sciendo

J Biomed Clin Res Volume 15 Number 2, 2022

DOI:10.2478/jbcr-2022-0022

Original Article

PROBIOTIC PROPHYLAXIS OF NEONATAL JAUNDICE

Tatyana D. Itova,
Victoria A. Georgieva[1]

Department of Neonatology, University Hospital Medika, Rousse, Bulgaria
[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Medical University – Pleven, Bulgaria

Corresponding Author:
Tatyana D. Itova
Department of Neonatology,
University Hospital Medika
35, Riga Str., 7013, Ruse, Bulgaria
e-mail: taniaitova@abv.bg

Received: June 16, 2022
Revision received: August 2, 2022
Accepted: October 24, 2022

Summary

We investigated the effect of the administration of probiotics (PB) for the prevention of neonatal jaundice (NJ) in 315 full-term newborns (NBs). We grouped them according to the type and duration of PB intake: A – 5 days L. rhamnosus; B – 5 days L. reteri; C – 5 days B. animalis; D – 30 days L. rhamnosus; E – 30 days L. reuteri, F – 30 days B. animalis; G – without PB. Bilirubin (BR) was measured from 1st to 5th, on 14th, and on 28th day. The incidence of pathologic NJ in groups A&D, B&E, C&F, and G was 37, 36, 29, and 44%, respectively. During first five days, the lowest BR levels were found in Group C, the highest in Group G. BR levels on 14th and 28th days were not significantly different between groups A and D, B and E, C and F. The lowest levels of BR on the same days were found in group F and the highest in group E. The prophylactic use of L. rhamnosus, L. reuteri and B. animalis in full-term NBs significantly reduced the incidence and continuance of NJ. Duration of taking PB significantly affects the development of NJ. The most pronounced effect was when B. animalis was added.

Keywords: jaundice, probiotic, newborn

Introduction

The intestines of a newborn (NB) at birth present with an aerobic environment and facultative anaerobes, and only the Enterobacteriaceae family could live there. After a few days, however, the intestinal lumen changes into anaerobic, allowing anaerobic bacteria, such as *Bifidobacterium*, *Clostridium*, and *Bacteroides*, to colonize it [1]. In the first few weeks, microorganisms in the intestine of the NB resemble those of the mother's skin and vagina: *Enterococcaceae*, *Streptococcaceae*, *Lactobacillaceae*, *Clostridiaceae* and *Bifidobacteriaceae* predominate. Birth mode is considered a factor that determines early colonization [2]. The microbiota of vaginally

born children is rich in *Lactobacillus*, *Bifidobacterium*, and *Bacteroides* [3]. In the first few months, *Bifidobacterium* dominates. Many bifidobacterial species are isolated from babies' intestines [4], which are the most common bacteria at this stage of life [5].

The intestinal microbiota regulates bilirubin (BR) metabolism in the enterohepatic circulation by dihydroxylation and dehydrogenation. It is a necessary condition for BR catabolism in the intestine. The change in its composition reduces the elimination of BR and increases its plasma levels [6]. No data suggested that *Bifidobacterium* can directly metabolize BR in humans, but this has been proven in a rat model [7]. BR catabolism can be achieved through the cooperation of bacteria in the human body, but the detailed mechanisms are not yet fully understood [6]. BR has been proven as potentially toxic to some Gram-positive bacteria, such as *Enterococcus faecalis*, *Bacillus cereus*, *Staphylococcus aureus*, and *Streptococcus agalactiae* [8]. Conversely, BR can be protective against *E. coli* [8].

The failure to convert conjugated BR into stercobiline due to the relative insufficiency of bacteria in the intestine in the first week of life, overactivity of the enzyme β-glucuronidase in the sterile intestine, and weak alkaline pH of the proximal part of the intestine are significant causes of neonatal hyperbilirubinemia (HB) [9]. Delivery of probiotics (PB) contributes to a pH decrease in the intestine and, on the other hand, reduces the enterohepatic circulation, altering the intestinal flora and suppressing the β-glucuronidase activity [10, 11].

Material and methods

A total of 315 full-term NBs with indirect HB only were randomized. We divided the NBs according to the type of added PB, and the duration of intake into six groups and a control Group G. Group A included 24 NBs, Group B – 16 NBs, and Group C – 18 NBs. They were given a PB from the first to the fifth day. Group D included 16 NBs, Croup E – 31 NBs, and Group F – 17 NBs. They were given a PB from the first to the 28th postnatal day. Groups A and D received *L. rhamnosus*, Groups B and E accept *L. reuteri*, and Groups B and E – *B. animalis*. The dose and route of administration are shown in (Table 1).

Transcutaneous measurement of total BR with a bilirubinometer KJ-8000 was performed as follows: from about the 12th postnatal hour daily until discharge, on the 12-14th and the 28th-30th postnatal day. The measurement was done on the forehead of the NB, avoiding areas of bruising or congenital skin defects. The mean value of three consecutive measurements of the total BR was recorded.

Table 1. Grouping of NBs according to the diet and mode of PB administration

Groups	Diet & Mode of PB administration	N	Days	Dose	Weight [g]	GA [GWs]
A	SF & L. rhamnosus	24	5	6x10*9 GFU/6dr	3266.7±408.0	38.8±1.3
B	SF & L. reuteri	16	5	100x10*6 CFU/5dr	3348.1±366.1	38.9±1.2
C	SF & B. animalis	18	5	1x10*9 CFU/6dr	3506.1±503.5	38.8±0.9
D	SF & L. rhamnosus	16	30	6x10*9 GFU/6dr	3476.9±320.5	38.8±1.2
E	SF, enriched with L. reuteri	31	30	115x10*6 CFU/ 100ml	3450.0±492.1	39.0±0.9
F	SF & B. animalis	17	30	1x10*9 CFU/6dr	3173.0±373.8	38.3±1.0
G	SF without supplementation of PB	193	30		3285.3±387.3	38.7±1.1
Total		315			3316.3±385.5	38.8±1.1

Newborn(s) – NB(s); Probiotic – PB; Gestational age – GA; Gestational week(s) – GW(s); Standard formula – SF (without PB); dr-drops; CFU- colony-forming unit

The choice of the PB was based on the following requirements:

- To be given easily, the dose amount and the administration route were suitable for NBs. The oral drops and factory-enriched with PB formula meet these requirements.
- To contain a single probiotic strain to facilitate objective comparison of effect and not contain other additives (prebiotics, postbiotics, vitamin D).
- The probiotic strain was recommended by the manufacturer as suitable for newborns with a fixed dose.
- The kind of drug PB-strains should be consistent with the typical microbiota settlement of the neonatal gastrointestinal tract.
- PB should be available for Bulgaria.

The initial intake of PB was up to the 12th postnatal hour after establishing enteral nutrition and spontaneous defecation. Application and dosing were performed according to the manufacturer's instructions. Children were given a single daily dose of PB for the first five days or throughout the neonatal period (Table 1).

The data were entered and processed with the statistical package SPSS 23.0 and Excel for Windows. A level of significance rejecting was chosen at $p<0.05$. Descriptive analysis and comparison of groups with t-tests (independent or independent summary samples) were used to evaluate the results. The parents of the patients had given written informed consent to participate in the study, following the requirements of the Ethics Committee of Medical University – Pleven and "Medica" University Hospital – Ruse.

Results

The mean weight and gestational age by group are shown in Table 1. There was no statistically significant difference in these values between the groups.

The difference in BR levels was significant from day 2 to day four between Groups A, B, C, and G with the addition of the first five days PB (2nd-day $p<0.001$; 3rd-day $p=0.010$; 4th-day $p=0.040$). The lowest transcutaneous levels of total BR were observed in Group C (Fig. 1) – from day 2nd to 5th day BR levels were 87.3±28.8µmol/L; 115.9±40.9µmol/L; 128.4±33.6µmol/L; 130.9±29.2µmol/L. The highest levels of BR for the same period were registered in group G – 143.8±35.8 µmol/L; 162.8±34.0 µmol/L; 160.6±29.0 µmol/L; 143.3± 30.0 µmol/L, respectively.

Groups D, E, and F (given probiotics throughout the whole neonatal period) compared to control group G showed a statistically significant difference in mean transcutaneous bilirubin levels from day 2nd to 4th, 14th, and 28th (2nd day – $p=0.001$; 3rd day – $p<0,001$; 4th day – $p=0.001$; 5th day – $p=0.061$; 14th day – $p=0.014$; 28th day – $p=0.029$). The highest levels of BR for the period were registered in group G (Figure 2).

We found a statistically significant difference depending on the duration of probiotic supplementation in transcutaneous bilirubin

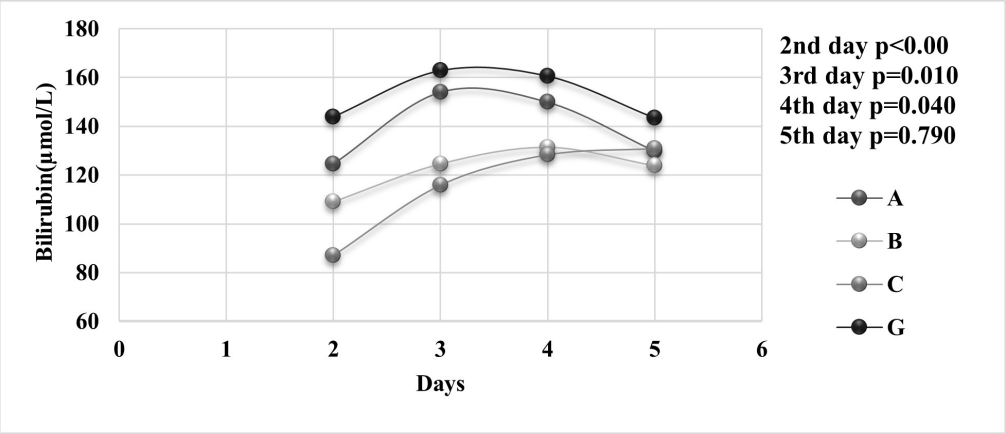

Figure 1. Comparison of mean levels of total bilirubin (µmol/L) between the groups with 5- day administration of probiotics - A, B, C and control Group G

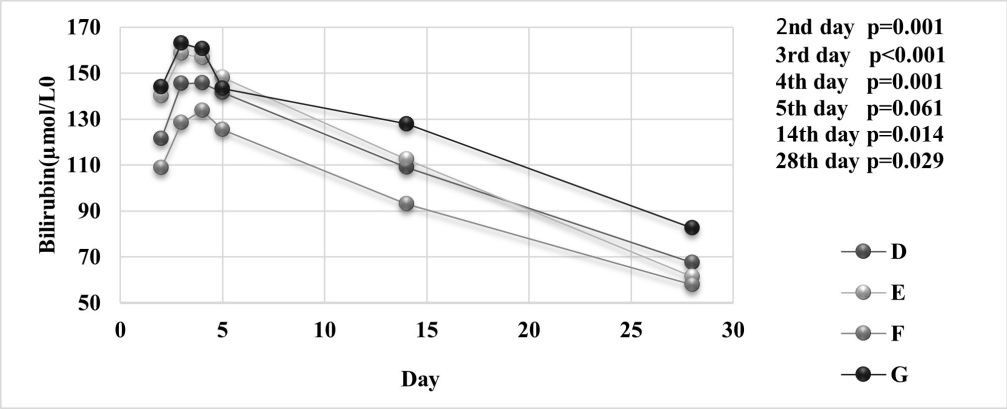

Figure 2. Comparison of mean levels of total bilirubin (µmol/L) between the groups with 30-day administration of probiotics – D, E, F and control Group G

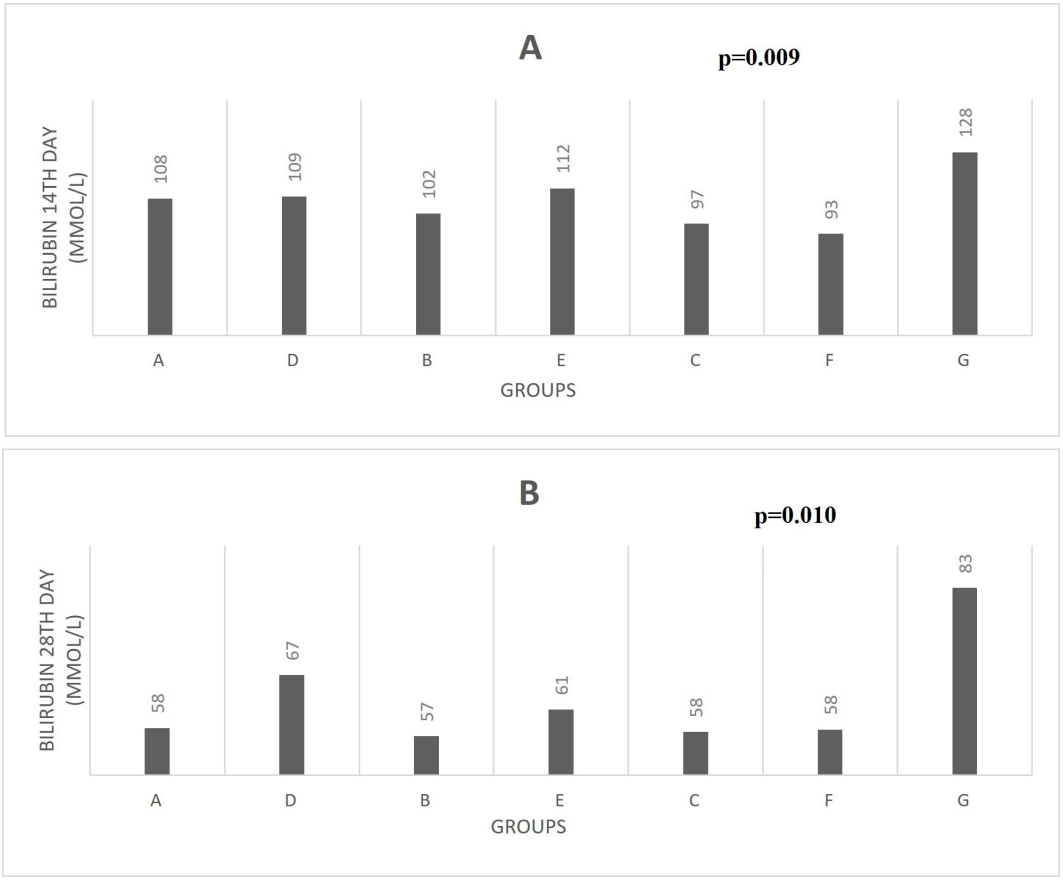

Figure 3. Average total bilirubin levels (µmol/L) on Day 14th (A) and Day28th (B) by groups from A to G.

levels on days 14 and 28. The highest levels were reported in the control group G – on day 14 (127.9±46.9µmol/L) and day 28 – (82.6±42.5µmol/L), and the lowest level – in group F – on day 14 (93.1±43.1 µmol/L) and day 28 (57.9±36.1µmol/L) (p=0.009; p=0.010). Among the groups with supplemented PB throughout the neonatal period, the highest levels were registered in group E – on day 14 (112.5±47.3µmol/L) and on day 28 (61.4±48.5µmol/L) (Figure 3).

We reported a difference in the percentage of children without neonatal jaundice (NJ) or with physiological NJ in the first five days by groups as follows: 63% in the groups given *L. rhamnosus*, 64% in the groups given *L. reuteri*,

71% in the Group given B. animalis, and 56% in control group G. The incidence of pathological NJ in groups A&D, B&E, C&F, and G were 37, 36, 29, and 44%, respectively.

The proportion of NBs treated with phototherapy in the first five days was: in Groups A & D - 37.5%, Groups B & E – 42.6%, Groups C & F – 25.7%, and Group G – 50.8% ($p<0.001$).

Discussion

The most commonly used strains of PB are those of the genus *Bifidobacterium (bifidum, animalis, lactis, longum), Streptococcus (thermophilus, boulardii), Lactobacillus (bulgaricus, acidophilus, rhamnosus, lactis, reuteri)*, and *Saccharomyces boulardii*. PB-supplementation in NBs is beneficial for accelerating intestinal colonization and inhibiting pathogenic microorganisms [12-14]. In NJ, the relative abundance of *Bifidobacterium* in breastfed patients is less represented [15, 16]. The choice of PB for the prevention and treatment of NJ is essential with the PB effects. One of the strains – *L. reuteri* DSM 17938, has been shown to improve the emptying of the stomach and intestines in preterm neonates [17]. *Saccharomyces boulardii*, administered in NJ, can help produce polyamines which improve intestinal maturity and function [18, 19].

Jaundice is usually a transient condition, but in pathological jaundice, the increase of bilirubin level is persistent and may reach dangerous toxic levels leading to kernicterus[20]. NJ is caused by an undeveloped intestinal microbiome, and increased enterohepatic circulation contributes to increased plasma bilirubin levels in the first days of life [21].

PB supplementation can affect neonatal hyperbilirubinemia by various potential mechanisms to balance the microbiota dysbiosis and reduce BR level: 1) by promoting colonization course; 2) by suppressing pathogenic processes; 3) by stimulating intestinal peristalsis and increasing stool frequency which reduce the enterohepatic circulation; 4) by enhancing the tight junction; 5) by increasing polyamines in the gut to improve the gut maturity[14].

Bifidobacterium spp. accelerates BR metabolism by inhibiting β-glucuronidase activity [22]. They can reduce the BR level by increasing stool frequency and decreasing enterohepatic circulation, leading to a significant reduction in PT time when PBs are used in combination with PT to treat NJ [12]. *Bifidobacterium* quadruple was used in a study on the prevention of NJ in China by Zuo Z. et al. (2015). The authors reported a decreased incidence of HB in the PB group (13.76% in the PB-taking group versus 28.70% in the control group) [23]. The groups in our study with supplemented B. animalis showed a significant effect on bilirubinemia in both the early and the entire neonatal period. Zuo Z et al. registered a smaller proportion of NBs who needed phototherapy. The proportion of children without NJ or physiological NJ was higher (71%) than in the control group (57%).

Lactobacilli, facultative anaerobic intestinal flora bacteria, predominantly colonize in regions with high endogenous β-glucuronidase activity, such as the stomach, duodenum, and jejunum [24]. Although lactobacilli were not the dominant microbiota in the early days of newborns, β-glucuronidase activity was suppressed by *L. rhamnosus* [25]. Mutlu M et al. conducted two studies on the prophylactics use of *Lactobacillus rhamnosus* GG against NJ. The first study included only full-term NBs who took PB immediately after birth until day 10. There was a proven significant difference in levels of mean total BR and an increased number of defecations between the control group and the PB intake group [26]. The other study involved only children with isoimmunisation aged from 35 to 42 GWs [14]. PB was administered immediately after birth until day 3, and serum BR was measured until 72 hours of life. The levels of BR were not influenced during the first 24 hours. The effect of the PB supplementation manifested at 36th hrs of life, then a difference in levels of BR between the groups was reported. These results are similar to those we found. We observed lower levels of BR in the groups receiving *L. rhamnosus* for prophylaxis compared to the control group levels from days 2 to 28. Nevertheless, these mean levels of BR were insignificantly_higher than those in the groups taking *B. animalis*.

PB with *L. reuteri* decreases bilirubin in neonates with NJ [27]. They provide minimal additional benefits in preventing PT admissions in full-term, healthy newborns. Their application

seems to provide neither additional nor economic benefits [28]. The prevalence of NJ was not reported as significantly different in low-birth-weight preterm infants if *L. reuteri* was used prophylactically. [29].

According to our results, the group given factory-enriched formula with *L. reiteri* registered the highest levels of bilirubin throughout the observed period and the highest need for phototherapy. This is probably due not only to the bacterial strain but also to the mode of intake and the number of probiotic bacteria taken per day.

Conclusion

According to our data, the prophylactic use of *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, and *Bifidobacterium animalis* in full-term NBs significantly reduces the frequency and duration of NJ and the duration of phototherapy for the whole period of supplementation. The duration of probiotic intake significantly affected the evolution of NJ in the full-term NBs. The most pronounced effect was that of *Bifidobacterium animalis*, and the least pronounced – was that of *Lactobacillus reuteri*.

References

1. Matamoros S, Gras-Leguen C, Le Vacon F, Potel G, De La Cochetiere MF. Development of intestinal microbiota in infants and its impact on health. Trends in microbiology. 2013;21(4):167-73.
2. Aagaard KM. Mode of delivery and pondering potential sources of the neonatal microbiome. EBioMedicine 2020;51:102554.
3. Perez-Muñoz ME, Arrieta MC, Ramer-Tait AE, Walter J. A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: implications for research on the pioneer infant microbiome. Microbiome. 2017;5(1):48.
4. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Scientific reports. 2016;6(1):1-3.
5. Wang M, Monaco MH, Donovan SM. Impact of early gut microbiota on immune and metabolic development and function. Semin Fetal Neonatal Med. 2016;21(6):380-87.
6. Chen K, Yuan T. The role of microbiota in neonatal hyperbilirubinemia. Am J Transl Res. 2020;12(11):7459-74.
7. Fahmy K, Gray CH, Nicholson DC. The reduction of bile pigments by faecal and intestinal bacteria. Biochim Biophys Acta. 1972;264(1):85-97.
8. Nobles CL, Green SI, Maresso AW. A product of heme catabolism modulates bacterial function and survival. PLoS Pathog. 2013;9(7):e1003507.
9. Watchko JF. Neonatal Hyperbilirubinemia and Kernicterus. In: Gleason CA, Devaskar SU, editors. Avery's diseases of the newborn. 10th ed. Philadelphia, PA: Elsevier; 2018. p. 1492-517.
10. Turroni F, Peano C, Pass DA, Foroni E, Severgnini M, Claesson MJ, et al. Diversity of Bifidobacteria within the infant gut microbiota. PLoS One. 2012;7(5):e36957.
11. Aires J, Thouverez M, Allano S, Butel MJ. Longitudinal analysis and genotyping of infant dominant bifidobacterial populations. Systematic and applied microbiology. 2011;34(7):536-41.
12. Deshmukh J, Deshmukh M, Patole S. Probiotics for the management of neonatal hyperbilirubinemia: a systematic review of randomized controlled trials. J Matern Fetal Neonatal Med. 2019;32(1):154-63.
13. Chen Z, Zhang L, Zeng L, Yang X, Jiang L, Gui G. Probiotics supplementation therapy for pathological neonatal jaundice: a systematic review and meta-analysis. Front Pharmacol. 2017;8:432.
14. Mutlu M, Aslan Y, Kader Ş, Acar FA. Preventive effects of probiotic supplementation on neonatal hyperbilirubinemia caused by isoimmunization. American journal of perinatology. 2020;37(11):1173-6.
15. Tuzun F, Kumral A, Duman N, Ozkan H. Breast milk jaundice: effect of bacteria present in breast milk and infant feces. J Pediatr Gastroenterol Nutr. 2013;56(3):328-32.
16. Zhou S, Wang Z, He F, Qiu H, Wang Y, Wang H, et al. Association of serum bilirubin in newborns affected by jaundice with gut microbiota dysbiosis. J Nutr Biochem. 2019;63:54-61.
17. Indrio F, Riezzo G, Raimondi F, Bisceglia M, Cavallo L, Francavilla R. The effects of probiotics on feeding tolerance, bowel habits, and gastrointestinal motility in preterm newborns. J Pediatr. 2008;152(6):801-6.
18. Serce O, Gursoy T, Ovali F, Karatekin G. Effects of Saccharomyces boulardii on neonatal hyperbilirubinemia: a randomized controlled trial. Am J Perinatol. 2015;32(2):137-42.
19. Costalos C, Skouteri V, Gounaris A, Sevastiadou S, Triandafilidou A, Ekonomidou C, et.al. Enteral feeding of premature infants with

Saccharomyces boulardii. Early Hum Dev. 2003;74(2):89-96.
20. Santosa I, Shoji H, Itoh S, Shimizu T. Roles of Probiotics in Reduction of Neonatal Jaundice in Term Newborns. Juntendo Medical Journal 2022;68(2):140-6
21. Liu W, Liu H, Wang T, Tang X. Therapeutic effects of probiotics on neonatal jaundice. Pak J Med Sci. 2015;31(5):1172-75.
22. An H, Park S, Lee D, Kim J, Cha M, Lee S, et al. Anti obesity and lipid-lowering effects of Bifidobacterium spp. in high fat diet-induced obese rats. Lipids Health Dis. 2011;10:116.
23. Zuo Z, Hai D, Wang Y, Wang C, Song L. Probiotics Protection Against Neonatal Hyperbilirubinemia. Chinese Journal of Medical Guide. 2015;11:1143-45.
24. Guarner F, Malagelada JR. Gut flora in healthy hand disease. Lancet 2003; 361(9356):512-19.
25. Ling WH, Korpela R, Mykkanen H, Salminen S, Hänninen O. Lactobacillus strain GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults. J Nutr 1994;124(1):18-23.
26. Mutlu M, Irmak E, Aslan Y, Kader Ş. Effects of lactobacillus rhamnosus GG as a probiotic on neonatal hyperbilirubinemia. Turk J Pediatr. 2018;60(5):482-87.
27. Makhoul G, Mardini J, Ojaimi MA, AbiFares G, Hanna P, Alameddine A, et al. Effect of Probiotic "L.Reuteri" Association on the Reduction of Serum Bilirubin in Neonatal Jaundice. ESJ. 2018;14(12):384.
28. Teran CG, Grandy G, Walker-Pizarro N. The role of probiotics in preventing neonatal hyperbilirubinemia. J Clin Neonatol. 2021;10(3):178-81.
29. Nouri Shadkam M, Jalalizadeh F, Nasiriani K. Effects of probiotic lactobacillus reuteri (DSM 17938) on the incidence of necrotizing enterocolitis in very low birth weight premature infants. IJN. 2015;6(4):15-20.