



Prevention of Necrotizing Enterocolitis in Very Low Birth Weight Preterm Infants with Probiotics: A Systematic Review and Meta-analysis

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Received July 31, 2019; Accepted October 20, 2019; Online Published December 24, 2019

Abstract

Introduction: Previous studies have shown that probiotics reduce the incidence of Necrotizing enterocolitis (NEC) and sepsis; while other studies have not shown a significant difference of NEC and sepsis incidence. To this date there is still no protocols or guidelines in regards to the use of routine probiotics in very low birth weight preterm infants.

Method: This meta-analysis was assessed according to PRISMA guidelines. Up to date RCTs were reviewed to see the effectiveness of probiotics in order to prevent necrotizing enterocolitis. Studies were searched through PubMed, ProQuest, and Cochrane Library searching engines from 2009 to 2019. The inclusion criteria were randomized controlled trials of probiotics for very low birth weight preterm babies; full text English articles; and having been published from 2009 to 2019.

Results: The findings of this study reveal that the incidence of NEC in the probiotic group was significantly low ($p < 0.00001$, $R=0.48$). In the subgroup analysis, the incidence of NEC was lower in the multiple strain group and Lactobacillus group with a P value of $p=0.0004$ and 0.006 respectively. The incidence of sepsis was lower in the probiotic group with a P value of $p=0.02$. Also, the incidence of all-cause mortality was lower in the probiotic group with a P value of $p=0.02$.

Conclusion: According to the results of the present study, it can be stated that it is beneficial to use multiple strain probiotics and Lactobacillus strain probiotics to prevent necrotizing enterocolitis in very low birth weight preterm babies.

Keywords: Probiotics, Necrotizing Enterocolitis, Preterm, Meta-analysis

Citation: Kurnia K, Rusli R. Prevention of necrotizing enterocolitis in very low birth weight preterm infants with probiotics: a systematic review and meta-analysis. Int J Med Rev. 2019;6(4):122-127. Doi:10.30491/IJMR.2019.100704.

Introduction

Necrotizing enterocolitis (NEC) is the most common complication of gastrointestinal system in Very Low Birth Weight (VLBW) preterm infants. The VLBW infants are at risk of NEC because they have abnormal bacterial colonization with a little amount of normal enteric bacterial species and have a delayed onset of bacterial colonization.¹ Probiotics is a live microbial that colonizes the gut and protects the neonates against NEC by upregulating local and systemic immunity, providing a barrier to bacterial migration across the mucosa and excluding potential pathogens competitively.² The use of probiotics still show controversial results. Some studies show that probiotics reduce the incidence of NEC and sepsis; while other studies did not show significant differences. It is worth mentioning that there is still no protocols or guidelines for the use of routine probiotics in VLBW preterm infants. The aim of the present study was to evaluate the efficacy of using probiotics to reduce NEC in preterm babies by comparing different randomized controlled trials (RCTs). All studies that met the inclusion criteria or eligibility criteria were.

Methods

Randomized Controlled Trials (RCT) were included in the review. Observational studies, systematic reviews, case reports, and Meta-analysis were excluded from this study. Only those RCTs with full text and those which had been published within the past 10 years were included in this research (2009-2019).

The preterm babies which had been born at gestational age ≤ 32 weeks or VLBW (≤ 1500 g) were the population of this study.

The intervention of this study was an oral administration of probiotic supplementation versus placebo as the control.

The primary outcome of the study was the occurrence of the stage \geq II NEC. The second outcome of the study was the all-cause mortality and sepsis.

This meta-analysis was assessed according to the PRISMA guidelines (<http://www.prisma-statement.org>). Any RCT studies which were in accordance to the eligible criteria of this study were included in the analysis. The databases of the studies were searched via PubMed, ProQuest, and Cochrane Library. The studies included in the analysis of this studied

included the published research from 2009 to 2019 with the following keywords: “probiotics”, “necrotizing enterocolitis or NEC”, and “preterm”. The search was limited to RCTs, research which had been published within the last 10 years, English text only and those studies with available full text. The quality of the studies were analyzed by JADAD score, and studies with a <3 score were excluded.

Data Extraction

There were two independent reviewers (BK and RR). The abstracts were obtained from an initial search and were read independently by two reviewers to identify the potential eligible studies. The reviewers obtained and assessed the full text articles for the eligibility criteria. Multiple publications of the same study were counted only once.

Statistical Analysis

Meta-analysis was conducted using Review Manager 5.3 (RevMan). The study used the fixed effects model (mantel-Haenszel method) as there was no significant heterogeneity between the trials with $I^2=25\%$ and $p=0.22$. The effect size was expressed as the risk ratio (RR) and 95% CI.

Results

The literature search was done through a systematic review

among 503 studies. After selecting the studies through the eligibility criteria (full text RCT and being published in the past 10 years), 12 studies were gathered. After reviewing the abstracts, 9 studies were included in this study. The flow diagram of the selection process are presented in [Figure 1](#). The characteristics of the 9 studies are shown in [Table 1](#).

Data on NEC has been reported in 9 studies ($n=3186$). According to these studies, there were higher populations of neonates in the control group experiencing NEC than neonates in the probiotic group with a p value <0.00001 . Meta-analysis using RevMan shows lower risk of NEC ($RR=0.48$) in the probiotic group. There was no significant heterogeneity between the studies ($I^2=25\%$, $p=0.22$). This result is presented in [Figure 2](#).

Subgroup analysis was done to see the analysis of the different types of probiotics being used. In the multiple strain probiotics trials, it can be seen that the multiple strain probiotics has a significant lower NEC incidence when compared to the placebo group with a p value of 0.0004. Meanwhile, in the single strain group (Saccharomyces group) no significant effect was seen in regards to the incidence of NEC when compared to the placebo group with a p value of 0.85. but there was a significant incidence of NEC in the single strain Lactobacillus group with p value 0.006. These results can be seen in [Figure 2](#).

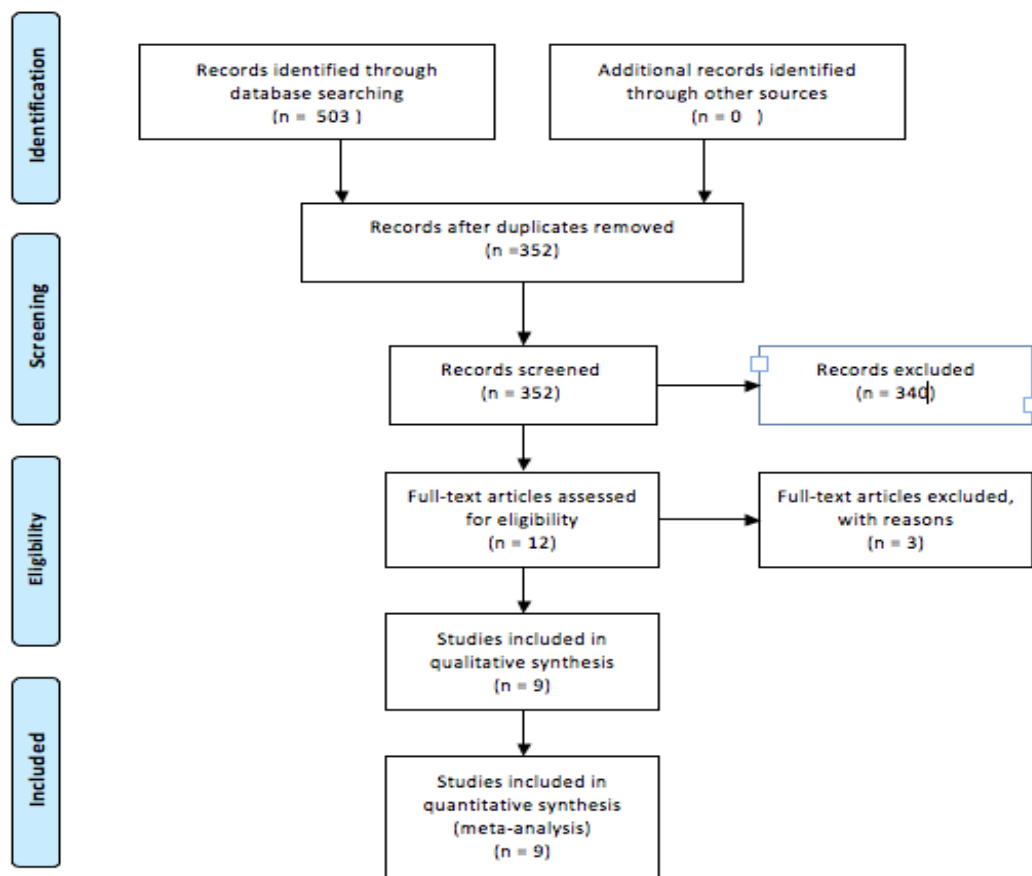


Figure 1. Flowchart of systematic reviews and reviewing processes

Table 1. Characteristics of the 9 included randomized controlled trials¹⁻⁹

NO	Name	Population	Intervention	Comparison	Outcome	Jadad Score
1.	Braga et al. 2010	Preterm infants with birth weight ≤ 1500 g at NICU Instituto de Medicina Integral Professor Fernando Figueira in Northeast Brazil	Human milk with supplementation (B.breve and L.casei) 3.5×10^7 - 3.5×10^9 CFU	Human milk containing no probiotics	- NEC 0/119 (probiotic) vs. 4/112 (placebo) with p value= 0.00 - Sepsis 40/119 (probiotic) vs. 42/112 (placebo) with p value= 0.90 - Death 26/119 (probiotic) vs. 27/112 (placebo) with p value= 0.91	4
2.	Damirel et al. 2013	Neonates born in ≤ 32 gestational weeks and birth weight ≤ 1500 gram At NICU Samsun Maternity and Child Health Hospital, Turkey	S, bouldarii supplementation 5×10^9 CFU added to human milk or formula milk	Human milk or formula milk with no probiotics	- NEC 6/135 (probiotic) vs. 7/136 (placebo) p value= 1.000 - Sepsis 47/135 (probiotic) vs. 65/136 (placebo) with p value= 0.030 - Death 5/135 (probiotic) vs. 5/136 (placebo) with p value= 1.000	5
3.	Sari et al. 2011	Preterm neonates with birth weight of < 1500 g or gestational age < 33 weeks at NICU of Zekai Tahir Burak Maternity Hospital in Turkey	L. sporogenes 3.5×10^8 CFU with breast milk or formula	Breast milk or formula without probiotics	- NEC 6/110 (probiotic) vs. 10/111 (placebo) with p value= 0.447 - Death 3/110 (probiotic) vs. 3/111 (placebo) with p value 1.000	5
4.	Oncel et al. 2013	Gestational age ≤ 32 weeks and birth weight ≤ 1500 g at NICU of Zekai Tahir Burak Maternity Teaching Hospital, Turkey	5 drops of probiotic L. reuteri 1×10^8 CFU	5 drops of Identical oil base placebo	- NEC 8/200 (probiotic) vs. 10/200 (placebo) with p value= 0.63 - Sepsis 13/200 (probiotic) vs. 25/200 (placebo) with p value= 0.041 - Death 12/200 (probiotic) vs. 16/200 (placebo) with p value= 0.27	5
5.	Manzoni et al. 2014	VLBW at multicenter (Italy and New Zealand	L.rhamnosus GG 6×10^9 CFU/day.	Placebo	- NEC 0/238 (probiotic) vs. 14/258 (placebo) with p value < 0.001 - Death 9/238 (probiotic) vs. 18/258 (placebo) with p value 0.11	4
6.	Serce el al. 2013	Preterm infants (GWs ≤ 32 , ≤ 1500 g) in NICU at Zeynep Kamil Maternity and Children's Research and Training Hospital, Turkey	S.bouldarii 0.5×10^9 CFU/kg per dose 2×1 added to breast milk or formula	Distilled water 1 ml per dose 2×1 added to breast milk or formula	- NEC 7/104 (probiotic) vs. 7/ 104 (placebo) with p value= 0.62 - Sepsis 19/104 (probiotic) vs. 25/104 (placebo) with p value= 0.29 - Death 4/104 (probiotic) vs. 5/104 (placebo) with p value of 0.74	5
7.	Jacobs 2013	Preterm infants (GWs < 32 , < 1500 g) multicenter in Australia and New Zealand	Bifidobacterium infantis 300×10^6 , Streptococcus thermophilus 350×10^6 , and Bifadobacterium lactis 350×10^6 added to breast milk or formula	Placebo (Maltodextrin) same color dan texture with the probiotics added to breast milk or formula	- NEC 11/548 (probiotic) vs. 24/551 (placebo) with p value- 0.03 - Sepsis 129/548 (probiotic) vs. 146/551 (placebo) with p value= 0.26 - Death 27/548 (probiotic) vs. 28/551 (placebo) with p value= 0.91	4
8.	Fernandez Carocera 2012	Preterm infants with birth weight ≤ 1500 g	L.acidophilus 1.0×10^9 CFU/g, L. rhamnous 4.4×10^8 CFU/g, L. casei 1.0×10^9 CFU/g, L. plantarum 1.76×10^8 CFU/g, B.infantis 2.76×10^7 CFU/g, S.thermophilus 6.6×10^5 CFU/g to human milk or preterm formula	Human milk or preterm formula without probiotics.	- NEC 6/75 (probiotic) vs. 12/75 (placebo) with p value= 0.142 - Death 1/75 (probiotic) vs. 7/75 (placebo) with p value= 0.063	5
9.	Varal 2016	Neonates born in ≤ 32 gestational weeks and birth weight ≤ 1500 gram at Uludag University Medical Faculty NICU,	Lactobacillus plantorum 4.1×10^8 cfu, Lactobacillus casei 8.2×10^8 cfu, Lactobacillus rhamnosus 4.1×10^8 cfu, Bifidobacterium animals 4.1×10^8 cfu	-	- NEC 0/70 (probiotic) vs. 4/40 (placebo) with p value= 0.016 - Sepsis 12/70 (probiotic) vs. 14/40 (placebo) with p value= 0.059 - Death 1/70 (probiotic) vs. 9/40 (placebo) with p value 0.001	3

Effect of Probiotics on $>$ Stage II NEC

Effect of Probiotics on Sepsis

Data on sepsis has been reported in 6 studies (n=2319). According to these studies, there was a higher population of neonates in the control group than neonates in the probiotic group with p value 0.02. This result can be seen in [Figure 3](#). Meta-analysis using RevMan shows a lower risk of sepsis (RR=0.66) in the probiotic group. There was a significant

heterogeneity between the studies ($I^2=50\%$, $p=0.07$); therefore, the random effect model has been used.

The Effect of Probiotics on All-Cause Mortality

Data related to all-cause mortality has been reported in 9 studies (3186). According to these studies, it can be stated that a significant difference of all-cause mortality exists between the two groups with the p value of 0.02. The result are presented in [Figure 4](#).

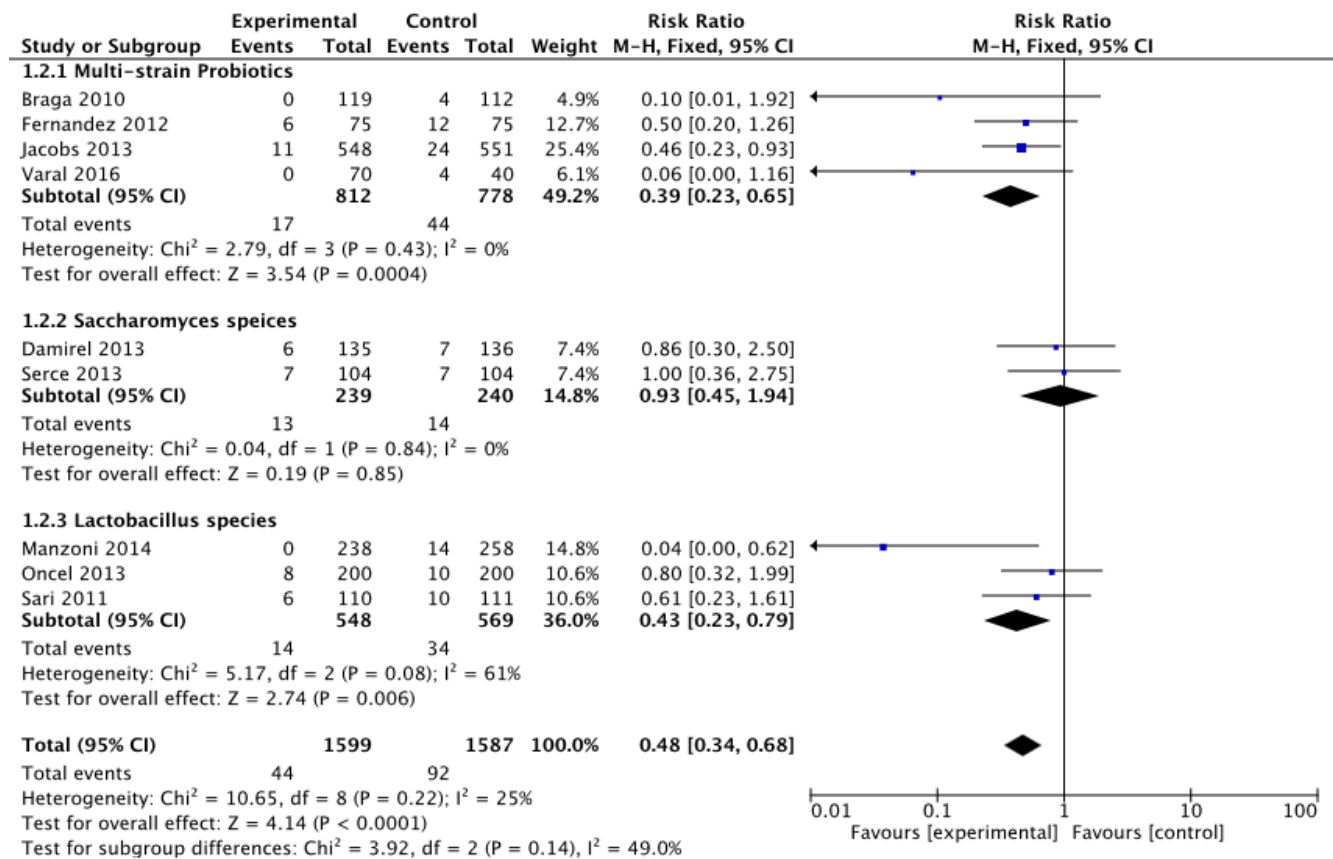


Figure 2. Forest plots of the effects of probiotics on NEC

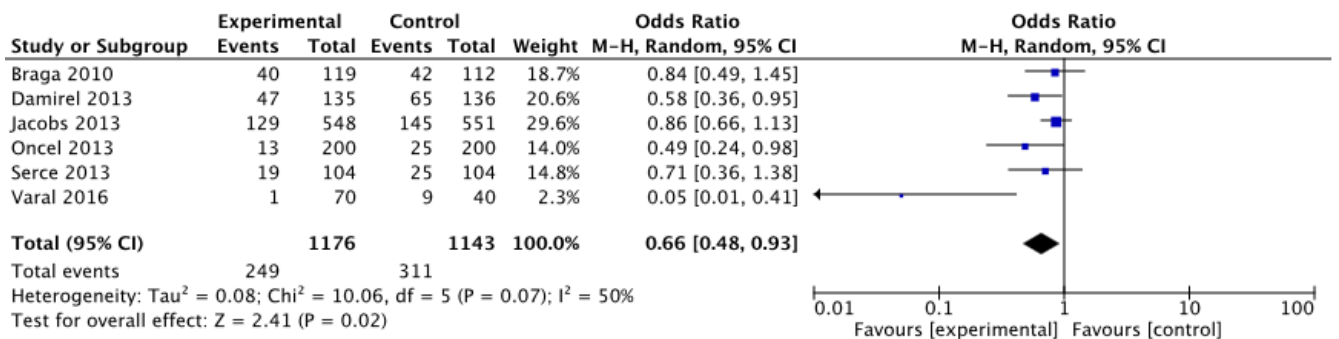


Figure 3. Forest plots of the effects of probiotics on sepsis

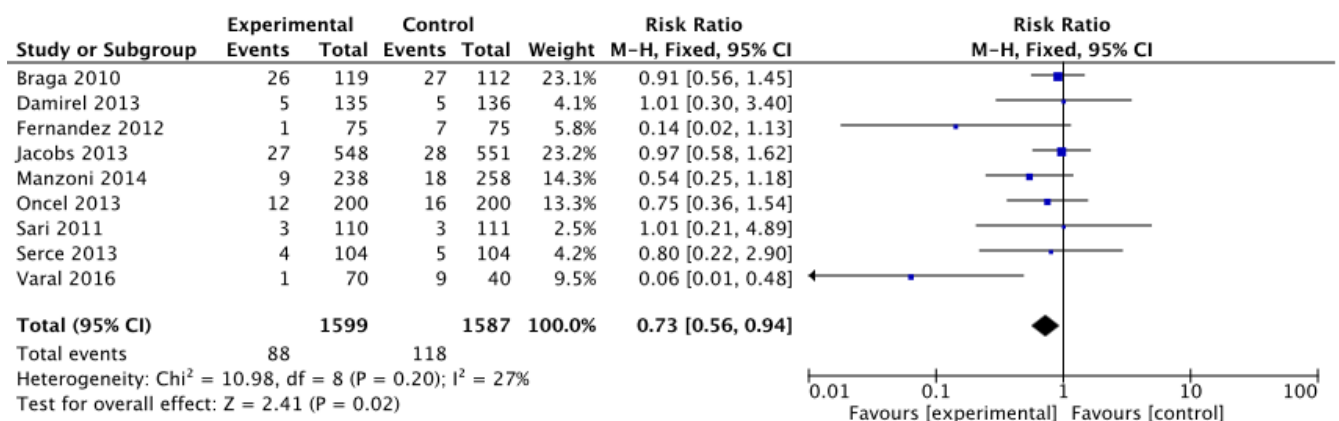


Figure 4. Forest plots of the effects of probiotics on all-cause mortality

Discussion

The results of this systematic review of 10 RCTs shows that probiotic supplementation in VLBW preterm neonates significantly reduces the risk of NEC and sepsis. The first finding of this meta-analysis study was the incidence of NEC. Findings reveal that there was a significant difference between the incidence of NEC in the prebiotic group and the placebo group with $p < 0.00001$. Also, significant differences were observed in regards to the incidence of NEC while using the multiple strain probiotics and single strain Lactobacillus group.

Over the last few years, probiotics have been studied by many researchers because of their beneficial effects. Probiotics are generally defined as containing live organisms that improve health. Probiotics were administered in an attempt to alter the composition of intestinal microbes. The colonization of the fetus intestinal tract begins during pregnancy. It is known that many preterm infants have been colonized with microbiota acquired from the amniotic fluid swallowed during labor. The amniotic fluid originally becomes colonized by microbiota from the maternal vagina.¹⁰ The etiology of NEC is multifactorial including intestinal immaturity; excessive inflammatory response to luminal microbial stimuli, and rapid increase in feeding. On the other hand, if the enteral feeds are withheld to prevent NEC, it will lead to a prolonged use of parenteral nutrition, causing intestinal atrophy, increased inflammation and late-onset sepsis.¹¹ Probiotics may prevent NEC as they promote the colonization of the good microbiota of the gut; therefore, prevent the pathogenic microbiota colonization; improve the maturity and function of gut mucosal barrier; and modulate the immune system.¹ Previous studies have shown no significant effect of probiotics on NEC.^{1,2}

Clinical trials which have intended to compare probiotic strains, doses and duration of administration are extremely rare. As a result, there is inadequate information in regards to which strain is superior to the other; and also which dose is considered to be the right dose for administration. There were several meta-analyses that had compared the combination of multiple strains with single strains. The following general principles have emerged that the combination products may have advantages over single organisms.¹⁰ This theory has been proven by this meta-analysis. There were still very few studies on the used single strain probiotic. This study only get 2 studies using the Saccharomyces strain and 2 studies using Lactobacillus strain when compared to 4 studies of mixed strain probiotics.

There was concern about bacterial translocation in preterm infants which will cause sepsis because of the infant intestinal barrier.^{10,12} This study uncovered a lower risk of sepsis in the probiotics group. Among the clinical trials of premature infants reporting mortality and/or culture negative clinical

sepsis, the incidences of both were either decreased or unchanged suggesting that the probiotic-induced sepsis is likely very rare.

The results of this study in regards to the incidence of NEC is similar to the study of Chang et al. carried out during 2017.¹³ In their study, it has been proven that the probiotics group has a lower risk of developing NEC compared to the placebo group with a p value of < 0.0001 . They also found the same results in regards to the Lactobacillus and Saccharomyces group with a p value of 0.05 and 0.52 respectively.¹³ Another study conducted in 2017, presented the same results of the lower risk of NEC in the probiotic group in comparison to the placebo group with a p value < 0.00001 . This is while they did not do subgroup analysis to investigate the difference of single strain administration of prebiotics with multiple strain in terms of the incidence of NEC.¹⁴

The results of this study are consistent with previous studies that show that probiotic groups have a lower incidence of sepsis with a p value of 0.01 and < 0.0001 respectively. In comparison to the incidence of all-cause mortality, the results of this study was similar to the study of Dermyshe 2017, Chi 2018 and Chang 2017 with p value of 0.003, 0.03, and 0.006 respectively. The incidence of all-cause mortality was lower in the probiotic groups.

The limitation of this study were we only include the full text manuscript of RCT; and we exclude the non-English manuscript and abstracts presented in conferences. The studies included were having different dosing form one another; therefore, we did not know the right optimal dosing of prebiotics.

Conclusion

Findings reveal that the use of probiotics can decrease the incidence of NEC, sepsis and all-cause mortality in VLBW preterm infants. The multiple strain probiotics and Lactobacillus species group has shown superior effects on decreasing the NEC incidence in comparison to single species Saccharomyces probiotics. Accordingly, the use of multiple strain probiotics should be considered in treating VLBW preterm babies in daily practice; and can also be included in the protocol/ guideline of treating VLBW babies. The combination of the multi-strain probiotics dosing still needs to be explored in future studies.

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

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