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The Impact of Administering Prophylactic Probiotics to Premature Infants in Neonatal Intensive Care Units on Healthcare-Associated Infections, Necrotizing Enterocolitis, and Mortality

Yenidoğan Yoğun Bakım Ünitelerinde Prematüre Bebeklere Profilaktik Probiyotik Verilmesinin Hastane İnfeksiyonları, Nekrotizan Enterokolit ve Mortalite Üzerine Etkisi

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ABSTRACT

Introduction: *Healthcare-associated infections (HCAIs) are an important cause of morbidity and mortality in hospitalized patients. In this study, we aimed to investigate the effect of prophylactic enteral probiotic supplementation on necrotizing enterocolitis (NEC), mortality, and HCAIs in infants hospitalized in the neonatal intensive care unit (NICU).*

Materials and Methods: *In the first six months of the one-year study, the >1000-gram infants hospitalized in the NICU constituted the probiotic-free group (group 1, n= 119). In the second six months of the study, the >1000-gram infants admitted to the unit were given daily routine prophylactic enteral probiotic (Bifidobacterium animalis (BB-12) 1 x 10^9 CFU and Streptococcus thermophilus (TH-4) 1 x 10^8 CFU, Bifiform®) supplementation (group 2, n= 78). HCAIs and mortality rates were compared between both groups.*

Results: *One hundred ninety-seven patients were included in the study and their demographic characteristics were analyzed. The incidence of NEC was significantly lower in group 2 (0/78 patients vs. 6/119 patients; p= 0.044). As a secondary outcome, the number of HCAIs (p= 0.039) and the proportion of HCAIs with the causative agent in culture (p= 0.018) were lower in group 2 compared to the group not receiving probiotics. The most common HCAI subgroups in NICU were bloodstream infection, central line-associated bloodstream infection, and NEC. The HCAIs incidence density was 22.05 in group 1 and 14.02 in group 2, which was not statistically significant. However, when HCAIs incidence densities were analyzed according to subgroups, a statistically significant difference was observed in the rate of central catheter-related bloodstream infections (38.78 vs. 5.69, p≤ 0.001). The rate of HCAIs caused by gram-negative microorganisms was lower in group 2 (15/119 vs. 3/78; p= 0.029).*

Conclusion: *We observed a statistically significant reduction in the incidence of NEC and overall mortality in premature infants given prophylactic probiotics. We also found a significant reduction in HCAIs and gram-negative sepsis in the same group.*

Key Words: *Probiotics; Neonatal intensive care unit; Necrotizing enterocolitis; Healthcare-associated infections; Dietary supplements*

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ÖZ

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Giriş: *Hastane infeksiyonları (Hİ), hastanede yatarak tedavi gören hastalarda önemli bir morbidite ve mortalite nedenidir. Bu çalışmada, yenidoğan yoğun bakım ünitesinde (YYBÜ) yatan bebeklerde profilaktik enteral probiyotik desteğinin nekrotizan enterokolit (NEK), mortalite ve Hİ üzerine etkisinin araştırılması amaçlanmıştır.*

Materyal ve Metod: *Bir yıllık çalışmanın ilk altı ayında, YYBÜ'de yatan >1000 gram bebekler probiyotiksiz grubu oluşturdu (grup 1, n= 119). Çalışmanın ikinci altı ayında üniteye başvuran >1000 gram bebeklere günlük rutin profilaktik enteral probiyotik (Bifidobacterium animalis (BB-12) 1 x 10^9 CFU ve Streptococcus thermophilus (TH-4) 1 x 10^8 kob, Bifiform®) desteği verildi (grup 2, n= 78). Her iki grup arasında hastane infeksiyonları ve mortalite oranları için karşılaştırma yapıldı.*

Bulgular: *Yüz doksan yedi hasta çalışmaya dahil edildi ve demografik özellikleri analiz edildi. Nekrotizan enterokolit insidansı grup 2'de anlamlı olarak daha düşüktü (0/78 hastaya karşı 6/119 hasta; p= 0.044). İkincil bir sonuç olarak, grup 2'de probiyotik almayan gruba göre Hİ sayısı (p= 0.039) ve kültürde etken üreyen Hİ oranı (p= 0.018) daha düşüktü. Yenidoğan yoğun bakım ünitesindeki en yaygın Hİ alt grupları kan dolaşımı infeksiyonu, kateterle ilişkili kan dolaşımı infeksiyonu ve NEK idi. Grup 1'de Hİ insidans dansitesi 22.05, grup 2'de ise 14.02 olarak bulundu, istatistiksel olarak anlamlı değildi. Ancak Hİ insidans dansiteleri alt gruplara göre incelendiğinde santral kateter ilişkili kan dolaşımı infeksiyonu oranlarında istatistiksel olarak anlamlı farklılık görüldü (38.78'e karşı 5.69, p≤ 0.001). Gram-negatif mikroorganizmaların sorumlu olduğu Hİ oranı grup 2'de daha düşük bulundu (15/119'a karşı 3/78; p= 0.029).*

Sonuç: *Profilaktik probiyotik verilen prematüre infantlarda, NEK insidansında ve genel mortalitede istatistiksel olarak anlamlı bir azalma gözlemledik. Ayrıca aynı grupta Hİ ve gram-negatif sepsiste anlamlı bir azalma bulduk.*

Anahtar Kelimeler: *Probiyotikler; Yenidoğan yoğun bakım ünitesi; Nekrotizan enterokolit; Sağlık ilişkili infeksiyonlar; Gıda takviyeleri*

INTRODUCTION

Healthcare-associated infections (HCAIs) occur during the course of receiving healthcare, typically manifesting in a hospital or other healthcare facility 48 hours or more after admission, or within approximately three days after receiving healthcare[1]. However, this timeframe may vary for patients who have undergone surgery, depending on the type of operation. While HCAIs in children share similarities with adults in many aspects, they also exhibit differences, particularly concerning age-related variances in the immune system, infection site, responsible microorganisms, and environmental conditions. Prematurity, low birth weight, invasive interventions, accompanying congenital anomalies, and immunosuppression increase the risk for HCAIs. These infections are more common in pediatric and neonatal intensive care units, where these risks are most commonly encountered, compared to other wards[2].

Necrotizing enterocolitis (NEC) is a multifactorial disease characterized by acute intestinal ischemic necrosis, presenting with symptoms such as abdominal distension, vomiting (including bile), bloody stools, lethargy, apnea, and bradycardia. It represents a typical gastrointestinal emergency in premature newborns. The incidence of NEC in premature infants ranges between 7-14%, with rates inversely proportional to the gestational week. The mortality rate associated with NEC is reported to be between 15-30%[3].

The intestinal microbiota of the premature infant plays an essential role in the development of NEC[4]. This microbiota, which remains sterile pre-birth, begins to reform due to the mother's vaginal and fecal materials ingested during birth and breastfeeding. This formation phase can vary according to a multitude of factors, including the medications and probiotics used by the mother, the mode of delivery, the stress level of the baby, whether postnatal nutrition

is provided through breast milk or formula, the type of nutrition received, invasive interventions, the baby's environment, and the medications administered. If pathogenic bacteria invade the microbiota, the inflammatory pathway is activated due to the effect of bacterial toxins, and the process can progress to inflammation, necrosis, and perforation of the intestinal wall[5]. At this stage, there are studies on using probiotics to reduce pathogenic microbiota formation and prevent the development of NEC in premature infants hospitalized in the neonatal intensive care unit (NICU).

In this study, we aimed to investigate the effect of oral probiotic (*Bifidobacterium animalis* (BB-12) 1 x 10^9 CFU/mL and *Streptococcus thermophilus* (TH-4) 1 x 10^8 CFU/mL) administration on healthcare-associated infections, healthcare-associated infections incidence density, NEC and mortality in NICU patients during hospitalization.

MATERIALS and METHODS

This registry-based prospective cohort study was conducted for one year in the third-level NICU of Süleyman Demirel University Research and Education Hospital. All premature infants weighing over 1000 grams were included in the study. The probiotic-free group was followed in the first six months of the one-year study period (group 1, n= 119). In the second six months of the study, probiotic drops (0.5 mL= *B. animalis* (BB-12) 1 x 10^9 CFU/mL and *S. thermophilus* (TH-4) 1 x 10^8 CFU/mL, Bifiform®) were routinely given internally to all premature infants followed in the NICU once daily until discharge (group 2, n= 78). Probiotic drops were started immediately after admission to the unit and continued until the patient was discharged.

Feeding was initiated with 10-20 mL/kg breast milk or gestational age-appropriate formula in infants with stable vital signs, no abdominal distension, normal bowel sounds, and no bile coming from the nasogastric tube. Probiotic-free premature formulas were used in infants who did not receive breast milk.

Patients were followed up with daily visits. Oral feeding was discontinued in infants with two or more signs of feeding intolerance (abdominal distension, gastric residue, and fecal occult blood positivity). The diagnosis of NEC was based on clinical findings and abdominal radiography results according to the modified Bell's classification; those classified in stage 2 and above were defined as NEC and included in the study[6].

To diagnose the HCAI cases, we used Centers for Disease Control and Prevention (CDC) definitions[1]. The HCAIs were documented using forms accessible on the website of the national healthcare-associated infections surveillance network of the Public Health Institution of Türkiye[7]. The HCAI rate was calculated as the number of HCAIs/number of patients admitted x 100, the HCAI incidence density was calculated as the number of HCAIs/patient day x 1000, Central line-associate bloodstream infection (CLABSI) incidence density was calculated as the number of CLABSI/patient day x 1000, ventilator-associated pneumonia (VAP) incidence density was calculated as the number of VAP/patient day x 1000, and catheter-associated urinary tract infection (CAUTI) was calculated as the number of UTI-CR/patient day x 1000. The updated diagnostic criteria for sepsis from 2020 were utilized[8].

Exclusion criteria included infants weighing under 1000 grams and those diagnosed with stage 1 NEC. Patients who were diagnosed with sepsis according to the criteria based on blood analyses obtained from patients with clinical deterioration were included in the study. Patients without a sepsis diagnosis were excluded.

The principles of the Declaration of Helsinki from 2008 guided our study. Approval for this study was obtained from the local ethics committee (date: 21.09.2023, no: 177). Written informed consent was obtained from the relatives of the patients who agreed to participate in the study.

Mean ± standard deviation values were used for normally distributed numerical variables and median (minimum-maximum) values for non-normally distributed numerical variables. The Chi-square test was used to compare the frequency of qualitative variables between different groups. Kolmogorov-Smirnov test was used to

assess the normality of the data. Risk analysis was calculated as odds ratio and confidence intervals. The Kruskal-Wallis test for non-normally distributed data was used to compare more than two groups, whereas the Mann-Whitney U test was used to compare two groups. The examination of Spearman correlation was carried out for nonparametric variables. A probability (p) of less than 0.05 was deemed significant for all tests conducted. IBM SPSS Statistics software (version 27.0 for Windows; SPSS, Chicago, IL, USA) was used for data analysis.

RESULTS

The study included 197 infants. Of these, 119 were in group 1, and 78 were in group 2. Perinatal characteristics of the two groups are given in Table 1.

In terms of perinatal variables, in contrast to group 2, the group 1 infants showed higher rates of preeclampsia, perinatal asphyxia, and small for gestational age (SGA), and displayed lower means of gestational week and birth weight. No significant difference was observed in other variables including gender, premature rupture of membranes (PRM), chorioamnionitis, antenatal steroid use, cesarean delivery rate, low APGAR score, and multipregnancy.

The clinical characteristics of the patients are given in Table 2. Surfactant use, antibiotic use between 2-4 weeks, umbilical catheter use, and mean umbilical catheter days were significantly higher in group 1.

Considering the nutritional status of the patients, 78 (65.5%) infants in group 1 and 41 (34.5%) infants in group 2 received total parenteral nutrition (TPN) (p= 0.047).

There was no significant difference between the groups in the number of cases with HCAIs. The rate of HCAIs proven by culture was higher in group 1 (group 1= 27/119, group 2= 8/78) (p= 0.018). Gram-negative agents were the predominant responsible microorganisms in group 1 (p= 0.029). The incidence of NEC was higher in group 1 (p= 0.044) (Table 3).

There was no difference between the two groups regarding the incidence density of HCAIs. However, when the subgroups were analyzed, the CLABSI incidence density was 38.78 in group 1 and 5.69 in group 2, and the difference was statistically significant (p< 0.001). The incidence density of VAP was similar between the groups. The incidence density of CAUTI could not be compared as there were no cases. The mortality rate was found higher in group 1 (12/119 infants vs. 2/78 infants; p= 0.037). There was no significant difference between the two groups regarding mortality. In group 1, the mortality was observed in one patient with concomitant HCAIs and NEC, while four died because of

Table 1. Perinatal characteristics of the cases

Variables	Group 1 (n= 119)	Group 2 (n= 78)	p
Male, n (%)	59 (49.6)	39 (50.0)	.95
PRM*, n (%)	17 (14.3)	9 (11.5)	.57
Preeclampsia, n (%)	19 (16.0)	5 (6.4)	**.04**
Chorioamnionitis, n (%)	1 (0.8)	0	.60
Antenatal steroids, n (%)	31 (26.1)	14 (17.9)	.18
C/S*, n (%)	85 (71.4)	52 (66.7)	.47
SGA*, n (%)	50 (42.0)	22 (28.2)	**.04**
APGAR score fifth minute <6, n (%)	6 (5.0)	3 (3.8)	.49
Perinatal asphyxia, n (%)	18 (15.1)	4 (5.1)	**.02**
Birth weight, gram (mean ± SD)	2568.9 ± 894.5	2917.2 ± 792.0	**.006**
Multipregnancy, n (%)	64 (53.8)	43 (55.1)	.85
Gestational week, (mean ± SD)	36.2 ± 3.4	37.2 ± 3.1	**.04**

*PRM: Premature rupture of membranes, C/S: Caesarean section, SGA: Small for gestational age, SD: Standard deviation.

Table 2. Clinical characteristics of the cases

Variables	Group 1 (n= 119)	Group 2 (n= 78)	p
Surfactant use, n (%)	16 (13.6)	2 (2.6)	**.009**
Pneumothorax, n (%)	3 (2.5)	3 (3.8)	.59
Antibiotic use, 0-2 weeks, n (%)	76 (63.9)	47 (60.3)	.60
Antibiotic use, 2-4 weeks, n (%)	23 (19.3)	6 (7.7)	**.02**
Antibiotic use, 4-6 weeks, n (%)	11 (9.2)	2 (2.6)	.65
UC* use, n (%)	34 (28.6)	9 (11.5)	**.005**
UC* days, ± SD, days	2.85 ± 5.57	1.00 ± 3.21	**.008**
Central catheter use, n (%)	7 (5.9)	2 (2.6)	.27
Central catheter days, (mean ± SD)	1.13 ± 5.19	0.19 ± 1.58	.12
Mechanical ventilator use, n (%)	27 (22.7)	10 (12.8)	.83
Mechanical ventilator days, (mean ± SD)	2.90 ± 7.86	1.64 ± 7.50	.26
Phototherapy, n (%)	66 (55.5)	37 (47.4)	.27

*UC: Umbilical catheter, SD: Standard deviation.

Table 3. Healthcare-associated infections, necrotizing enterocolitis, and mortality

Variables	Group 1 (n= 119)	Group 2 (n= 78)	p
Number of cases with HCAI, n (%)	25 (21)	10 (12.8)	.099
Number of HCAI episodes, n (%)	41 (34.4)	17 (21.7)	**.039**
HCAIs, culture proven, n (%)	27 (22.6)	8 (10.2)	**.018**
HCAIs, gram-positive, n (%)	9 (7.5)	4 (5.1)	.35
HCAIs, gram-negative, n (%)	15 (12.6)	3 (3.8)	**.029**
HCAIs, Fungus, n (%)	3 (2.5)	1 (1.2)	.48
Number of NEC* (Bell stage ≥2), n (%)	6 (5)	0	**.044**
Number of NEC (Bell stage 3), n (%)	1 (0.8)	0	.60
HCAI incidence density	22.05	14.02	.54
Central line-associated bloodstream infection	38.78	5.69	**<.001**
Ventilator associated pneumonia	6.15	5.31	.69
Mortality, n (%)	12 (10.08)	2 (2.56)	**.037**
Non-HCAIs mortality, n (%)	7 (5.8)	2 (2.5)	.234
HCAIs-attributable mortality, n (%)	4 (3.3)	0	.13
HCAIs + NEC attributable mortality, n (%)	1 (0.8)	0	.604
Days of NEC development, ± SD, days	15 ± 4.3	0	.00

*HCAIs: Healthcare-associated infections, NEC: Necrotizing enterocolitis, SD: Standard deviation.

HCAIs alone, and seven died of non-HCAIs causes. In group 2, two patients died due to non-HCAI causes.

No significant difference was found between the groups regarding infection site. The most common healthcare-associated infections in the NICU were bloodstream infections, CLABSI, NEC, VAP, urinary tract infections, and meningitis, respectively. The distribution of HCAIs according to infection site and responsible microorganisms was presented in Table 4.

Table 4. Responsible microorganisms of health-care associated infections

Variables	Group 1 (n= 41)	Group 2 (n= 17)	p
HCAIs episode	41	17	**.039**
Bloodstream infections	14	9	.183
MRCoNS	1	2	
Acinetobacter baumannii	1	0	
Candida parapsilosis	1	0	
Enterobacter spp.	1	0	
Klebsiella oxytoca	1	0	
Bacillus licheniformis	0	1	
Klebsiella pneumoniae (ESBL+)	0	1	
Serratia marcescens	0	1	
Central line-associated bloodstream infection	14	2	.083
MRCoNS	6	1	
A. baumannii	5	0	
C. parapsilosis	1	0	
Enterococcus faecalis	1	0	
Enterococcus spp.	1	0	
Candida albicans	0	1	
Necrotizing Enterocolitis	6	0	.096
MRCoNS	1	0	
A. baumannii	1	0	
K. oxytoca	1	0	
Ventilator-associated pneumonia	2	2	.34
MRCoNS	1	0	
Stenotrophomonas maltophilia	1	0	
Urinary tract infection	1	2	.14
Candida spp.	1	0	
Enterobacter spp.	0	1	
Meningitis	3	0	.25
A. baumannii	1	0	
K. pneumoniae (ESBL+)	1	0	
Other infections (URTI*, STI*, Pneumonia)	1	2	.14

*HCAIs: Healthcare-associated infections, MRCoNS: Methicillin-resistant coagulase-negative staphylococcus, ESBL: Extended-spectrum beta-lactamase, URTI: Upper respiratory tract infection, STI: Soft tissue infection.

Regardless of the type of infection, the incidence of *A. baumannii* was significantly high in group 1 (p= 0.016).

DISCUSSION

Our study showed that prophylactic probiotic use decreased the incidence of NEC. Furthermore, in line with existing literature, our study stands out as one of the few to examine rates of HCAIs rates, infection-site, and responsible microorganisms. There was no difference between the two groups regarding the incidence density of overall HCAIs. However, when the subgroups were analyzed, the CLABSI incidence density was 38.78 in group 1 and 5.69 in group 2 and the difference was statistically

significant (p< 0.001). The incidence density of VAP was comparable between the groups. Once more, probiotic use correlated with a significant decrease in the mortality rate. Additionally, there was no significant difference observed between the groups regarding the site of HCAIs.

We selected the specific probiotics utilized in our study due to particular reasons. *Bifidobacterium*, abundantly found in the gut microbiota of infants fed regular breast milk, was chosen for its beneficial attributes. *S. thermophilus* was included as it enhances the colonization of beneficial microorganisms, working synergistically with *Bifidobacterium*[9]. Molecular studies have shown that 60-90% of the fecal microbiota in exclusively breastfed infants consists of *Bifidobacterium* strains *Bifidobacterium bifidum, Bifidobacterium longum*, and *Bifidobacterium breve*[10]. *Bifidobacterium infantis, S. thermophilus*, and *B. bifidum* were used in a study by Bin-Nun et al. examining the effect of probiotic use on the development of NEC in premature infants[11]. Although no adverse effects were reported with the strains we used, probiotic-associated bacteremia has been documented in certain studies, particularly in immunocompromised patients using *Lactobacillus* group probiotics[12]. During our study, we did not observe any probiotic-associated adverse effects or cases of bacteremia.

Upon reviewing the studies, it was noted that probiotic doses were typically administered within the range of 1.5-3 x 10^9[13]. In our research, we administered 2 x 10^9 of two distinct strains. The duration of probiotic initiation and administration varied between the studies. In general, the most effective period for reducing the frequency of NEC was found to be 4-6 weeks, while the most effective duration for preventing sepsis and mortality was probiotic administration for over six weeks or until discharge[14]. In our study, probiotics were initiated on the first day to observe the development of HCAIs and mortality, and they were continued until the patient was discharged or deceased.

The initial and crucial stage in the development of NEC involves the binding of pathogenic bacteria to the intestinal epithelial wall[15]. At this stage, an intact intestinal microbiota consisting of commensal bacteria is vital to the host immune system. Animal studies have shown that the administration of *Bifidobacterium*, a type of commensal bacteria, before the formation of pathogenic microbiota reduces NEC lesions in mouse intestines[16]. Numerous human studies have similarly indicated that the oral use of probiotics leads to a decrease in the occurrence of NEC[17]. In a study designed with a similar methodology in our country in 2017, a significant reduction in the frequency of NEC and mortality rates was found in a one-year follow-up period among the participants receiving the same probiotic strains compared to the control group[18]. In a multicenter randomized controlled study conducted in 2015 involving 400 infants, *Bifidobacterium lactis* was administered. An 80% decrease in the frequency of NEC was observed (p≤ 0.001), while no significant difference was found in mortality[19]. In a similar study by Lin et al. (2008) and Hoyos (1999), prophylactic probiotic use was shown to reduce NEC and mortality in the neonatal intensive care unit[3,20]. In a retrospective cohort study by Uberos et al., a notable reduction was identified in Stage II and higher NEC (p= 0.033), and mortality rates (p= 0.029) within the probiotic group[21]. A meta-analysis conducted by Deshpande et al., based on 11 studies, demonstrated that if the probiotic treatment was started within the first ten days of life in very low birth-weight (<1500 g) infants, the incidence of NEC decreased by 30%, the risk of death decreased in infants with stage II and higher NEC. The risk of sepsis did not change[22]. In a randomized controlled study by Mihatsch et al. involving 183 infants, *B. lactis* was given to premature infants of under 30 weeks and 1500 grams, and the change in healthcare-associated infection incidence density was investigated. The authors found no difference in the incidence density of healthcare-associated infections. In our study, the incidence density of HCAIs was not different between the groups, but the incidence density of CLABSI was higher in group 1. The difference was statistically significant (p< 0.001). As a secondary outcome, there was a 50% decrease in the frequency of NEC in the probiotic group compared to the

placebo group[23]. Similarly, our study found a significant decrease in NEC and overall mortality rates through probiotic administration, aligning with existing literature (p= 0.044, p= 0.037).

Some studies have suggested that probiotic use decreases the incidence of sepsis in the newborn. In our country, a significant decrease in the incidence of nosocomial sepsis was observed in the study by Dilli et al (p= 0.004). Still, no difference was found in the incidence of proven sepsis[19]. Studies conducted internationally reported a reduction in the occurrence of sepsis[24]. In a study carried out in Spain in 2017, employing a methodology similar to ours and involving 261 infants, a significant decrease in the incidence of sepsis was observed[21]. In our study, the number of HCAIs (p= 0.039), the number of proven sepsis cases (group 1; 27/119, group 2; 8/78) (p= 0.018), and the incidence of gram-negative sepsis (group 1; 15/119, group 2; 3/78) (p= 0.029) decreased significantly in the probiotic group, aligning with existing literature. Conversely, no alteration was observed in instances of gram-positive and fungal sepsis, consistent with the findings reported by Sarı et al[25].

With a rising number of studies exploring the correlation between prophylactic probiotic administration in neonates and outcomes like NEC, mortality, and healthcare-associated infections, there has been a subsequent increase in the publication of meta-analyses in recent years. A systematic meta-analysis conducted by Olsen et al. in 2016, consisting of 12 studies and involving 10.800 premature infants, demonstrated that the use of prophylactic probiotics led to a substantial reduction in both NEC (45% reduction) and mortality among premature infants hospitalized in the neonatal unit[26]. In the meta-analysis of Xue Jiao et al. in 2019, 16 studies were analyzed, and probiotic use was found to reduce the frequency of NEC[27]. In 2020, two articles were published, one being a Cochrane meta-analysis involving a total patient pool exceeding 25.000, investigating the impact of probiotic utilization. These articles revealed a noteworthy decrease in mortality, a significant reduction in severe NEC with moderate to high levels of evidence, and a lowered risk of invasive infection[28,29]. The meta-analysis of 31 studies, published in 2021, reaffirmed the finding of a reduced occurrence of NEC[30]. Finally, a meta-analysis of 27 studies published in 2023 concluded that administering bovine Lactoferrin and probiotics exhibited greater effectiveness in reducing the occurrence of NEC[31].

Studies and meta-analyses in the literature generally indicate that *Lactobacillus* and *Bifidobacterium* species are more effective. However, more evidence is required to formulate precise recommendations regarding the optimal initiation time, dosage, and duration of administration.

We anticipate that our study will guide new discussions on prophylactic probiotic use, probiotic selection, effective dose, duration, and single or combined probiotic use to reduce NEC, mortality, and HCAIs in neonatal intensive care units. Nonetheless, definitive reports and recommendations necessitate new, more homogeneous studies encompassing larger patient populations.

Limitations

Our study has several limitations, including the somewhat uneven prenatal and clinical characteristics of the compared groups, the inclusion of infants weighing over 1000 grams exclusively, the follow-up of both groups being conducted at distinct periods, an inadequate number of cases, and the absence of laboratory conditions to track alterations in stool microbiota among patients receiving varying dosages of the probiotic. Furthermore, the results cannot be generalized for all probiotics at different doses.

ETHICS COMMITTEE APPROVAL

This study was approved by the Süleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee (Decision no: 177, Date: 21.09.2023).

CONFLICT of INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

AUTHORSHIP CONTRIBUTIONS

Concept and Design: AE, MÖ

Analysis/Interpretation: AE, MÖ

Data Collection or Processing: AE, MÖ

Writing: AE

Review and Correction: MÖ, MA

Final Approval: MÖ, MA

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