Prevention of necrotizing enterocolitis in premature infants – an updated review

Yu-Ting Jin, Yue Duan, Xiao-Kai Deng, Jing Lin

Abstract

Necrotizing enterocolitis (NEC) is among the most common and devastating diseases encountered in premature infants, yet the true etiology continues to be poorly understood despite decades of research. Recently, gut bacterial dysbiosis has been proposed as a risk factor for the development of NEC. Based on this theory, several best clinical practices designed to reduce the risk of NEC have been proposed and/or implemented. This review summarizes the results of recent clinical trials and meta-analyses that support some of the existing clinical practices for reducing the risk of NEC in premature infants. It is evident that human milk feeding can reduce the incidence of NEC. While most of the studies demonstrated that probiotic supplementation can significantly reduce the incidence of NEC in premature infants, there are still some concerns regarding the quality, safety, optimal dosage, and treatment duration of probiotic preparations. Antibiotic prophylaxis does not reduce the incidence of NEC, and prolonged initial empirical use of antibiotics might in fact increase the risk of NEC for high-risk premature infants. Lastly, standardized feeding protocols are strongly recommended, both for prevention of postnatal growth restriction and NEC.

Key words: Necrotizing enterocolitis; Prevention; Human milk feeding; Probiotics; Empiric antibiotics; Standardized feeding protocols

Core tip: In this review, we summarize some of the clinical practices recommended to reduce the risk of necrotizing enterocolitis (NEC) in premature infants. Firstly, it is
evident that human milk feeding can reduce the incidence of NEC. Secondly, while most of the studies demonstrated that probiotic supplementation can significantly reduce the incidence of NEC in premature infants, there are still some concerns regarding the quality of probiotic preparations, safety, optimal dosage, and treatment duration. Thirdly, initial empiric antibiotic use should be restricted in daily practice to reduce the incidence of NEC. Lastly, standardized feeding protocols are recommended both for prevention of postnatal growth restriction and NEC.

Citation: Jin YT, Duan Y, Deng XK, Lin J. Prevention of necrotizing enterocolitis in premature infants – an updated review. World J Clin Pediatr 2019; 8(2): 23-32
URL: https://www.wjgnet.com/2219-2808/full/v8/i2/23.htm
DOI: https://dx.doi.org/10.5409/wjcp.v8.i2.23

INTRODUCTION
Necrotizing enterocolitis (NEC) is among the most common and devastating diseases encountered in premature infants, yet the true etiology continues to be poorly understood, despite decades of research. Prematurity remains the most consistent risk factor, although term babies can develop NEC with a much lower incidence. Based on a recent large study from the Canadian Neonatal Network, approximately 5.1% (1.3%-12.9%) of infants with a gestational age < 33 wk develop NEC, and the incidence increases with decreasing gestational age[1]. Despite advances in care in the neonatal intensive care unit (NICU), the estimated mortality rate associated with NEC ranges between 20% and 30%, with the highest rate among infants requiring surgery. Following recovery from the acute phase of NEC, long term complications include intestinal strictures and short bowel syndrome[2-4].

The classical presentation of NEC includes feeding intolerance, abdominal distension, and bloody stools after 8-10 d of age when feeding enterally. The signs and symptoms are quite variable, ranging from feeding intolerance to evidence of a fulminant intra-abdominal catastrophe with peritonitis, sepsis, shock, and death. Many theories have attempted to elucidate the true pathogenesis since Santulli et al[5] first described a series of NEC cases in premature infants with respiratory distress syndrome. Most theories about the pathogenesis of NEC have focused on the most important risk factors, such as immaturity, formula feeding, and the presence of bacteria[6]. More recently, gut bacterial dysbiosis has been proposed as the main risk factor for the development of NEC[7]. Based on this theory, several best clinical strategies are being recommended to reduce the risk of NEC. These include breast milk feeding, restrictive use of antibiotics, supplementation with probiotics, and standardized feeding protocols (SFPs). The purpose of this review is to summarize the results of the recent clinical trials that provide evidence supporting these practices in premature infants as methods to reduce the risk of NEC.

LITERATURE REVIEW
A search was conducted in PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) for studies published before 15 June 2018. The search included all terms related to NEC and preventive interventions, including human milk feeding, probiotics, prophylactic antibiotics, and SFPs, utilizing PubMed MeSH terms and free-text words and their combinations through the appropriate Boolean operators. Similar criteria were used for searching MEDLINE. The review was limited to clinical studies involving human subjects. All relevant articles were accessed in full text following PRISMA guidelines. The manual search included references of retrieved articles. We reported the results in tables and text.

HUMAN MILK FEEDING
The unique properties of human milk promote an improved host defense and gastrointestinal function. Several well controlled clinical trials have demonstrated that human milk feeding can reduce the incidence of NEC. The results of the recent
randomized trials are summarized in Table 1[8-12]. Cristofalo et al[8] and Schanler et al[9] demonstrated that human milk feeding could reduce the incidence of NEC in premature infants compared to those fed with preterm formula in their randomized trials. Sullivan et al[10] studied a total of 207 infants and found that feeding with an exclusively human milk-based diet is associated with a significantly lower rate of NEC than a diet of human milk fortified with bovine milk-based products. Human milk feeding also reduces the incidence of late onset sepsis in premature infants[12].

Human donor milk is considered a safe alternative when the mother’s own milk is not available. When the mother’s breast milk supply was deficient, the short-term outcomes related to safety and efficacy were similar in very low birth weight (VLBW) infants who were fed with pasteurized donor milk or with preterm formula in the first 10 d of life[40]. For feeding extremely preterm infants, donor milk offered little short-term preponderance over preterm formula[40-43]. However, by using the Cochrane Neonatal search strategy, Quigley et al[44] performed a systematic review and meta-analysis of formula versus donor breast milk for feeding preterm or low birth weight (LBW) infants. They identified 11 randomized or quasi-randomized trials in which 1809 infants participated, and they concluded that donor milk feeding decreased the risk of NEC based on the meta-analysis. However, formula-fed infants had higher in-hospital rates of weight gain, linear growth and head growth[45]. Furthermore, infants fed with donor human milk-based fortifier had approximately 64% lower odds of developing NEC compared to those fed with bovine-based fortifiers[14]. It is clear that human milk feeding can reduce the risk of NEC. Recently, the policy statement from the American Academy of Pediatrics on the use of human breast milk states that preterm infants should only receive their own mother’s milk or pasteurized human donor milk when their own mother’s milk is not available[46].

Human milk colostrum is high in protein, fat-soluble vitamins, minerals, and immunoglobulins. The benefit of colostrum for newborn infants has been well established. However, most extremely premature infants are usually not ready to be fed in the first few days of life for a variety of reasons. Several studies support the use of colostrum for oral care to provide immunotherapy in preterm infants. The efficacy of oropharyngeal colostrum therapy (OCT) in the prevention of NEC in VLBW infants has been reviewed, and a meta-analysis on this topic was recently published[47]. Only randomized controlled trials and quasi-randomized trials performed in VLBW infants or preterm infants with gestational age < 32 wk were included for the meta-analysis. As a result, a total of 148 subjects (77 in OCT arm and 71 in control arm) in four trials were analyzed, and no statistically significant difference in the incidence of NEC was demonstrated. The authors concluded that the current evidence was not sufficient to enable the recommendation of OCT as a routine clinical practice in the prevention of NEC[48].

**ADMINISTRATION OF PROBIOTICS**

Establishment of a normal intestinal microbial colonization after birth is vital for proper maturity of the innate immune system and maintenance of intestinal barrier function. It has been proposed that disruption of the normal gut microbiota formation may play a major role in the pathogenesis of NEC in premature infants[49]. Probiotics are live micro-organisms that, upon ingestion at certain amounts, confer health to the host. It is known that probiotics can produce bacteriostatic and bactericidal substances, thus having immunomodulatory effects; furthermore, they prevent colonization of pathogens by competing for adhesion to the intestinal mucosa[50]. One strategy to prevent NEC is oral administration of probiotics to alter the balance of the gut microbiome in favor of non-pathogenic bacteria. In the past two decades, multiple randomized clinical trials in preterm infants have been performed to evaluate the effect of probiotic administration on NEC prevention. The results of these studies are summarized in Table 2. A total of 10520 infants have now been enrolled in probiotic-NEC studies, and a cumulative pooled meta-analysis of the effects of probiotics on NEC was recently published[51]. In these trials, a wide variety of probiotic strains, dosages, and durations were used. Despite the clinical heterogeneity, the conclusion of the cumulative meta-analysis was that probiotic treatment decreased the incidence of NEC (average estimate of treatment effect, relative risk: 0.53; 95%CI: 0.42-0.66)[51]. Therefore, it is clear that some oral probiotics can prevent NEC and decrease mortality in preterm infants. However, it is unclear whether a single probiotic or a mixture of probiotics is most effective for the prevention of NEC. Furthermore, some questions remain unanswered regarding the quality of probiotic products, safety, optimal dosage, and treatment duration.

Probiotics are not all equally effective in preventing NEC in preterm infants. A
Table 1  Summary of five randomized controlled trials of human milk feeding on the risk of necrotizing enterocolitis

| Ref.          | Yr | GA  | BW     | N   | Study   | Control | NEC ≥ stage II, % | Mortality, % | LOS, % |
|---------------|----|-----|--------|-----|---------|---------|-------------------|--------------|--------|
| Corpeleijn et al[8] | 2016 | <1500 | 373 | DM | PF | 9.3 | 8.9 | No | 13.7 | 12.7 | No | 36.6 | 34.7 | No |
| Cristofalo et al[9] | 2013 | 500-1250 | 53 | HM | PF | 3 | 21 | Yes | 0 | 8 | No | 55 | 79 | No |
| Sullivan et al[10] | 2010 | 500-1250 | 207 | HM | Bovine milk | 5.8 | 15.9 | Yes | - | - | No | 19 | 13 | No |
| Schanler et al[11] | 2005 | <30 | - | 243 | DM | PF | 6 | 9 | No | 3 | 3.6 | No | 1 | 4.8 | No |
| Schanler et al[12] | 1999 | 26-30 | - | 108 | HM | PF | 1.6 | 13 | Yes | - | - | No | 31 | 48 | Yes |

BW: Birth weight; LOS: Late onset sepsis; NEC: Necrotizing enterocolitis.

A detailed analysis of the published data on the effects of probiotics for preterm infants regarding specific probiotic strains was recently performed by the ESPGHAN Working Group on Probiotics, Prebiotics and Committee on Nutrition[20]. They concluded that both Lactobacillus rhamnosus GG and Bifidobacterium lactis Bb-12/B94 appeared to be effective in reducing NEC. Both the combination of Lactobacillus rhamnosus GG with Bifidobacterium longum BB536 and the combination of Bifidobacterium lactis Bb-12 with Bifidobacterium longum BB536, however, showed no measurable effect. They suggest that we need to more precisely define the optimal treatment strategies before the routine clinical use of probiotics in preterm infants for NEC prevention can be recommended. Another recent meta-analysis concluded that multiple strains of probiotics were associated with a significantly lower incidence of NEC, with a pooled OR of 0.36 (95%CI: 0.24-0.53; P < 0.00001)[21]. As probiotics are neither drugs nor devices, they fall into a peculiar category of medical intervention and are therefore not strictly regulated. Because the cost of probiotics is low and the consequences of NEC can be devastating, given the available evidence and safety profile of probiotics from the large number of infants studied, a strong argument can be made for the routine use of probiotics in all preterm infants during their NICU stay[22].

RESTRICT EMPIRIC ANTIBIOTIC USE

Empiric antibiotics are commonly used in preterm infants immediately after birth due to the possibility that infection caused preterm labor and the relatively high risk for sepsis in VLBW infants. Because the presence of bacteria is one of the main risk factors
Table 2  Summary of 23 randomized controlled trials of probiotics on the risk of necrotizing enterocolitis

| Ref. | Yr  | GA  | BW   | N   | Study                                                                 | NEC ≥ stage II, % | Mortality, % | LOS, % |
|------|-----|-----|------|-----|----------------------------------------------------------------------|-------------------|--------------|--------|
|      |     |     |      |     |                                                                     | Study            | Control | P < 0.05 | Study | Control | P < 0.05 | Study | Control | P < 0.05 |
| Shashidhar et al[25] | 2017 | -   | 750-1499 | 104 | L. acidophilus, L. rhamnosus, B. longum and S. boulardii            | 4.1              | 12.5       | No     | 1.9   | 5.7       | No     | -        | -        |
| Güney-Varal et al[26] | 2017 | ≤ 32 | ≤ 1500  | 110 | L. rhamnosus, L. casei, L. plantarum and B. animalis                | 0                | 10         | Yes    | 1.4   | 22.5      | Yes    | 17.1     | 35       | No      |
| Xu et al[27]          | 2016 | 30-37| 1500-2500| 125 | S. boulardii                                                           | 0                | 0          | No     | -     | -         | -      | 7.8      | 12.2     | No      |
| Hays et al[28]        | 2016 | 25-31| 700-1600| 199 | B. lactis, B. longum, B. lactis and B. longum.                      | 5.5              | 5.8       | No     | -     | -         | -      | -        | -        |
| Costelo et al[29]     | 2016 | 23-30| -      | 1310| B. breve                                                              | 9.4              | 10.0      | No     | 8.3   | 8.5       | No     | 11.2     | 11.7     | No      |
| Patole et al[30]      | 2014 | < 33 | -     | 159 | B. breve                                                              | 0                | 1.3       | No     | 0     | 0         | No     | 22       | 16       | No      |
| Totsu et al[31]       | 2014 | -    | < 1500 | 283 | B. bifidum                                                            | 0                | 0         | No     | 1.3   | 0         | No     | 8.5      | 13.1     | No      |
| Benor et al[32]       | 2014 | ≤ 32 | ≤ 1500 | 58  | L. acidophilus and B. lactis                                         | 4                | 18.2      | No     | 1     | 2         | NO    | 24       | 18       | No      |
| Oncel et al[33]       | 2014 | ≤ 32 | ≤ 1500 | 454 | L. reuteri                                                            | 4.0              | 5.0       | No     | 7.5   | 10        | No     | 6.5      | 12.5     | Yes     |
| Jacobs et al[34]      | 2013 | < 32 | < 1500 | 1099| B. infantis, S. thermophilus and B. lactis                           | 2.0              | 4.4       | Yes    | 4.9   | 5.1       | No     | 14.2     | 16.5     | No      |
| Serce et al[35]       | 2013 | ≤ 32 | ≤ 1500 | 208 | S. boulardii                                                          | 6.7              | 6.7       | No     | 3.8   | 4.8       | No     | 24.3     | 18.3     | No      |
| Fernández-Carrocerra et al[36] | 2013 | -    | < 1500 | 150 | L. acidophilus, L. rhamnosus, L. casei, L. plantarum, B. infantis and S. thermophilus | 8.0              | 16.0      | No     | 1.3   | 9.3       | No     | -        | -        |
| Demirel et al[37]     | 2013 | ≤ 32 | ≤ 1500 | 271 | S. boulardii                                                          | 4.4              | 5.1       | No     | 3.7   | 3.6       | No     | 14.9     | 15.4     | No      |
| Rojas et al[38]       | 2012 | ≤ 2000 | 750   | 24  | L. reuteri                                                            | 2.4              | 4.0       | No     | 5.9   | 7.4       | No     | 9.1      | 10.6     | No      |
| Al-Hosni et al[39]    | 2012 | -    | 501-1000| 101 | L. rhamnosus and B. infantis                                         | 6.0              | 7.8       | No     | 6     | 7.8       | No     | 26.0     | 31.4     | No      |
| Beaga et al[40]       | 2011 | -    | 750-1499| 231 | B. breve and L. casei                                                | 0                | 3.6       | Yes    | 21.8 | 24.1       | No     | 33.6     | 37.5     | No      |
BW: Birth weight; LOS: Late onset sepsis; NEC: Necrotizing enterocolitis.

for NEC, some believe that the use of prophylactic antibiotics may decrease the risk of NEC. Others feel that the opposite is true and that the altered normal postnatal gut colonization due to antibiotic use may contribute to the pathogenesis of NEC\textsuperscript{[17]}. Several randomized controlled clinical trials have been performed to evaluate the effect of prophylactic antibiotic administration on the risk of NEC. The results of randomized controlled trials are summarized in Table 3\textsuperscript{[47-51]}. Although Siu et al\textsuperscript{[50]} found that prophylactic oral vancomycin conferred some protection against NEC in VLBW infants, Tagare et al\textsuperscript{[47]}, Kenyon et al\textsuperscript{[48]} and Owen et al\textsuperscript{[51]} found no protective effect of routine antibiotic use in low risk preterm neonates. Rather, their data suggest that antibiotic may increase the risk of NEC. The efficacy of prophylactic antibiotic usage in the prevention of NEC in premature infants was reviewed, and a meta-analysis on this topic was recently published\textsuperscript{[52]}. Only randomized controlled trials or retrospective cohort studies in LBW infants or preterm infants were included in the meta-analysis. As a result, a total of 5207 infants were included in nine studies. Based on their meta-analysis, the authors conclude that the current evidence does not support the use of prophylactic antibiotics to reduce the incidence of NEC for high-risk premature infants\textsuperscript{[52]}.

On the other hand, restricting the use of initial empiric antibiotics course may be important. There is increasing recognition that prolonged empirical antibiotic use might increase the risk of NEC for high-risk premature infants. Cotton et al\textsuperscript{[53]} investigated initial empirical antibiotic practices for 4039 extremely low birth weight (ELBW) infants, and 2147 infants in the study cohort received initial empirical antibiotic treatment for more than 5 d. The data suggest that the administration of empiric antibiotics for more than 4 d when the blood culture is negative increases odds of NEC or death in ELBW infants. They suggest that prolonged initial empirical antibiotic therapy for infants with sterile cultures may be associated with increased risk of subsequent death or NEC and should be used with caution. In another retrospective 2:1 control-case analysis from Yale, 124 cases of NEC were matched with 248 controls. Infants with NEC were less likely to have had respiratory distress syndrome ($P = 0.018$) and more likely to have achieved full enteral feeding ($P = 0.028$) than were the controls. The risk of NEC significantly increased with duration of antibiotic exposure when infants with culture-confirmed sepsis were removed from the cohort, and exposure to antibiotics for more than 10 d resulted in an approximately three-fold increase in NEC risk\textsuperscript{[54]}.  

| Study          | Year | BW (cm) | Age (cm) | N | Species                  | BWG | AgeG | Duration (d) | Recovery Rate | Nutritional Rate | Infection Rate | Outcome Rate |
|----------------|------|---------|----------|---|--------------------------|-----|------|--------------|----------------|-----------------|----------------|--------------|
| Sari et al\textsuperscript{[41]} | 2011 | < 33    | < 1500   | 221 | L. sporogenes            | 6.5 | 9    | No           | 2.7            | 3.6             | No             | 26.4         | 23.4         | No           |
| Mihatsch et al\textsuperscript{[18]} | 2010 | < 30    | < 1500   | 183 | B. lactis                | 2.2 | 4.5  | No           | 2.2            | 1.1             | No             | -            | -            | -            |
| Samanta et al\textsuperscript{[42]} | 2009 | < 32    | < 1500   | 274 | B. infantis, B. bifidum, B. longum and L. acidophilus | 5.5 | 15.8 | Yes          | 4.4            | 14.7            | Yes           | 14.3         | 19.5         | Yes          |
| Lin et al\textsuperscript{[43]}     | 2008 | < 34    | < 1500   | 434 | B. bifidum and L. acidophilus | 1.8 | 6.5  | Yes          | 0.9            | 4.1             | No            | 19.8         | 11.5         | No           |
| Lin et al\textsuperscript{[44]}     | 2005 | -       | < 1500   | 367 | L. acidophilus and B. infantis | 1.1 | 5.3  | Yes          | 3.9            | 10.7            | Yes           | 12.3         | 19.3         | Yes          |
| Bin-Nun et al\textsuperscript{[45]} | 2005 | - ≤ 1500| < 1500   | 145 | B. infantis, S. thermophilus, and B. bifidus | 4   | 16.4 | Yes          | 0              | 20.5            | Yes           | -            | -            | -            |
| Dani et al\textsuperscript{[46]}    | 2002 | < 33    | < 1500   | 585 | L. rhamnosus             | 1.4 | 2.8  | No           | -              | -               | -             | 4.7          | 4.1          | No           |
Table 3  Summary of five randomized controlled trials of prophylactic antibiotics on the risk of necrotizing enterocolitis

| Ref. | Yr GA BW | Study | NEC ≥ stage II, % | Mortality, % | LOS, (%) |
|------|----------|-------|-------------------|--------------|---------|
|      |          |       |                   | Study | Contro | P < 0.05 | Study | Contro | P < 0.05 | Study | Contro | P < 0.05 |
| Tagare et al[47] | 2010 < 37 - 140 | Amoxicillin clavulanic acid and amikacin | 13.0 | 4.2 | Yes | 2.9 | 2.8 | No | 1.4 | 14.1 | Yes |
| Kenyon et al[48] | 2002 < 37 - 4809 | Co-amoxiclav | 1.6 | 0.3 | Yes | 5.7 | 6.2 | No | 6.2 | 7.9 | No |
| Oei et al[49] | 2001 ≤ 32 - 43 | Erythromycin | 4.5 | 4.8 | No | 4.5 | 4.8 | No | - | - | - |
| Siu et al[50] | 1998 < 1500 | Vancomycin | 12.7 | 27.5 | Yes | 15.5 | 18.8 | No | - | - | - |
| Owen et al[51] | 1993 24-33 - 117 | Ampicillin | 14 | 3.5 | Yes | 6.8 | 12 | No | 3.4 | 10 | No |

BW: Birth weight; LOS: Late onset sepsis; NEC: Necrotizing enterocolitis.

STANDARDIZED FEEDING PROTOCOL

A current challenge in clinical NEC research is the high variation in feeding practices. It is clear that consistency in approach to feeding intolerance, feeding advancement and breast milk promotion all impact NEC. SFPs address a consistent approach to the: (1) preferred feeding substance; (2) advancement and fortification of feeding; (3) criteria to stop and specifying how to re-start feedings once held; (4) identification and handling of feeding intolerance; and (5) initiation and duration of trophic feeding. SFPs are simple, inexpensive, effective, and transmissible methods for prevention of postnatal growth restriction in premature infants.

In 2015, a total of 482 infants were enrolled in a feeding bundle study, which was a prospective quality improvement project to standardize a protocol for initiating and advancing enteral feeds, and to improve the nutritional care of neonates admitted to the NICU[55]. In this study, the feeding bundle included breast milk feeding, initiating feedings within 24 h of birth, fortification of breast milk with additional calcium, phosphorus and vitamin D, and the use of trophic feeding for 5 d for ELBW infants followed by daily increases of 10 to 20 mL/kg per day if criteria for tolerance are met. The rate of NEC after bundle implementation was decreased compared to the baseline rate of NEC prior to bundle implementation. Therefore, the authors suggest that early initiation and advancement of enteral feedings does not increase NEC risk, but may actually improve the outcomes[5]. In 2016, Gephart et al[14] reviewed papers published and found that studies consistently showed lower or unchanged NEC rates when SFPs were used. They combined data from nine observational studies of infants with birth weight < 1500 g and showed overall reduced odds of NEC by 67% (OR = 0.33, 95%CI: 0.17, 0.65, P = 0.001) when SFPs were used. Therefore, it is possible that SFPs reduce the risk of NEC.

CONCLUSION

In this review, we summarize the results of the recent clinical trials and meta-analyses that support some of the common clinical practices to reduce the risk of NEC in premature infants. Firstly, it is evident that human milk feeding can reduce the incidence of NEC. We suggest enhanced lactation support in all NICUs, as well as the establishment of more human milk banks in NICUs. Secondly, while most of the studies demonstrated that probiotic supplementation can significantly reduce the incidence of NEC in premature infants, there are still some concerns in regards to the quality of probiotic preparations, safety, optimal dosage, and treatment duration. Thirdly, antibiotic prophylaxis does not reduce the incidence of NEC, and prolonged empirical use of antibiotics may in fact increase the risk of NEC for high-risk premature infants. Therefore, restricting initial empiric antibiotic use should be implemented in daily practice. Lastly, SFPs are recommended both for prevention of postnatal growth restriction and NEC.

ACKNOWLEDGEMENTS
We would like to thank Dr. Robert Green for critical review of the manuscript.

REFERENCES

1. Yee WH, Sororiamsh AS, Shah VS, Aziz K, Yoon W, Lee SK; Canadian Neonatal Network. Incidence and timing of presentation of necrotizing enterocolitis in preterm infants. *Pediatrics* 2012; 129: e298-e304 [PMID: 22271701 DOI: 10.1542/peds.2011-2022]

2. Fitzgibbon SC, Ching Y, Yu D, Carpenter J, Kenny M, Weldon C, Lillehei C, Valim C, Horbar JD, Jakicis T. Mortality of necrotizing enterocolitis expressed by birth weight categories. *J Pediatr Surg* 2009; 44: 1072-1075; discussion 1075-6 [PMID: 19524719 DOI: 10.1016/j.jpedsurg.2009.02.013]

3. Hull MA, Fisher R, Gutierrez IM, Jones BA, Kang KH, Kenny M, Zurakowski D, Modi BP, Horbar JD, Jakicis T. Mortality and management of surgical necrotizing enterocolitis in very low birth weight neonates: a prospective cohort study. *J Am Coll Surg* 2014; 218: 1148-1155 [PMID: 24468227 DOI: 10.1016/j.jamcollsurg.2013.11.015]

4. Thyoka M, de Coppi P, Eaton S, Khoo S, Hall NJ, Curry J, Kiely E, Drake D, Cross K, Pierro A. Advanced necrotizing enterocolitis part 1: mortality. *Eur J Pediatr Surg* 2012; 22: 8-12 [PMID: 22434227 DOI: 10.1055/s-0032-1306263]

5. Santulli TV, Schullinger JN, Heird WC, Gongaware RD, Wigger J, Barlow B, Blane WA, Berdon WE. Acute necrotizing enterocolitis in infancy: a review of 64 cases. *Pediatrics* 1975; 55: 376-387 [PMID: 1143976]

6. Gibbs K, Lin J, Holzmar IR. Necrotising enterocolitis: the state of the science. *Indian J Pediatr* 2007; 74: 67-72 [PMID: 17264459 DOI: 10.1007/s12098-007-0031-0]

7. Warraer BB, Deych E, Zhou Y, Hall-Moore C, Weinstock GM, Sodergren EJ, Shaikh N, Hoffmann JA, Linneman LA, Hannavas A, Khanna G, Rouggy-Nickless LC, Ndad M, Shands BA, Escobedo M, Sullivan JE, Radmacher PG, Shannon WD, Tarr PL. Gut bacteria dysbiosis and necrotising enterocolitis in very low birthweight infants: a prospective case-control study. *Lancet* 2016; 387: 1928-1936 [PMID: 26969089 DOI: 10.1016/S0140-6736(16)00881-7]

8. Corpeís de la We, van de Waard M, Christmann V, van Goudoever JB, Szajewska H, Embleton ND, Hojsak I, Reid D, Shamir R; Neonatal Research Network of Japan. Comparison of mortality and morbidity of very low birth weight infants: The Early Nutrition Study Randomized Clinical Trial. *JAMA Pediatr* 2016; 170: 654-661 [PMID: 27315598 DOI: 10.1001/jamapediatrics.2016.0183]

9. Cristofalo EA, Schanler RJ, Blanco CL, Sullivan S, Traegerová R, Kiechl-Kohlendorfer U, Dudell G, Rechtman DJ, Lee ML, Lucas A, Abrams S. Randomized trial of exclusive human milk versus preterm formula diets in extremely premature infants. *J Pediatr* 2013; 163: 1592-1595 [PMID: 23968744 DOI: 10.1016/j.jpeds.2013.07.011]

10. Sullivan S, Schanler RJ, Kim HH, Patel AL, Traegerová R, Kiechl-Kohlendorfer U, Chan GM, Blanco CL, Abrams S, Cotten CM, Larue N, Ehrenkranz RA, Dudell G, Cristofalo EA, Meier P, Lee ML, Rechtman DJ, Lucas A. An exclusively human milk-based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk-based products. *J Pediatr* 2010; 156: 562-567.e1 [PMID: 20056378 DOI: 10.1016/j.jpeds.2009.10.040]

11. Schanler RJ, Lau C, Hurst NM, Smith EO. Randomized trial of donor human milk versus preterm formula as substitutes for mothers' own milk in the feeding of extremely premature infants. *Pediatrics* 2005; 116: 400-406 [PMID: 16601595 DOI: 10.1542/peds.2004-1974]

12. Schanler RJ, Shulman RJ, Lau C. Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. *Pediatrics* 1999; 103: 1150-1157 [PMID: 10353922 DOI: 10.1542/peds.103.6.1150]

13. Quigley M, Embleton ND, McGuire W. Formula or donor breast milk for feeding preterm or low birth weight infants. Cochrane Database Syst Rev 2016; 6: CD002971 [PMID: 29926476 DOI: 10.1002/14651858.CD002971.pub4]

14. Gephart SM, Hanson C, Wetzell CM, Fleiner M, Umberger E, Martin L, Rao S, Agrawal A, Marin T, Kirmani K, Quinn M, Quinn J, Dudding KM, Clay T, Sauberan J, Eskenazi Y, Porter C, Msowoya AL, Kirmani K, Quinn M, Quinn J, Dudding KM, Clay T, Sauberan J, Eskenazi Y, Porter C, Msowoya AL, Linneman LA, Hamavas A, Khanna G, Rouggy-Nickless LC, Ndad M, Shands BA, Escobedo M, Sullivan JE, Radmacher PG, Shannon WD, Tarr PL. Gut bacteria dysbiosis and necrotising enterocolitis in very low birthweight infants: a prospective case-control study. *Lancet* 2017; 387: 1928-1936 [PMID: 26969089 DOI: 10.1016/S0140-6736(16)00881-7]

15. Section on Breastfeeding. Breastfeeding and the use of human milk. *Pediatrics* 2012; 129: e827-e841 [PMID: 22571471 DOI: 10.1542/peds.2011-3553]

16. Garg BD, Balasubramanian H, Kabra NS, Bansal A. Effect of oropharyngeal colostomy therapy in the prevention of necrotising enterocolitis among very low birth weight neonates: A meta-analysis of randomised controlled trials. *J Hum Nutr Diet* 2018; 31: 612-624 [PMID: 30073712 DOI: 10.1111/jhn.12585]

17. Huang XZ, Zhu LB, Li ZR, Lin J, Lin J. Bacterial colonization and intestinal mucosal barrier development. *World J Clin Pediatr* 2013; 2: 46-53 [PMID: 25254174 DOI: 10.5490/wjcp.v2.i4.46]

18. Mihatsch WA, Vossbeck S, Eikmanns B, Hoegel J, Pohlandt F. Effect of Bifidobacterium lactis on the incidence of nosocomial infections in very-low-birth-weight infants: a randomized controlled trial. *Neonatology* 2010; 98: 156-163 [PMID: 20234148 DOI: 10.1159/000280291]

19. Patel RM, Underwood MA. Probiotics and necrotizing enterocolitis. *Semin Pediatr Surg* 2018; 27: 39-46 [PMID: 29275816 DOI: 10.1053/j.sempedsurg.2017.11.008]

20. van den Akker CHP, van Goudoever JB, Szajewska H, Embleton ND, Hojsak I, Reid D, Sharmir R; ESfPHAN Working Group for Probiotics, Prebiotics & Committee on Nutrition. Probiotics for Preterm Infants: A Strain-Specific Systematic Review and Network Meta-analysis. *J Pediatr Gastroenterol Nutr* 2018; 67: 103-122 [PMID: 29384838 DOI: 10.1097/MPG.0000000000001897]

21. Chang HY, Chen JH, Chang JH, Lin HC, Lin CY, Peng CC. Multiple strains probiotics appear to be the most effective probiotics in the prevention of necrotizing enterocolitis and mortality: An updated meta-analysis. *PLoS One* 2012; 7: e1017579 [PMID: 22818264 DOI: 10.1371/journal.pone.0101759]

22. Taylor RS. Probiotics to prevent necrotizing enterocolitis: Too cheap and easy? *Paediatr Child Health* 2014; 19: 351-352 [PMID: 25332671 DOI: 10.1093/pch/19.7.351]

23. Isayama T, Lee SK, Mori R, Kusuda S, Fujimura M, Ye XY, Shah PS; Canadian Neonatal Network; Neonatal Research Network of Japan. Comparison of mortality and morbidity of very low birth weight
infants between Canada and Japan. Pediatrics 2012; 130: e957-e965 [PMID: 22966031 DOI: 10.1542/peds.2012-0336]

24 Escribano E, Zozaya C, Madero R, Sánchez L, van Goudoever J, Rodríguez JM, de Pipaon MS. Increased incidence of necrotizing enterocolitis associated with routine administration of Infloran™ in extremely preterm infants. Benef Microbes 2018; 9: 683-690 [PMID: 29886655 DOI: 10.3920/BM2017.0098]

25 Shashidhar A, Suman Rao PN, Nesargi S, Bhat S, Chandrakala BS. Probiotics for Promoting Feed Tolerance in Very Low Birth Weight Neonates - A Randomized Controlled Trial. Indian Pediatr 2017; 54: 363-367 [PMID: 28360269 DOI: 10.1007/s13312-017-1106-2]

26 Gánev-Varal I, Kšíkal N, Ōzkhan H, Bağcı O, Değan P. The effect of early administration of combined multi-strain and multi-species probiotics on gastrointestinal morbidities and mortality in preterm infants: A randomized controlled trial in a tertiary care unit. Turk J Pediatr 2017; 59: 13-19 [PMID: 29168358 DOI: 10.24953/turkped.2017.01.003]

27 Xu L, Wang Y, Wang Y, Fu J, Sun M, Mao Z, Vandenplas Y. A double-blinded randomized trial on growth and feeding tolerance with Saccharomyces bouardiou CNCM I-745 in formula-fed preterm infants. J Pediatr (Rio J) 2016; 92: 296-301 [PMID: 26946967 DOI: 10.1016/j.jped.2015.08.013]

28 Hays S, Jacquot A, Gauthier H, Kempf C, Beissel A, Pidoux O, Jumass-Bilik E, Decullier E, Lachambrel E, Beck L, Cambonie G, Patot E, Claris O, Picard JC. Probiotics and growth in preterm infants: A randomized controlled trial, PREMAPRO study. Clin Nutr 2016; 35: 802-811 [PMID: 26220763 DOI: 10.1016/j.clun.2015.06.006]

29 Costeles K, Bowler U, Brocklehurst P, Hardy P, Heal P, Juszczak E, King A, Panton N, Stacey F, Whiteley A, Wilks A, MILLAR MR. A randomised controlled trial of the probiotic Bifidobacterium breve BBG-001 in preterm babies to prevent sepsis, necrotising enterocolitis and death: the Probiotics in Preterm infants (PiPS) trial. Health Technol Assess 2016; 20: 1-194 [PMID: 27594381 DOI: 10.3310/hta20640]

30 Patole S, Keil AD, Chang A, Nathan E, Doherty D, Simmer K, Emmar S, Conanow P. Effect of Bifidobacterium breve M-16V supplementation on fecal bifidobacteria in preterm neonates—a randomised double blind placebo controlled trial. PLoS One 2014; 9: e89511 [PMID: 24594833 DOI: 10.1371/journal.pone.0089511]

31 Totou S, Yamasaki T, Terahara M, Uchiyama A, Kusuda S. Probiotics Study Group in Japan. Bifidobacterium and enteral feeding in preterm infants: cluster-randomized trial. Pediatr Int 2014; 56: 714-719 [PMID: 24617812 DOI: 10.1111/ped.12330]

32 Benos S, Moran R, Ben Tov A, Aronovska K, Zaidenberg-Israeli G, Dillborg S. Probiotic supplementation in mothers of very low birth weight infants. Am J Perinatol 2014; 31: 497-504 [PMID: 23994538 DOI: 10.1055/s-0033-1353400]

33 Oncel MY, Sari FN, Arayici S, Güzoglu N, Erdeve O, Uras N, Oguz SS, Dilmen U. Lactobacillus Reuteri for the prevention of necrotising enterocolitis in very low birthweight infants: a randomised controlled trial. Arch Dis Child Fetal Neonatal Ed 2014; 99: F110-F115 [PMID: 24309022 DOI: 10.1136/archdischild-2013-304745]

34 Jacobs SE, Tobin JM, Opie GF, Donath S, Tabrizi SN, Pirotta M, Morley CJ, Garland SM; PrePrems Study Group. Probiotic effects on late-onset sepsis in very preterm infants: a randomised controlled trial. Pediatrics 2013; 132: 1055-1062 [PMID: 24249817 DOI: 10.1542/peds.2013-1339]

35 Sercé O, Benzer D, Gursoy T, Karatekin G, Ovalli F. Efficacy of Saccharomyces bouardiou on necrotizing enterocolitis or sepsis in very low birth weight infants: a randomised controlled trial. Acta Paediatr 2013; 102: e560-e565 [PMID: 23701204 DOI: 10.1111/aped.12330]

36 Fernández-Carrocer A, Solis-Herrera A, Cabanillas-Ayón M, Gallardo-Sarmiento RB, García-Pérez CS, Montaño-Rodríguez R, Echániz-Aviles MO. Double-blind, randomised clinical assay to evaluate the efficacy of probiotics in preterm newborns weighing less than 1500 g in the prevention of necrotising enterocolitis. Arch Dis Child Fetal Neonatal Ed 2013; 98: F5-F9 [PMID: 22556209 DOI: 10.1136/archdischild-2011-300435]

37 Demird E, Erdeve O, Celik IH, Dilmen U. Saccharomyces bouardiou boulardii for prevention of necrotizing enterocolitis in preterm infants: a randomized, controlled study. Acta Paediatr 2013; 102: e560-e565 [PMID: 24028629 DOI: 10.1111/apa.12416]

38 Rojas MA, Lozano JM, Rojas MX, Rodríguez VA, Rondon MA, Bastidas JA, Perez LA, Rojas C, Ovalle O, Garcia-Harker JE, Tamayo ME, Ruiz GC, Ballester A, Arahila MM, Arevalo M. Prophylactic probiotics to prevent death and nosocomial infection in preterm infants. Pediatrics 2012; 130: e1113-e1120 [PMID: 23071204 DOI: 10.1542/peds.2011-3584]

39 Al-Hosni M, Duenas M, Hawk M, Stewart LA, Borghese RA, Cahoon M, Atwood L, Howard D, Ferrelli K, Soll R. Probiotics-supplemented feeding in extremely low-birth-weight infants. J Perinatol 2012; 32: 253-259 [PMID: 21549042 DOI: 10.1038/jp.2011.51]

40 Braga TD, da Silva GA, de Lira PI, de Carvalho Lima M. Efficacy of Bifidobacterium breve and Lactobacillus casei oral supplementation on necrotizing enterocolitis in very-low-birth-weight preterm infants: a double-blind, randomized, controlled trial. Am J Clin Nutr 2011; 93: 81-86 [PMID: 20980486 DOI: 10.3945/ajcn.2010.29799]

41 Sari FN, Dizdar EA, Oguz S, Erdeve O, Uras N, Dilmen U. Oral probiotics: Lactobacillus sporogenes for prevention of necrotizing enterocolitis in very low birthweight infants: a randomized, controlled trial. Eur J Clin Nutr 2011; 65: 434-439 [PMID: 21245887 DOI: 10.1038/ejcn.2010.278]

42 Samanta M, Sarkar M, Ghosh P, Ghosh JK, Sinha MK, Chatterjee S. Prophylactic probiotics for prevention of necrotizing enterocolitis in very low birthweight newborns. J Trop Pediatr 2009; 55: 128-131 [PMID: 18842610 DOI: 10.1093/tropej/fmm091]

43 Lin HC, Su BH, Chen HC, Chung MY, Hsu JF, Lien RJ, Tsao LY, Chen CH, Su BH. Oral probiotics prevent necrotising enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. Pediatrics 2008; 122: 693-700 [PMID: 18527980 DOI: 10.1542/peds.2007-3004]

44 Lin HC, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, Oh W. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. Pediatrics 2005; 115: 1-4 [PMID: 15829973 DOI: 10.1542/peds.2004-1463]

45 Bin-Nun A, Brromker R, Wilkanchski M, Kaplan M, Rudensky B, Kaplan M, Hammerman C. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. J Pediatr 2005; 147: 192-196 [PMID: 16126048 DOI: 10.1016/j.jpeds.2005.03.054]

46 Dani C, Biadaiolo R, Bertiin G, Martelli E, Ruballesi FF. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. Biol Neonate 2002; 82: 103-108 [PMID: 12169832 DOI: 10.1159/00006399]

47 Tagare A, Kadam S, Vaidya U, Pandit A. Routine antibiotic use in preterm neonates: a randomised
controlled trial. *J Hosp Infect* 2010; 74: 332-336 [PMID: 19926166 DOI: 10.1016/j.jhin.2009.09.010]

48 Kenyon S, Taylor DJ, Tarnow-Mordi WG; ORACLE Collaborative Group. ORACLE—antibiotics for preterm prelabour rupture of the membranes: short-term and long-term outcomes. *Acta Paediatr Suppl* 2002; 91: 12-15 [PMID: 12200889 DOI: 10.1111/j.1651-2227.2002.tb00153.x]

49 Oei J, Lui K. A placebo-controlled trial of low-dose erythromycin to promote feed tolerance in preterm infants. *Acta Paediatr* 2001; 90: 904-908 [PMID: 11529540 DOI: 10.1111/j.1651-2227.2001.tb02455.x]

50 Siu YK, Ng PC, Fung SC, Lee CH, Wong MY, Fok TF, So KW, Cheung KL, Wong W, Cheng AF. Double blind, randomised, placebo controlled study of oral vancomycin in prevention of necrotising enterocolitis in preterm, very low birthweight infants. *Arch Dis Child Fetal Neonatal Ed* 1998; 79: F105-F109 [PMID: 9828735 DOI: 10.1136/fn.79.2.F105]

51 Owen J, Groome LJ, Hauth JC. Randomized trial of prophylactic antibiotic therapy after preterm amnion rupture. *Am J Obstet Gynecol* 1993; 169: 976-981 [PMID: 8238160 DOI: 10.1016/0002-9378(93)90038-K]

52 Fan X, Zhang L, Tang J, Chen C, Chen J, Qu Y, Mu D. The initial prophylactic antibiotic usage and subsequent necrotizing enterocolitis in high-risk premature infants: a systematic review and meta-analysis. *Pediatr Surg Int* 2018; 34: 35-45 [PMID: 29128874 DOI: 10.1007/s00385-017-4207-z]

53 Cotten CM, Taylor S, Stoll B, Goldberg RN, Hansen NI, Sánchez PJ, Ambalavanan N, Benjamin DK; NICHD Neonatal Research Network. Prolonged duration of initial empirical antibiotic treatment is associated with increased rates of necrotizing enterocolitis and death for extremely low birth weight infants. *Pediatrics* 2009; 123: 58-66 [PMID: 19117861 DOI: 10.1542/peds.2007-3423]

54 Alexander VN, Northrup V, Bizzarro MJ. Antibiotic exposure in the newborn intensive care unit and the risk of necrotizing enterocolitis. *J Pediatr* 2011; 159: 392-397 [PMID: 21489560 DOI: 10.1016/j.jpeds.2011.02.035]

55 Graziano PD, Tauber KA, Cummings J, Graffunder E, Horgan MJ. Prevention of postnatal growth restriction by the implementation of an evidence-based premature infant feeding bundle. *J Perinatol* 2015; 35: 642-649 [PMID: 25880797 DOI: 10.1038/jp.2015.35]
