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Meta-analysis

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

Bella Kurnia [1]*, Richardo Rusli [1]

[1] Faculty of Medicine, Kristen Krida Wacana University, Jakarta, Indonesia

* **Corresponding Author:** Bella Kurnia, Faculty of Medicine, Kristen Krida Wacana University, Jakarta, Indonesia. E-mail: bellakurnia12@gmail.com

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Abstract

Background: Some 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. There are still no protocols or guidelines for the use of routine probiotics in Very Low Birth Weight (VLBW) preterm infants.

Objective: A meta-analysis was assessed by reviewing up to date Randomized Controlled Trials (RCTs) to investigate the effectiveness of probiotics to prevent NEC.

Data Sources: The Pubmed, Proquest, and Cochrane Library databases were searched from 2009 to 2019.

Study selection: This meta-analysis was assessed according to PRISMA guidelines.

Data extraction: The inclusion criteria included RCTs of probiotics for very low birth weight preterm babies; full text English articles; and those manuscripts published within the year of 2009 to 2019.

Results: The present research included nine studies. The incidence of NEC in the probiotic group were significantly lower (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 P = 0.0004 and 0.006 respectively. The incidence of sepsis was lower in the probiotic group with P = 0.02; and the incidence of all-cause mortality was lower in the probiotic group with P = 0.02.

Conclusion: According to findings it can be stated that it is beneficial to use multiple strain probiotics and *lactobacillus* strain probiotics to prevent NEC in VLBW preterm babies.

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

Introduction

Necrotizing enterocolitis (NEC) is the most common complication of the gastrointestinal system in Very Low Birth Weight (VLBW) preterm infants. 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 shows controversial results. Some studies show that probiotics reduce the incidence of NEC and sepsis; while other studies have not shown significant differences of NEC and sepsis incidence. There is still no protocols or guidelines for the use of routine probiotics in VLBW preterm infants. to the aim of this study is to evaluate the efficacy of probiotics use in order to reduce NEC in preterm infants by comparing different Randomized Controlled Trials (RCTs). All studies that met the inclusion criteria or eligibility criteria were evaluated.

Materials and Methods

Eligibility Criteria

Types of Studies

RCTs were included in this review. Observational studies, systematic reviews, case reports, and meta-analysis were excluded. Only those RCTs with full text which were published within the past 10 years were included (2009-2019).

Population of the Study

Preterm babies born at gestational age ≤ 32 weeks or VLBW (≤1500 g).

Intervention and Comparison

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

Outcome of the Study

The primary outcome of the study was occurance of stage ≥II NEC. The secondary outcome of the study was all-cause mortality and sepsis.

Study Selection

This meta-analysis was assessed according to the PRISMA guidelines (http://www.prisma-statement.org). Any RCT studies which matched the inclusion criteria was included in the analysis. The databases of the studies were searched via Pubmed, Proquest, and Cochrane Library. The studies included in the analysis was published from 2009 to 2019 with the following keywords: "probiotics", and "necrotizing enterocolitis or NEC", and "preterm". The search was limited to only RCTs, those published in the last 10 years, only English text studies and those which had available full text. The quality of the studies were analyzed by the JADAD score. Studies with scores <3 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 potential eligible studies. The two reviewers assessed the full text articles for eligibility criteria. Multiple publications of the same study were only counted once.

Statistical Analysis

Meta-analysis was conducted using Review Manager 5.3 (RevMan). The study used the fixed effects model (mantel-Haenszel method) because there were no significant heterogeneity between the trials. The effect size was expressed as risk ratio (RR) and 95% CI.

Results

The literature search was done through a systematic review of 503 studies. After selecting the manuscripts, 12 studies were finally selected. After reviewing the abstracts, nine studies were included in this study. The flow diagram of the selection process is presented in Figure 1. Also, the characteristics of all the nine studies are shown in Table 1.

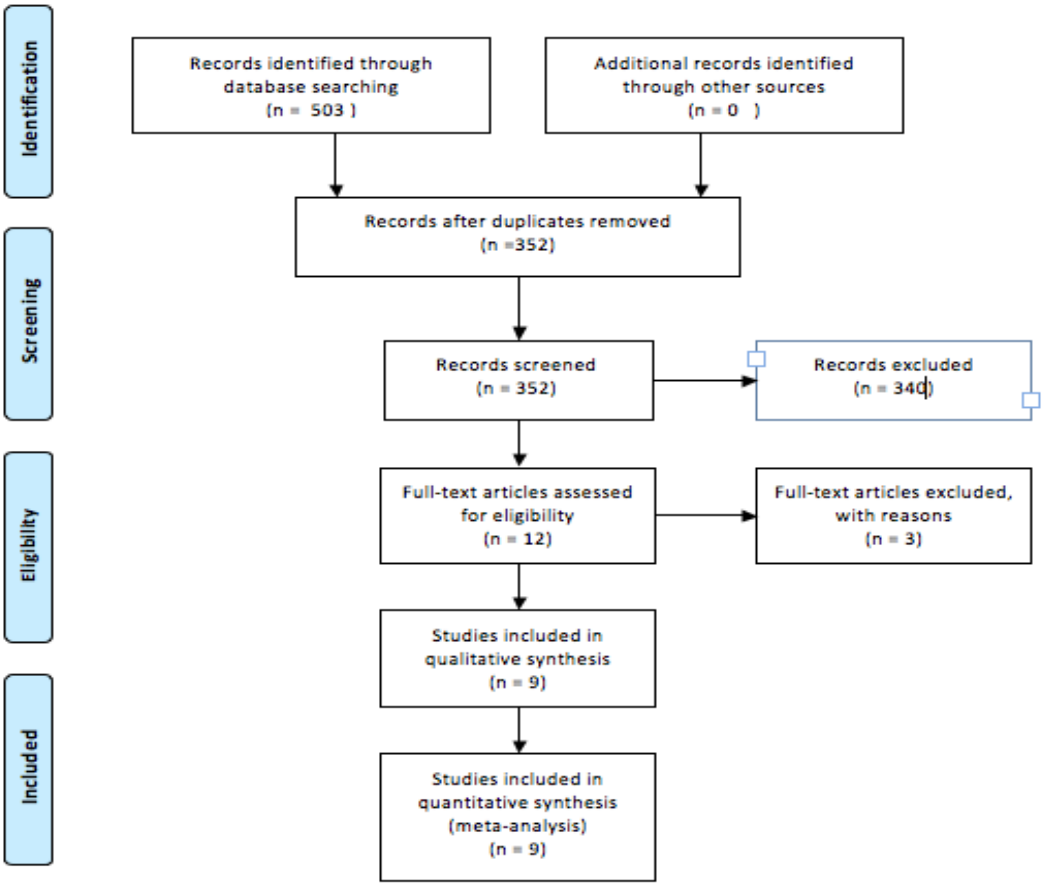

Figure 1. Flowchart of Systematic Reviews and Representing the Reviewing Process.

Table 1. Characteristics of the Nine Included RCTs [1-9]

No	Name	Population	Intervention	Comparison	Outcome	Jadad Score
1.	Braga et al 2010	Preterm infants with birth weight ≤1500g at NICU Instituto de Medicina Integral Professor Fernando Figueira in Northeast Brazil	Human milk with supplementation (B.breve and L.casei) $3.5x10^7$-$3.5x10^9$ 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, boulardii supplementation 5x 10^9 CFU added to human milk or formula milk	Human milk or formula milk with no pobiotics	- 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 <1500g or gestational age <33 weeks at NICU of Zekai Tahir Burak Maternity Hospital in Turkey	L. sporogenes $3.5x10^8$ 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 $1x10^8$ 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 $6X10^9$ 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 $0.5x10^9$CFU/kg per dose 2x1 added to breast milk or formula	Distilled water 1 ml per dose 2x1 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 $300x10^6$, Streptococcus thermophilus $350x10^6$, and Bifadobacterium lactis 350 $x10^6$ added to breast milk or formula	Placebo (Maltodextrin) same color dan texture with the probiotics aaded 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 Carrocera 2012	Preterm infants with birth weight ≤1500g	L.acidophilus $1.0x10^9$ CFU/g, L. rhamnous $4.4x10^8$ CFU/g, L. casei $1.0 x10^9$ CFU/g, L. plantarum $1.76x10^8$ CFU/g, B.infantis $2.76x10^7$CFU/g, S.thermophilus $6.6x10^5$ 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 $4.1x10^8$cfu, Lactobacillus casei $8.2x10^8$ cfu, Lactobacilus rhamnosus $4.1X10^8$ cfu, Bifidobacterium animalis $4.1x10^8$ cfu	Human milk or preterm formula without probiotics.	- 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

Data on NEC was reported by nine studies (n = 3186). From these studies, there was a higher population of neonates in the control group experiencing NEC than neonates in the probiotic group with a p value <0.00001. Meta-analysis using RevMan showed a lower risk of NEC (RR = 0.48) in the probiotic group. There was no significant heterogeneity between the studies (I2 = 25%, *P* = 0.22). This result can be seen in Figure 2.

Sub group analysis was done in order to observe the analysis of different types of probiotics being used. In the multiple strain probiotics trials, the multiple strain probiotics had significant lower NEC incidence when compared to the placebo group with p value of 0.0004. Meanwhile, in the single strain group (Saccharomyces group), no significant effect was observed on 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 a *P* value of 0.006. These results have been presented in Figure 2.

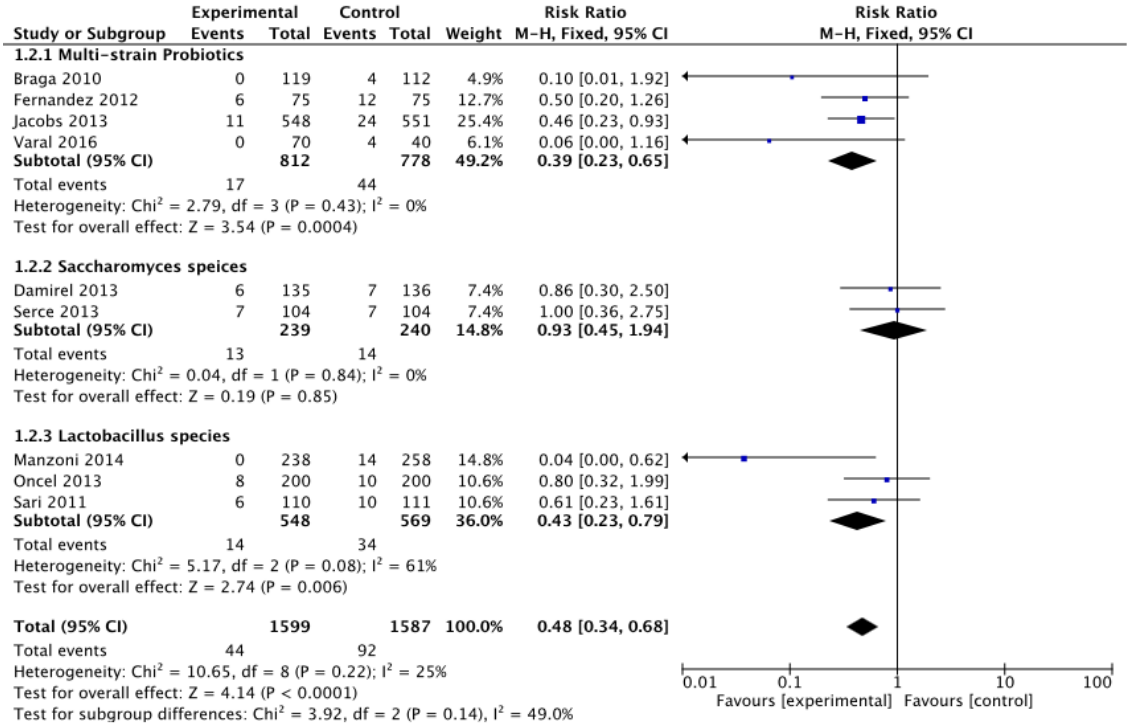

Figure 2. Forest Plots of the Effects of Probiotics on NEC.

Effect of Probiotics on Sepsis

Data on sepsis was reported by six studies (n = 2319). According to these studies, there were higher populations of neonates in the control group than neonates in the probiotic group with a p value of0.02 (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 (I2 = 50%, *P* = 0.07); therefore, the random effect model has been used.

Effect of Probiotics on All-Cause Mortality

Data on all-cause mortality was reported by nine studies (3186). According to these studies, there was a significant difference of all-cause mortality between the two groups with the *P* value of 0.02 (Figure 4).

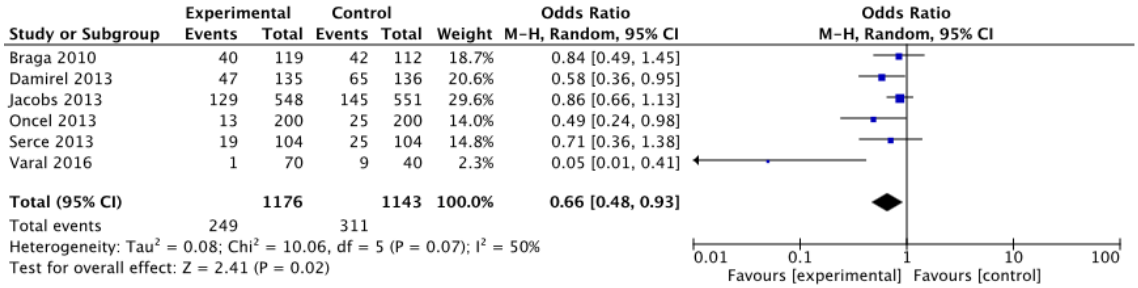

Figure 3. Forest Plots of the Effects of Probiotics on Sepsis.

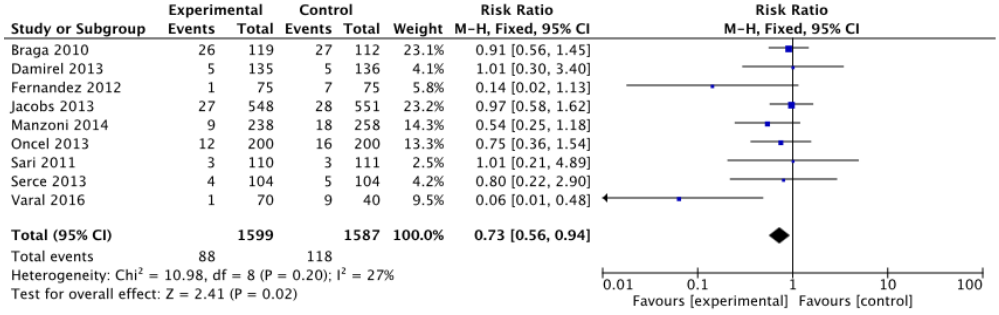

Figure 4. Forest Plots of the Effects of Probiotics on All-cause Mortality.

Discussion

The result of our 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 was the incidence of NEC. Our updated meta-analysis shows that there was a significant difference between the incidence of NEC in the prebiotic group and the placebo group with $P<0.00001$; and there were significant differences of NEC incidence using multiple strain probiotics and single strain Lactobacillus group.

Over the last few years, probiotics have been studied by many researchers because of its many beneficial effects. Probiotics are generally defined as containing live organisms that improve health. Probiotics were administered with the attempt to alter the composition of intestinal microbes. Colonization of the fetus intestinal tract begins during pregnancy. It is known that many preterm infants are colonized with microbiota acquired from the amniotic fluid swallowed during labor. Amniotic fluid become colonized by microbiota originally 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 we withhold enteral feeds to prevent NEC, it will lead to prolonged use of parenteral nutrition, causing intestinal atrophy, increased inflammation and late-onset sepsis.[11] Probiotics may prevent NEC as it promotes the colonization of good microbiota of the gut; therefore, preventing pathogenic microbiota colonization; improving the maturity and function of gut mucosal barrier; and modulating the immune system.[1] There are many different benefits of probiotics and there are also strain-specific effects of probiotics. According to previous studies, it can also be stated that probiotics have no significant effect on NEC.[1,2]

Clinical trials comparing probiotic strains, doses and duration of administration are rarely done. This result in the inadequate information of which strain was superior than the other; and which dose was the right dose for administration. There were several meta-analyses that compare a combination of multiple strains with single strain. The following general principles have emerged that combination products may have advantages over single organisms.[10] This theory was proven by this meta-analysis. There were still few studies on the single strain probiotic used. 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 that will cause sepsis because the immature of the infant intestinal barrier.[10,12] This study found 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 are either decreased or unchanged suggesting that probiotic-induced sepsis is likely to be extremely rare.

In comparison of the incidence of NEC, the result of this study was similar to the study of Chang et al., where they found that the probiotics group had a lower risk of developing NEC than the placebo group with a *P* value <00001. Also, no significant difference of NEC incidence was found in the Lactobaccilus and Saccharomyces group with a p value of 0.05 and 0.52 respectively.[13] From Dermyshi study in 2017, the have the same result of lower risk of NEC in probiotic group than placebo group with *P* value <0.00001; but they did not do subgroup analysis to see the difference of single strain administration of prebiotics with multiple strain in terms of NEC incidence.[14]

In comparison of the incidence of sepsis, the results of this study are similar to the study of Dermyshi 2017 and Chi 2018 that revealed that probiotic groups have a lower incidence of sepsis with a *P* value 0.01 and <0.0001 respectively. In comparison of the incidence of all-cause mortality, the result of this study was similar to the study of Dermyshi 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 limitations of this study were that we only included full text manuscripts of RCT; and we excluded the non-English manuscripts and abstracts presented in conferences. Also, the included studies had different dosing from one another; therefore, we did not know the right optimal dosing of probiotics.

Conclusion

According to the findings of this study, it can be stated that the use of probiotics can decrease the incidence of NEC, sepsis and all-cause mortality in

VLBW preterm infants. Multiple strain probiotics and lactobacillus species group has shown superior effects on decreasing the incidence of NEC than single species Saccharomyces probiotics. From this study and other meta-analysis, the use of multiple strain probiotics should be considered in treating VLBW preterm babies in daily practice; and can be included in the protocol/ guideline of treating VLBW babies. The combination of the multi strain probiotics still need to be explored in future studies.

Acknowledgement

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