01
0.1
1
10
100
RR
B
SE(log[RR])
0
0.5
1
1.5
2
0.01
0.1
1
10
100
RR
C
SE(log[RR])
0
0.5
1
1.5
2
0.01
0.1
1
10
100
RR
D
SE(log[RR])
0
0.5
1
1.5
2
0.01
0.1
1
10
100
RR

Supplementary Table 1: Risk of bias in individual studies

Study	Sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessors	Incomplete outcome data	Selective outcome data	Other sources of bias / Comments
Al-Hosni 2012	unclear	unclear	low	low	low	low	randomisation process not defined, hence unclear
Bin-Nun 2005	unclear	unclear	low	low	low	low	randomisation process not defined, hence unclear
Costeloe 2015	low	low	low	low	low	low	
Dani 2002	unclear	low	low	unclear	low	low	used envelopes but not reported how sequence was generated, says double blind but blinding procedures not described, likely most relevant for outcome assessment
Demirel 2013	low	low	low	low	low	low	
Fernandez-Carrocera 2013	low	unclear	low	low	low	low	
Jacobs 2013	low	low	low	low	low	low	
Lin 2008	low	unclear	low	unclear	low	low	assignment sent to principal investigator when eligible patient so 'unclear'; NEC as an outcome defined blind but not stated for other outcomes and local PI knew assignment
Lin 2005	unclear	low	low	unclear	low	low	NEC as an outcome was defined blind but not stated for other outcomes and local PI
Manzoni 2006	low	unclear	high	high	low	low	clinical staff do not appear to have been blinded
Oncel 2014	low	low	unclear	unclear	low	low	
Rouge 2009	low	low	unclear	unclear	low	low	
Saengtawesin 2014	low	unclear	unclear	unclear	low	low	
Sari 2011	low	low	low	low	low	low	low for NEC, unclear for other outcomes
Serce 2013	low	low	low	unclear	low	low	
Tewari 2015	low	low	low	low	low	low	
Totsu 2014	low	unclear	unclear	unclear	low	low	Cluster randomized, stated double-blind but hospital allocation concealment not clear
Van Niekerk 2015	low	unclear	low	low	low	low	
Xu 2016	unclear	unclear	medium	medium	low	low	Staff administering product not involved in care but not clear who assessed outcomes