



The Effect of Probiotics on Late-Onset Sepsis in Very Preterm Infants: A Randomized Clinical Trial

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Abstract

Background

Late onset sepsis is a frequent complication of prematurity, associated with increased mortality and morbidity. Probiotics may prevent late onset sepsis in premature infants. The aim of this study was to determine prophylactic effect of oral probiotics in prevention of late onset sepsis of very preterm infants.

Materials and Methods

This study was a randomized, double blinded, placebo controlled trial. Eighty preterm infants born at < 32 weeks gestation weighing 1,000- 1,500 gr randomly assigned in intervention and control groups. From soon after the start of feeds, intervention group received Pedilact drop, which was a probiotic and control group received distilled water (DW) as placebo, 1 drop per kg of body weight every 12 hours, made by Zist-Takhmir, Iran Company. After data collection incidence of late onset sepsis, mortality, time to establish full enteral feeding and duration of hospitalization were compared between two groups.

Results

Cause of hospitalization in all patients was respiratory distress and prematurity. The incidence of late sepsis and death in the intervention group was lower than the control group, which was significant. The mean time to establish full enteral feeding in the probiotic group was lower than the control group. No case of necrotizing enterocolitis was observed. There was no difference in terms of days of hospitalization among two groups ($P>0.05$).

Conclusion

According to the results, usage of prophylactic probiotics can reduce the incidence of late onset sepsis and its mortality. By consuming probiotics preterm infants could reach the full enteral feeding in a shorter period of time, but the duration of hospitalization not reduced.

Key Words: Infant, Probiotic, Randomized Clinical Trial, Sepsis.

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1- INTRODUCTION

Terms like early-onset sepsis and late-onset sepsis refer to various neonatal periods, in which infection has begun. The former is the incidence of sepsis in the first week of life and the latter means the incidence of sepsis after the first week. The early-onset sepsis is transmitted before or during the childbirth (mother-to-fetus vertical transmission), and the late-onset sepsis is caused after childbirth through the organisms in the hospital and society. The incidence of infantile bacterial sepsis varies from one to four per 1,000 live births in developed countries; while in poor and developing countries, it is reported almost ten times more frequently (1). In 2009, the Australian and New Zealand Neonatal Association reported 15.7% prevalence of late-onset sepsis in the preterm infants with the gestational age of below 32 weeks and weight of below 1,500 gr (2).

However, progress in medical technology has improved the survival of very-low-birth-weight (VLBW) infants with birth weight of below 1500 gr (1, 2); but they have still remained at high risk for sepsis (3, 4). Also, 20% of all deaths in VLBW infants are due to sepsis with the risk of death three times as high as infants without sepsis. Infants infected with sepsis, even after adjusting for the gestational age, gender, and other diseases, have the mortality rate of nearly 3 times as the infants without sepsis (4, 5). The probability of death among these infants is increased due to diseases such as pulmonary dysplasia, prolonged hospitalization, and neurological disorders (5, 6). Due to the incomplete immune system and need for intravenous and prolonged hospitalization (8, 14), the VLBW infants are at the risk of late-onset sepsis, which is caused by Gram-positive organisms (1, 5, 8). Despite the improvement in the quality of neonatal care, the incidence of late-onset sepsis is

still reported significantly higher (5, 9, 10). Preterm infants in the neonatal intensive care unit (NICU) are also at high risk for intestinal disorders caused by pathogen microflora, which is caused by antibiotic treatment and total parenteral nutrition (TPN) with incubator care, which disrupts or delays the intestinal colonization process. Lack of coexist microorganisms in the intestines, such as Bifidobacterium and lactobacilli, can lead to the increased risk of intestinal colonization with pathogenic microbes for the delayed oral nutrition. Due to all the causes, the digestive system is one of the most important centers for colonization, pathogen storage, and subsequent sepsis in preterm infants. However, for high prevalence of neurodevelopmental disorders in the infants who survive sepsis, the prevention of infection, instead of its treatment, is considered a key solution in this group of patients. The current strategy for dealing with infection in NICU among the high-risk patients includes two solutions: 1. Treatment of infants with sepsis, 2. Use of drugs and bioactive substances that prevent the infection by certain microorganisms (4). Probiotics are the living microorganisms, if consumed sufficiently, which have health benefits for the host body (1, 12).

Nearly all probiotics have been multiplied and isolated from human microbiomes. The probiotic supplements are available in the form of oral and vaginal suppositories. Certain bacterial and fungal organisms are used as probiotics. The bacterial probiotics include Bifidobacterium, lactobacilli, streptococci, enterococci, and Escherichia coli (E.coli) (1). The living organisms are used for changes in intestinal flora, excess growth suppressing, transmission of pathogens in the intestine, and as a result, prevention of life-threatening infections (13, 14). The mechanisms that protect host probiotics against intestinal and urinary tract infections are: 1. Increasing resistance

of mucous membrane to bacterial migration and penetration of toxins through strengthening the connection of intestinal cells, 2. Changing host response to microbial products, 3. Strengthening mucosal Immunoglobulin A (IgA) response, 4. Strengthening intestinal defense by inhibiting growth of pathogens, 5. Producing Bacteriocins (small proteins which kill bacteria), and 6. Excluding potential pathogens through competitive duplication; theoretically, it is noted that the very-preterm infants that have a low variety of microorganisms in their intestine might benefit from the administration of probiotics. Based on the strain, these probiotics can cause the population induction of microorganisms in the intestine similar to the term infants or adults (7). Although there is no doubt about reducing the risk of stage I and II of Necrotizing enterocolitis (NEC) in preterm infants by probiotics, their effect on the late-onset sepsis (LOS) can still be discussed. The studies that evaluate the effect of probiotics on the late-onset sepsis (LOS) show different and conflicting results. Moreover, the studies have not been sufficiently solid to prove or reject the effect of probiotics on LOS (5, 9).

Researchers have reported that use of live probiotic bacteria with nutrient prebiotics, if used even for a long period of time, has no harmful effect on the host (6). In spite of 15.7% prevalence of the late-onset sepsis in developed countries and higher prevalence in developing countries; unfortunately, there is no accurate estimate for the late-onset sepsis in Iran. Nouri Shadkam et al. in Yazd city (2015) concluded that the prevalence of necrotizing enterocolitis in preterm infants is decreased from 66.7% to 20% using probiotics (8). Since the results of different studies on the effects of probiotics in preterm neonatal diseases have been significant, exaggerated, and contradictory and since all of these studies have

emphasized the use of probiotics (6, 7); therefore, this study aimed to evaluate the effect of probiotics on the incidence of late infections in the very-preterm infants at NICU of Motahari Hospital, Urmia, Iran.

2- MATERIALS AND METHODS

This study was a double-blind clinical trial that was conducted in NICU of Motahari Hospital, Urmia, Iran, from March to September 2016. To observe ethical issues, an approval with the number IR.RCC.UMSU.1395.480 was obtained from the ethics committee of Urmia University of Medical Sciences, and the study was registered in Iranian Registry of Clinical Trial site with the number (IRCT-code) IRCT20171218037936N1. After being approved by the Research Council for Supervision of thesis as well as the Ethics Committee, and providing the necessary explanations, a written consent was obtained from the parents of the infants for participation in the study.

The primary outcome was the incidence of at least one episode of definite late-onset sepsis in premature infants. The population consisted of very low weight infants with the gestational age of below 32 weeks and weight of 1,000 to 1,500 gr, who were hospitalized at first 48 h after birth at NICU of Motahari Hospital, Urmia. The qualified infants were randomly assigned to the intervention and control groups. Intervention group received Pedilact drop and control group received distilled water as placebo.

The inclusion criteria were 1,000 to 1,500 gr preterm infants below 32 weeks gestational age and hospitalization during the first 48 h after birth; the exclusion criteria were major congenital malformations (Esophageal atresia, omphalocele and gastroschisis, and imperforate anus), major cardiac disorders, genetic disorders such as trisomy 21 or other trisomies, likelihood of infant death during the first 72 h after birth, death

before minimal enteral feeding (10-20cc/kg/day), parental dissatisfaction, initial sepsis during hospitalization (C-reactive protein (CRP), above 10 in the first day of hospitalization), Asphyxia with grade II and III, maternal chorioamnionitis, and In Vitro Fertilization (IVF) infants. The sampling method was convenience sampling (random appointment). Based on previous studies (7, 11, 13, 15), and regarding $d = 10\%$, $P=0.7$, $\alpha=0.05$, the sample size was considered 40 people per group, so that, at first, 40 A cards and 40 B cards were mixed; then, they were put into the envelope and allocated for each hospitalized infant in the order of one card from the envelope. The infants with A card formed the probiotic group and those with B cards formed the placebo group.

Based on the policy of using antibiotics at NICU for all the infants in both groups, the antibiotics were used immediately after admission and intravenous. In the intervention group, with the first oral feeding, the oral probiotics (Pedilact drop, which was a probiotic made by Zist-Takhmir, Iran Company) were given by one drop per kg of body weight every 12 h; in the control group, the placebo was given by one drop per kg of body weight every 12 h. The placebo looks identical to the probiotic, consists of distilled water, and is identically packaged by The Zist-Takhmir, Iran Company. The probiotic and placebo solution already prepared in the same glasses was prescribed by the trained nurse for the infant and the evaluations were done by the executive assistant that was not aware of the drugs. The duration of administration of probiotics and placebo was up to 36 weeks of post menstrual age or discharge time from the hospital (whichever occurred earlier). The required data were given

to the pre-prepared checklists. The oral probiotics used in this study was a Pedilact drop manufactured by Zist-Takhmir, Iran Company, where no similar study had been carried out, and each drop contained *Lactobacillus ruteri* 2×10^9 CFU, *Lactobacillus Rumnosus* 1×10^{10} CFU, and *Bifidobacterium infantis* 1.5×10^9 CFU.

The first result of the incidence was at least 1 episode of late-onset sepsis, as CRP of above 10 mg/dl, or clinical sepsis prior to 40 weeks (regulated age for pregnancy) or discharge from the hospital, whichever had been the earlier. For the negative results of the blood culture test in this center, in most cases even in the infants with sepsis clinical criteria, we decided to use the laboratory criteria for CRP with high sensitivity for the diagnosis of sepsis. The number of leukopenia or leukocytosis blood cells and thrombocytopenia was the laboratory criteria for the diagnosis of sepsis. The clinical sepsis was considered as the intolerance to oral nutrition, frequent vomiting, abdominal distension, sclerema or severe skin, unstable vital signs and oxygen saturation (SO₂), fever, hypothermia, respiratory distress, decreased consciousness, decreased neonatal reflexes, reduced spontaneous movement of the infant and frequent apnea.

The secondary outcomes were: the incidence of necrotizing enterocolitis (NEC), which was graded by Bell Criteria (15), number of deaths from sepsis, number of days from first admission, number of days for full enteral feeding, and full enteral feeding as the time when the infant feeding was as low as 100 cc/kg/day. NEC intensity was determined as follows: Stage I (suspected NEC); apnea, lethargy, bloody stool,

abdominal distention, intolerance to nutrition, temperature instability, and intestinal distention in abdominal radiology. Stage II (approved NEC); signs of stage I plus thrombocytopenia \pm mild metabolic acidosis, abdominal tenderness, and pneumatosis intestinalis or gas in the portal vein. Stage III (advanced NEC): signs of stage II plus hypotension, bradycardia, severe and refractory apnea, metabolic acidosis, disseminated intravascular coagulation (DIC), abdominal tenderness, neutropenia and pneumoperitoneum (15). The information was collected using SPSS version 13.0 software. The comparison of the qualitative variables was conducted by Chi-square test; while the quantitative variables were compared using t-test and, if necessary, a nonparametric equivalent test (The results were significant at ($p \leq 0.05$). Odds ratio (OR), and 95% confidence interval (CI) were computed for the outcomes.

3- RESULTS

This study was a randomized clinical trial that was performed on 80 preterm infants at the first 48 h of birth at NICU of Motahari Hospital, Urmia, Iran, in both groups of intervention and control. The number of 40 infants was selected for each group, and 8 and 6 infants were died in the intervention group and control group prior to the enteral feeding, respectively. Therefore, the analyses were performed on 32 remaining infants in the intervention group and 34 surviving infants in the control group. Based on the findings, the cause of hospitalization for the infants was prematurity and respiratory distress with the diagnosis of respiratory distress syndrome (RDS). In terms of sexual distribution ($P=0.59$), and delivery type ($P=0.54$), there was no statistically

significant difference between the two groups. Also, in terms of birth weight ($P=0.18$), fifth minute Apgar score ($P=0.640$), gestational age (based on LMP) ($P=0.240$), duration of admission ($P=0.854$), and time of full enteral feeding ($P=0.188$), there was no statistically significant difference between the mean of both groups. In the study period, the mortality rate ($P = 0.004$), infection to sepsis ($P=0.015$), and administration of surfactant ($P=0.011$) in the control group were higher than the intervention group, which was statistically significant. On the other hand, the distribution of ventilator usage in the studied infants was not significant in the two groups ($p=0.185$) as shown in **Table.1**.

The odds ratio of death in the intervention group was 0.07 of the odds of death for infants in the control group, and the odds ratio of 0.77 was obtained (0. 647- 0.009). In order to use the surfactant, the odds ratio of 0.263 (0. 751-0.092) was obtained, so it can be said that the odds of using surfactant in the intervention group was 0.263 of the odds of using the surfactant in the control group. For infection to sepsis, the odds ratio of 0.260 (0. 791- 0.085) was obtained, so it can be said that the odds of having sepsis in the intervention group was one quarter of the control group.

The odds ratio of death, use of surfactant, and infection to sepsis between the two groups was statistically significant, and with 95% confidence interval, it can be said that the death rate, use of surfactant, and infection to sepsis in the intervention group were less than the control group. To use the ventilator, the odds ratio of 0.242 (2. 291- 0.026) was obtained, so it can be said that the use of ventilator in the intervention group was less than the control group, but this difference was not statistically significant, As seen in the **Table.2**.

Table-1: Socio-demographic characteristics of participants by study groups.

Variables	Intervention group	Control group	P-value
	Number (Percent) n = 32	Number (Percent) n = 34	
Gender			0.59 [†]
Male	17(53.1)	18(52.9)	
Female	15(46.9)	16(47.1)	
Delivery type			0.54 [†]
Vaginal	7(21.9)	8(23.5)	
Cesarean section	25(78.1)	26(76.5)	
Ventilator usage			0.185 [†]
Yes	1(1.3)	4(11.8)	
No	31(96.9)	30(88.2)	
Mortality rate			0.004 [†]
Death	1(1.3)	10(29.4)	
Alive	31(96.9)	24(70.6)	
Infection to sepsis			0.015 [†]
Yes	6(18.8)	16(47.1)	
No	26(81.3)	18(52.9)	
Administration of surfactant			0.011 [†]
Yes	8(25)	19(55.9)	
No	24(75)	15(44.1)	
Birth weight (g), mean (SD)	1225.29 (206.49)	1225.29 (206.49)	0.18*
Fifth minute Apgar score, mean (SD)	7.78 (0.85)	7.66 (1.01)	0.64*
Gestational age (based on LMP) , mean (SD)	29.67(1.74)	30.29(2.42)	0.240*
Duration of admission(day) , mean (SD)	17.85(11.46)	17.31(12.29)	0.854*
Time of full enteral feeding(day) , mean (SD)	10.48(5.22)	8.81(4.21)	0.188*

† Chi-square test; * t-test; SD: Standard deviation.

Table-2: Odds ratio of prophylactic effect of oral probiotics in prevention of late onset sepsis in intervention and control groups

Variables	OR	95% CI
Mortality rate	0.077	(0.647 to 0.009)
Ventilator usage	0.263	(0.751 to 0.092)
Infection to sepsis	0.260	(0.791 to 0.085)
Use of surfactant	0.242	(2.291 to 0.026)

CI: Confidence interval; OR: Odds ratio.

4- DISCUSSION

Treatment with probiotics is based on the healthy "flour microbial hypothesis". The use of specific flour microbial of the healthy intestine has formed the logic of

probiotic treatment for modification of endogenous cluster flour microbial (9, 13, 16). Preterm infants, due to the small variety of gastrointestinal microflora, might benefit from colonization by oral probiotics (16). The studies carried out in

recent years have shown the useful effect of supplementary probiotics on reducing the incidence of NEC and mortality in preterm infants (17, 18). The other useful effects that have been already reported are the reduced time of reaching the full enteral feeding, improved weighting, improved colonization of useful intestinal bacteria, and immune response in the probiotic consuming infant (19, 20). Late-onset sepsis with the incidence of 15.7% in preterm infants causes high mortality and morbidity (2). Effect of probiotics on the incidence or severity of sepsis has been unclear (11-14).

The differences might be due to the recent clinical trials differences in using different microbial strains, prescription, (dosage, frequency of usage per day, and duration of use, etc.), and differences in clinical guidelines in the local midwifery for the diagnosis and treatment of sepsis. In 1994, Norishadkam et al. studied the effect of oral probiotics on the NEC using the Pedilact drop; however, no similar study has been performed in Iran to evaluate the effect of probiotics in the late-onset sepsis (8). We also studied the effect of prophylactic effect of this probiotic compound in the late-onset sepsis with the similar dosage to the one used by Norishadkam et al. (8), based on the results, in this study, 6 cases (18.8%) of the sepsis was seen in the probiotic group compared to 16 cases (47.1%) in the control group, which was consistent with results by Bosante et al. in 2013 (10.7% incidence of sepsis in the probiotic group and 16.6% in the control group), and Kanic et al. (2008) (40% occurrence of sepsis in the probiotic group in contrast to 72% in the control group) (7, 11-13). However, in some other works, such as Jacobs et al. in 2013 and Tewari et al. in 2014, there has been no statistical difference in the occurrence of late-onset sepsis (Los) between the probiotic and placebo groups (13.1% versus 16.1% in the study by Jacobs et al.

and 13% versus 10% by Tewari et al.) (11, 14). The mortality rate caused by the late-onset sepsis in the very low birth weight infants has been reported 20%, which is considered a major medical problem (4, 5). The results of this study showed that 10 cases of death (29.4%) were caused by the sepsis in the control group compared to 1 case (1.3%) in the probiotic group, which was consistent with the findings by Bonsante et al. in 2013. This researcher also concluded that consuming prophylactic probiotics reduces the mortality rate (10.7% in the probiotic group compared to 16.6% in the control group); however, the results of other studies such as Rojas et al. (2008), Jacobs et al. (2013), Kanic et al. (2008), and Tewari et al. (2014) have demonstrated no significant effect on the prevention of mortality followed by consumption of probiotics (9, 11, 13, 14).

In this study, in the control group, 55.9% of the infants received the surfactant and 11.8% were under mechanical ventilation. However, the receiving rate of surfactant and mechanical ventilation in the intervention group was 25% and 3.1%, which can be the sign of inferior health condition of the infants in the control group compared to the intervention one before probiotics, so the higher level of sepsis and mortality in the control group compared to the intervention group can be somehow justified by their inferior health condition before the intervention. However, by looking at the average weight, gestational age, and Apgar score at birth, it can be found that both groups were selected quite randomly, since there was no significant difference between them in terms of the three above parameters. Necrotizing enterocolitis is one of the common causes of mortality in the premature infants. Although in the studies by Norishadkam et al. (2015), Rojas et al. (2011), Kanic et al. (2011), and Bonsante et al. (2013), the significant effect of

probiotics has been shown on reducing NEC and mortality (8, 9, 12, 13), no case of NEC has been seen during 7 months among the patients in the probiotic and placebo groups. Therefore, the evaluation of probiotic effect on the incidence of NEC is not possible in this study. Lack of NEC at NICU in our center was due to the protocol of low weight infant's nutrition and sufficient care was taken for preventing this complication in the department. One of the most common problems in preterm infants is intolerance to nutrition. Recent studies have shown that the probiotic products containing *Lactobacillus reuteri* found in our product are effective in increasing the intestinal movements and bearing nutrition. Regarding the earlier studies, we concluded that the time required for reaching the full enteral feeding in infants in the intervention group was less than the control group (10.48 days for the control group in contrast to 8.8 days for the intervention group).

However, this difference was not statistically significant ($P=0.188$); but it was consistent with the results of other similar studies such as Norishadkam et al. (2015), Kanic et al. (2011), and Tewari et al. (2015) (8, 13, 14). Achieving the full enteral feeding could be effective in the rapid increase of infant weight and early discharge from the hospital, thus the prevention of complications such as sepsis. Duration of hospitalization of the infants in both groups was not different in this study (17.8 ± 11.46 days for the control group in contrast to 17.3 ± 12.29 days for the probiotic group); while the Bonsante (2013) and Kanic (2011) have represented that consumption of probiotics reduces the duration of hospitalization (12, 13).

5- CONCLUSION

Considering the high prevalence of late-onset sepsis in preterm infants and high rate of mortality, and high prevalence of

the neurological disorders in the infants who have survived from sepsis, prevention from sepsis could be an appropriate strategy to avoid morbidity and mortality. Results of this study showed that the use of oral probiotics (such as Pedilact drop) could reduce the incidence of late-onset sepsis and its mortality in the preterm infants and increase the survival rate of this group of infants. By consuming probiotics, preterm infants could reach the full enteral feeding in a shorter period of time, but the duration of hospitalization is not reduced. Moreover, their consumption has no harmful effects on the infants. Since no severe and life-threatening complications have been reported so far from the consumption of probiotics in preterm infants, as well as relatively low prices of these products compared to conventional antibiotic treatments, as a recommendation, probiotics could be used in the neonatal departments and NICU for preterm infants as a dietary supplementation at the same time with oral nutrition. Larger clinical trials should be done using different probiotic strains to evaluate the impact and harmlessness of these products in the neonatal population of the country.

6- CONFLICT OF INTEREST: None.

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8- REFERENCES

1. Kliegman R, Behrman RE, Nelson WE. Nelson textbook of pediatrics. Elsevier; 2016.
2. Australian and New Zealand Neonatal Network. Report of the Australian and New Zealand Neonatal 2008 and 2009. Sydney: Australian and New Zealand Neonatal Network; 2012.
3. Hornik CP, Fort P, Clark RH, Watt K, Benjamin D, Smith PB, et al. Early and late

- onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. *Early human development*. 2012; 88: 69-74.
4. Manzoni P, Rizzollo S, Decembrino L, Ruffinazzi G, Ricci AR, Gallo E, et al. Recent advances in prevention of sepsis in the premature neonates in NICU. *Early human development*. 2011; 87: 31-3.
 5. Zhang G-Q, Hu H-J, Liu C-Y, Shakya S, Li Z-Y. Probiotics for Preventing Late-Onset Sepsis in Preterm Neonates: A PRISMA-Compliant Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Medicine*. 2016; 95(8):e2581.
 6. Dilli D, Aydin B, Fettah ND, Özyazıcı E, Beken S, Zenciroğlu A, et al. The prospective study: effects of probiotics and prebiotics alone or combined on necrotizing enterocolitis in very low birth weight infants. *The Journal of pediatrics*. 2015;166(3):545-51.
 7. Garland SM, Tobin JM, Pirotta M, Tabrizi SN, Opie G, Donath S, et al. The ProPrems trial: investigating the effects of probiotics on late onset sepsis in very preterm infants. *BMC infectious diseases*. 2011;11(1): 210.
 8. Norishadkam M, Naghibifard F, Mostaghasi M. The Effect of Oral Probiotics in Prevention of Necrotizing Enterocolitis in Premature Neonates. *SSU_Journals*. 2015;23(3):2032-9.
 9. Rojas MA, Lozano JM, Rojas MX, Rodriguez VA, Rondon MA, Bastidas JA, Perez LA, Rojas C, Ovalle O, Garcia-Harker JE, Tamayo ME. Prophylactic probiotics to prevent death and nosocomial infection in preterm infants. *Pediatrics*. 2012;130(5):e1113-20.
 10. Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. *Neonatology*. 2002; 82(2):103-8.
 11. Jacobs SE, Tobin JM, Opie GF, Donath S, Tabrizi SN, Pirotta M, et al. Probiotic effects on late-onset sepsis in very preterm infants: a randomized controlled trial. *Pediatrics*. 2013;132(6):1055-62.
 12. Bonsante F, Iacobelli S, Gouyon J-B. Routine probiotic use in very preterm infants: retrospective comparison of two cohorts. *Am J Perinatol*. 2013; 30(1):41-6.
 13. Kanic Z, Turk DM, Burja APS, Kanic V, Dinevski D. Influence of a combination of probiotics on bacterial infections in very low birthweight newborns. *Wien Klin Wochenschr*. 2015; 127 Suppl 5:S210-5.
 14. Tewari VV, Dubey SK, Gupta G. *Bacillus clausii* for prevention of late-onset sepsis in preterm infants: a randomized controlled trial. *Journal of tropical pediatrics*. 2015; 61(5):377-85.
 15. Bloom BT, Kamper J, Delmore P, Glass RI. Necrotizing enterocolitis in preterm neonates. *J Perinatal* 2008; 33:532-7.
 16. Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. 2001. FAO: Available at: <http://www.fao.org/3/a-a0512e.pdf>.
 17. Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, et al: Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. *Ann Surg* 1978, 187(1):1-7.
 18. Mohan R, Koebnick C, Schildt J, Mueller M, Radke M, Blaut M. Effects of *Bifidobacterium lactis* Bb12 supplementation on body weight, fecal pH, acetate, lactate, calprotectin, and IgA in preterm infants. *Pediatr Res* 2008, 64(4):418-22.
 19. Awad H, Mokhtar H, Imam SS, Gad GI, Hafez H, Aboushady N. Comparison between killed and living probiotic usage versus placebo for the prevention of necrotizing enterocolitis and sepsis in neonates. *Pak J Biol Sci* 2010; 13(6):253-62.
 20. Cukrowska B, LodInová-Zádníková R, Enders C, Sonnenborn U, Schulze J, Tlaskalová-Hogenová H. Specific proliferative and antibody responses of premature infants to intestinal colonization with nonpathogenic probiotic *E. coli* strain Nissle 1917. *Scand J Immunol* 2002; 55(2):204-9.