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

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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\textsuperscript{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\textsuperscript{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\textsuperscript{3-6}. Some commentators have asserted that it is ‘almost unethical’ to withhold probiotic administration to all preterm infants in order to prevent NEC\textsuperscript{6}. As the type/strain, dose, duration and timing of probiotics is not standardized, others find the evidence less compelling\textsuperscript{7}. The American Pediatric Surgical Association Outcomes and Clinical Trials Committee\textsuperscript{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-co-lis)) 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-Reuters) 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 $\alpha$ error of 5% and $\beta$ 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 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; 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 (I²=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, of which 12/19 explicitly reported Bell’s stage 3; 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 (I²=0%, p=0.73).

Surgery for NEC

Data on surgery for NEC were available from only 8/19 studies of which only 6/19 explicitly reported incidence of surgery for NEC. 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 (I²=0%, p=0.83)

Deaths attributable to NEC

Data on death attributable to NEC were available from 15/19 studies, of these 13/19 explicitly reported death attributable to NEC. 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. 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 (I²=0%, p=0.85).

Analysis by probiotic product

We repeated the analyses, excluding those using only a fungal probiotic product. 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 NEC3-6. 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 staging51.

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\(^3\)\(^-\)\(^5\) 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\textsuperscript{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\textsuperscript{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**

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**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

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Probiotic used</th>
<th>Placebo</th>
<th>No of patients</th>
<th>Primary outcome defined</th>
<th>Bells stage 3</th>
<th>Surgery for NEC</th>
<th>Death from NEC</th>
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<td>Al-Hosni</td>
<td>2012</td>
<td>Lactobacillus rhamnosus GG + Bifidobacterium infantis in milk</td>
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<td>50</td>
<td>Weight</td>
<td>x</td>
<td>x</td>
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<td>NEC</td>
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<td></td>
<td>&lt;1500g BW</td>
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<td>Costeloe</td>
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<td>Unsupplemented formula</td>
<td>654</td>
<td>NEC (stage 2 or 3)</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<td>23-30 weeks GA</td>
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<td>2002</td>
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<td>Maltodextrins in milk</td>
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<td></td>
<td>x</td>
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<td></td>
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<tr>
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<td>548</td>
<td>late-onset sepsis</td>
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<td>&lt;32 weeks GA and BW &lt; 1500 g</td>
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<td>2005</td>
<td>Lactobacillus acidophilus and Bifidobacter infantis</td>
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<td>x</td>
<td>x</td>
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<td></td>
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<td></td>
<td></td>
<td>&lt;1500g</td>
<td></td>
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<td>Year</td>
<td>Treatment Details</td>
<td>Method</td>
<td>Survival Rate</td>
<td>NEC, Death, Sepsis, Growth</td>
<td>GA and BW</td>
<td>Treatment Details</td>
<td>Survival Rate</td>
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<td>Oncel</td>
<td>2013</td>
<td>Lactobacillus reuteri in oil base</td>
<td>Oil base</td>
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<td>200</td>
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<td>Bifidobacterium longum and Lactobacillus rhamnosus</td>
<td>Maltodextrin</td>
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<td>49</td>
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<td>Saengtawe sin</td>
<td>2014</td>
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<td>No addition</td>
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<td>GA and &lt;1500g BW</td>
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<td>Sterile water</td>
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<td>121</td>
<td>&lt;34 weeks</td>
<td>GA</td>
<td>x</td>
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<td>2014</td>
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<td>130</td>
<td>&lt;1500g</td>
<td></td>
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<td>Van Nierkerk</td>
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<td>Lactobacillus rhamnosus and Bifidobacterium infantis</td>
<td>Medium chain triglyceride oil</td>
<td>91</td>
<td>93</td>
<td>NEC, sepsis</td>
<td>&gt;500 g and &lt;1250 g, breast milk fed</td>
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<td>2016</td>
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<td>62</td>
<td>30-37 weeks</td>
<td>1500-2500g BW</td>
<td>x</td>
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</tbody>
</table>

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


Figure 1

Identification
- 169 records identified through database searching
- 1 additional record identified through other sources

170 records after duplicates removed

Screening
- 170 records screened
- 127 records excluded

Eligibility
- 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

Included
- 19 studies included in quantitative synthesis (meta-analysis)
### Supplementary Table 1: Risk of bias in individual studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Sequence generation</th>
<th>Allocation concealment</th>
<th>Blinding of participants and personnel</th>
<th>Blinding of outcome assessors</th>
<th>Incomplete outcome data</th>
<th>Selective outcome data</th>
<th>Other sources of bias / Comments</th>
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<td>unclear</td>
<td>unclear</td>
<td>low</td>
<td>low</td>
<td>low</td>
<td>low</td>
<td>randomisation process not defined, hence unclear</td>
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<td>Bin-Nun 2005</td>
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<td>unclear</td>
<td>low</td>
<td>low</td>
<td>low</td>
<td>low</td>
<td>randomisation process not defined, hence unclear</td>
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<td>Costeloe 2015</td>
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<td>used envelopes but not reported how sequence was generated, says double blind but blinding procedures not described, likely most relevant for outcome assessment</td>
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<td>Demirel 2013</td>
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<td>low</td>
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<td>NEC as an outcome was defined blind but not stated for other outcomes and local PI knew assignment</td>
</tr>
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<td>Manzoni 2006</td>
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<td>clinical staff do not appear to have been blinded</td>
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<td>low</td>
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<td>low</td>
<td>low for NEC, unclear for other outcomes</td>
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<tr>
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<td>unclear</td>
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<td>Cluster randomized, stated double-blind but hospital allocation concealment not clear</td>
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<td>unclear</td>
<td>medium</td>
<td>medium</td>
<td>low</td>
<td>low</td>
<td>Staff administering product not involved in care but not clear who assessed outcomes</td>
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