Clinical and Experimental Health Sciences

# *Lactobacillus* Species in Breast Milk: Do They Get Affected by Birth Style?

Aya Daif, Yasemin Zer, Mehmet Erinmez

Gaziantep University, Faculty of Medicine, Department of Medical Microbiology Gaziantep, Türkiye.

**Correspondence Author:** Mehmet Erinmez
**E-mail:** mehmeterinmez92@hotmail.com
**Received:** 14.04.2021 **Accepted:** 04.02.2022

**ABSTRACT**

**Objective:** Breast milk has an important function in the formation of the intestinal flora. Cesarean section bypasses the vertical transition of vaginal flora to the baby also usually causes the late start of lactation. The difference is in birth style and lactation period may affect the microbiota of breast milk. In this study, it was aimed to investigate how Lactobacillus species found in breast milk differ by the birth style and stages of milk.

**Methods:** Milk samples were taken from 72 mothers who had a vaginal birth (n:36) and cesarean (n:36) were divided into three groups as colostrum (n:12), early milk (n:12), and mature milk (n:12). *Lactobacillus* species were investigated from milk samples by real-time PCR.

**Results:** While Lactobacillus was detected in 70 (97.2%) of the samples, it was not detected in 2 (2.8%) of the samples taken from women with cesarean delivery. *L. acidophilus* and *L. rhamnosus* were detected simultaneously in all transitional milk samples of women who had a vaginal birth, and 82 *Lactobacillus* species were detected. The species identified were 33(39.3%) *L. rhamnosus*, 25(29.8%) Lactobacillus spp., and 24(28.6%) L. acidophilus. The rate of detection of *L. acidophilus* in milk samples taken from women who gave birth was found to be significantly higher than that found in milk samples taken from women who gave birth by cesarean section ($p < 0.05$).

**Conclusion:** Breast milk is not only a nutritional source but an important source of probiotics. *Lactobacilli* were found to be concentrated in breast milk. Also, *Lactobacillus* species detected in breast milk may differ according to the mode of delivery.

**Keywords:** Cesarean, *Lactobacillus,* microbiota, colostrum

## 1. INTRODUCTION

The microorganism assemblage, found in a certain ecological place or environment is called 'microbiota'. The microorganisms in the human body which are commensal, symbiotic, and pathogenic form the microbiota. The 'microbiome' usually used as the same meaning as microbiota, represents the gene pool of microbiota in a certain area and its relationship with the environment (1,2). In recent years, with the developments in molecular methods detection and identification of microbiota and microbiome of the human body becomes easier and more rapid (3). At present, developments in bacterial detection techniques especially those which are unrelated to culture methods and microbiome approach showed much more bacterial diversity in breast milk than predicted (4).

Human breast milk consists of high amounts of carbohydrates, essential fatty acids, proteins, vitamins, and minerals which are very important for feeding a baby, therefore it is recognized as the gold standard in baby nutrition (5-7). Breast milk plays a crucial role in the survival of the baby and baby development, not only with the nutrient source but also with the transfer of microflora (8). Breast milk is shown to be a consistent commensal, mutualistic, and probiotic bacterial source to baby intestines including *Staphylococcus, Streptococcus, Bifidobacteria*, and lactic acid bacteria (8-10).

*Lactobacillus, Pediococcus,* and *Lactococcus* species are belong to lactic acid bacteria (LAB) and strains of these bacteria often used in the production and preservation of many foods or used as probiotics for humans and animals (11,12). LAB like *L. gasseri, L. salivarius, L. rhamnosus, L. plantarum*, and *L. fermentum* which are found in breast milk and are considered to be probiotic species become more attractive targets (13). In this study, detection, and identification of *Lactobacillus* species in colostrum, transition, and mature breast milk from randomly selected mothers who are in the lactation period, also the determination of



*DOI: 10.33808/clinexphealthsci.915721*

effects of factors like delivery method (vaginal or cesarean) and lactation duration was aimed.

## 2. METHODS

Between August 2019 and March 2020, women aged between 18 to 45 years old and in the lactation period who had full-time pregnancy and have 0-240 days lactation duration was randomly selected for this study. This research was reviewed and approved by the Gaziantep University Clinical Research Ethics Committee (protocol code: 2019/240, date: 19.06.2019), and participation involved informed consent.

### *2.1. Research Sample*

Two groups were formed based on the delivery method. Two groups were formed based on vaginal and cesarean delivery and the minimum number for each group was defined as 36 based on statistical analysis, according to the literature, with 3% of expected prevalence, 5% precision, and %95 confidence interval.

Breast milk samples were classified into three different groups; breast milk between the 0-5 days after the birth was classified as colostrum, breast milk between the 6-15 days after the birth was classified as early milk, breast milk between the 15-240 days after the birth was classified as mature milk (14). Twelve colostrums, 12 early milk samples, and 12 mature milk samples, 36 in total, were collected from both groups consisting of women who gave birth by vaginal delivery and Cesarean section.

### *2.2. Exclusion Criteria*

– Women who took antibiotics for any reason before 15 days

– Women with gestational diabetes, hypertension, heart disease, acute infectious diseases, and postpartum depression

– Women with breast-related diseases

– Breastfeeding women who received hormonal therapy during the three months prior to starting the study or who had inadequate skills in understanding study questionnaires were not included in the study

– Women using commercial probiotic supplements

### *2.3. Data and Sample Collection*

Verbal instructions for standard sampling were given to mothers before sampling. Milk collection was taken by manually expressing the woman's breast. After the areola and its surroundings were cleaned with soap and sterile water and soaked in chlorhexidine and hand asepsis of the woman was achieved, the first few drops (0.5-1 mL) of milk were wiped off with a dry sterile sponge and 2-3 mL of milk was extracted from the subsequent milk sample and collected with sterile gloves in a sterile container.

### *2.4. Determination of demographic characteristics*

The mother's level of education, working status, family type (core & large family), smoking/alcohol use, number of children, birth style, weight gained at birth, whether or not she received breastfeeding education during pregnancy, profession, where the family lives (city & rural), monthly income, sex of the baby, birth weight, birth height, weight and height of the baby during sample intake, sleep patterns of baby, gastrointestinal complaints (colic, vomiting) of baby, defecation count of the baby were questioned with a questionnaire form. While families consisting of a mother, father and children were accepted as core families, families living with adults in addition to these individuals were accepted as large families. No amount was questioned about alcohol and cigarette use, but the presence of habit was questioned. Babies with a birth weight of less than 2500 grams were considered low birth weight, while babies between 2500-4000 grams were considered normal birth weight, and those with a birth weight over 4000 grams were considered high birth weight. Male babies with a birth length of 48 cm or less were classified as short birth length, while those 48-58 cms were considered normal birth length and those larger than 58 cm were considered longer than normal birth lengths. Female babies with a birth length of 45 cm or less were classified as short birth length, while those with a normal birth length of 45-55 cm and those larger than 55 cm were considered as longer than normal birth lengths. Infants with bouts of restlessness, spasms, and crying for at least 3 days a week and lasting approximately 3 hours during the day were considered colic.

### *2.5. Species Identification with Real-Time PCR*

All samples were kept frozen in the laboratory until work was carried out at −20 °C. DNA isolation was carried out using the Qiagen Stool Fast Kit (Qiagen, Hilden) protocol. Post-isolation DNA concentration was measured with the NanoDrop device (Thermo, USA). Using primary arrays (Primer Design Genesig (UK)) available from literature (15) according to brand species identification kits; *L. casei, L. acidophilus, L. delbrueckii, L. gasseri, L. reuteri, L. plantarum, L. rhamnosus,* species were defined, the species that are left out of these species were defined as Lactobacillus spp, negative and positive controls used according to producers recommendations. Amplification was run as 40 cycles on the Rotor-gene (Qiagen, Germany) PCR device.

### *2.6. Statistical Methods*

Data suitability for normal distribution was tested by the Shapiro-Wilk test. Student t or Mann-Whitney U tests were used to compare numerical data in 2 independent groups. Correlation analysis of the relationships between numerical variables and relationships between categorical variables was tested with Chi-Square. SPSS 22 Windows version was used in the analysis and it was considered statistically significant that the P-value was smaller than 0.05.

## 3. RESULTS

The mean age of the women included in the research, in Group I (vaginal delivery) and Group II (cesarean section) was similar and was found to be 30.02±3.60 and 30.97±4.10, respectively. Statistical evaluation of the demographic characteristics of study groups is given in Table 1. Statistical analysis revealed a significant difference between vaginal and cesarean delivery based on family type only (p:.028). The rate at which mothers who gave birth by Caesarean section had a core family structure was significantly higher than those who gave vaginal birth (Table 1).

*Lactobacillus* was detected in 70 (97.2%) of the samples in the study, while 2 (2.8%) of the samples taken from women with cesarean birth Lactobacillus was not detected. *L. acidophilus and L. rhamnosus* were detected simultaneously in all early milk samples of women who gave vaginal birth and 82 Lactobacillus species were identified (Table 2).

As a result of statistical analysis to determine whether there was a difference between PCR results of women who gave vaginal birth (Group I) and cesarean (Group II), it was found that the rate of detection of *L. acidophilus* in vaginal birth (Group I) was significantly higher than in cesarean (Group II) (p:.000), with no significant difference in other types of bacteria (p>0.05) (Table 2). When we investigated the relationship between Lactobacillus species detected in breast milk and the birth weight of babies, no statistically significant correlation was found (p:.0809).

***Table 1.*** *Comparison of demographic characteristics of vaginal and cesarean delivery groups*

| | Vaginal Delivery | Cesarean Section | P |
|---|---|---|---|
| **Age** (Av ± sd) | 30.02 ± 3.60 | 30.97 ± 4.10 | 0.303 |
| **Number of children** n(%) | | | |
| 1 child | 9 (25.0) | 7 (19.4) | 0.571 |
| 2 and more children | 27 (75.0) | 29 (80.6) | |
| **Smoking or alcohol use** n (%) | | | |
| Yes | 5 (13.9) | 2 (5.6) | 0.233 |
| No | 31 (86.1) | 34 (84.4) | |
| **Mother's Working Status** n (%) | | | |
| Housewife | 36 (100.0) | 34 (84.4) | 0.151 |
| Working | 0 (0.0) | 2 (5.6) | |
| **Family Type** n (%) | | | |
| Large family | 18 (50.0) | 9 (25.0) | 0.028 |
| Core family | 18 (50.0) | 27 (75.0) | |
| **Breastfeeding Training in Pregnancy** n (%) | | | |
| Yes | 8 (22.2) | 8 (22.2) | 1.000 |
| No | 28 (77.8) | 28 (77.8) | |
| **Weight Gain during Pregnancy** n (%) | | | |
| <10 kg | 11 (30.6) | 11 (30.6) | 1.000 |
| 10-15 kg | 16 (44.4) | 16 (44.4) | |
| >15 kg | 9 (25.0) | 9 (25.0) | |
| **The sex of the baby** n (%) | | | |
| Female | 20 (55.6) | 19 (52.8) | 0.813 |
| Male | 16 (44.4) | 17 (47.2) | |
| **Baby's Birth Weight** n (%) | | | |
| Low | 2 (5.6) | 0 (0.0) | 0.068 |
| Normal | 31 (86.1) | 36 (100.0) | |
| High | 3 (8.3) | 0 (0.0) | |
| **Baby's Birth Height** n (%) | | | |
| Low | 5 (13.9) | 3 (8.3) | 0.753 |
| Normal | 29 (80.6) | 31 (86.1) | |
| High | 2 (5.6) | 3 (8.3) | |
| **Colic Complaint** n (%) | | | |
| Have | 17 (47.2) | 23 (63.9) | 0.155 |
| No | 19 (52.8) | 13 (36.1) | |

***Table 2.*** *Distribution of Lactobacillus species among different milk types*

| | *Lactobacillus spp.* | *L. casei-group d* | *L. acidophilus* | *L. delbrueckii* | *L. gasseri* | *L. reuteri* | *L. plantarum* | *L. rhamnosus* |
|---|---|---|---|---|---|---|---|---|
| **Vaginal Delivery** | | | | | | | | |
| Colostrum n(%) | 8 (88.9) | 0 (0.0) | 4 (20) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 4 (19.1) |
| Early Milk n(%) | 0 (0.0) | 0 (0.0) | 12 (60) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 10 (47.6) |
| Mature Milk n(%) | 1(11.1) | 0 (0.0) | 4 (20) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 7 (33.3) |
| **Cesarean Section** | | | | | | | | |
| Colostrum n(%) | 10 (62.5) | 0 (0.0) | 1 (100) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 |
| Early Milk n(%) | 1 (6.3) | 0 (0.0) | 0 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 11 (73.3) |
| Mature Milk n(%) | 5 (31.2) | 0 (0.0) | 0 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 4 (26.7) |
| Total n (%) | 25 (30.5) | 0 (0.0) | 21 (25.6) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 36 (43.9) |
| p | 0.083 | 1.000 | 0.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.157 |

## 4. DISCUSSION

Breast milk has a protein content that has a special structure and that is easy to digest and protects against infections. Recent studies on breast milk, which is known to be a living and biological mixture, show that microbial content varies between individuals in terms of both species and numbers. There are many different microorganisms in breast milk microbiota (16,17).

Soto et al. (18) examine the lactobacilli population in the breast milk of healthy women and investigate the effects of various factors (antibiotherapy during pregnancy and breastfeeding, country and date of birth, type of birth, or infant age), including 160 healthy women from Germany and Austria. The three most common Lactobacillus species in milk are *L. salivarius* (35%), *L. fermentum* (25%), and *L.gasseri* (21.88%), as well as *L. reuteri* in 11.88% and *L. plantarum* in 10.63%, *L. ramnosus* in 8.13%, and *L. casei* in 4.38% of the milk samples were detected. Pregnancy, breastfeeding period and antibiotherapy have been reported as the main factors affecting the detection rates of lactobacilli.

Regardless of the geographical location and the analysis method used, it has been reported that there are most *Staphylococci* and *Streptococci* in breast milk. The limited type of microorganism, which will be named as the main microbiota of breast milk, constitutes 50% of the entire microbiota, while the remaining 50% is specific to the mother and varies according to environmental conditions (19). Factors such as the mother's health status, the living environment, diet, obesity, immunological status, gestational age, mode of delivery, antibiotic use, and lactation stage are the main factors affecting breast milk microbiota (20-22). Healthy microbiota development in infants is associated with a healthy maternal microbiota (23). In studies on the subject, microorganisms in breast milk have been shown to be effective in the development of the baby's microbiota (24).

Martin et al. (25) compared the *Lactobacillus* groups in five breast milk, vaginal swabs, and feces of babies in order to examine the effect of breast milk and vagina on intestinal colonization of the newborn. As a result of the study, none of the bacterial species detected in the vaginal samples were detected in breast milk samples, while a few species in the feces were detected in breast milk. Murphy et al. (20) compared breast milk with infant feces in the first three months and 10 months in their study. As a result of the study, it was seen that there were common bacterial species in both. The results obtained from the studies can be said that there is a vertical bacterial transmission from mother to baby through breastfeeding, and this affects the development of the baby's intestinal microbiota.

The transition of breast milk microbiota to the baby occurs as a result of extremely complex and developed processes (26). Especially in the first 3-4 months period is extremely important in terms of microbiota development (27). Bacteria that pass through the mother's vaginal, fecal, and skin microbiota are the first microorganisms to colonize in the neonatal intestine (3,28). The microbiota of the baby born with vaginal birth consists of facultative anaerobic bacteria such as *Staphylococcus, Streptococcus*, and *E. coli*, which are first transferred to the baby during passage through the birth canal. These bacteria multiply and form an anaerobic environment after a few days, which allows for the colonization and becoming dominance of *Bacteroides* and *Bifidobacteria* that pass from breast milk to the baby and can only reproduce in an anaerobic environment (27,29). Studies have reported that a variety of bacteria in the intestines of newborns who do not receive breast milk is higher and the amount of *Bifidobacteria* is less (27,30).

The first dietary factor affecting the microbiota is breastfeeding. Because it is accepted that breast milk is one of the most important factors affecting the formation of intestinal microbiota in babies. Breast milk is a symbiotic food that contains prebiotics (breast milk oligosaccharides) and probiotics (*Lactobacillus, Bifidobacterium*) together (31).

Studies have also shown that the milk microbiome can be affected by the mode of delivery (32). Also, maternal health can affect the breast milk microbiome. Bifidobacterium levels in the milk of mothers with problems such as obesity and allergies have been found to be lower than healthy mothers (33). The nutritional status of the mother affects microorganisms and immunomodulatory factors in milk (34).

After the presence of bacteria in breast milk was understood, the question of where these bacteria came from came to mind. In the first studies on this subject, it was suggested that the microorganisms in the breast skin are caused by the passage of the microorganisms into the milk during sucking, while some researchers have suggested that the bacteria in the infant oral mucosa pass into the milk (25). However, the detection of anaerobic bacterial cells or DNA in breast milk, which are mostly related to the intestinal environment and cannot live in aerobic regions, has led to the emergence of different opinions about the origin of milk-related bacteria. Because, despite the oxygen stress of the anaerobic *Bifidobacteria*, it is unlikely to be transferred from the mouth of the baby to the breast skin of the mother (4). Martin et al. (25) found that the bacteria in breast milk were not the bacteria found in the breast skin, but the fecal microbiota of the mother and baby. In this study, the researchers examined the mouth swabs and feces of eight babies who were breastfed, and samples were taken from their mother's milk, breast areola, and skin. They reported that lactic acid bacteria were isolated in all samples as a result of the examination. However, the RAPD (Random Amplified Polymorphic DNA) profile of the lactic acid bacteria found in the breast skin was found to be different from the other samples. As a result, it has been suggested that the presence of lactic acid in breast milk may not be due to the contamination of the milk with the breast skin environment and may have an endogenous origin. In the mechanism known as the Enteromammary Pathway, dendritic cells are advanced to have important functions. According to this view, dendritic cells can spread directly from the lumen to the intestinal epithelium to take a sample of bacteria. In addition, it can open tight junction points between the intestinal epithelium cells, extend beyond the epithelium through dendrites and directly sample bacteria without disrupting the epithelium cluster. After the bacteria bind to dendritic cells, they can travel to other regions including the milk glands via the monocyte circulation in the lymphocyte system associated with the mucosa (19). According to this knowledge, we formed the hypothesis that states mode of delivery can affect the bacteria species in breast milk because cesarean delivery may result in a late-onset of breastfeeding in the first hour of life and a reduction in the maintenance of breastfeeding in the first year of life (35). Failure to intake bacteria such as *Lactobacilli* and *Bifidobacteria* in breast milk, which are physiological stimulators of the physiological intestinal microbiota of babies born by cesarean section, may lead to a lower colonization level in the baby's intestinal flora (36, 37).

As limitations of our study, although we instructed the participants to collect milk samples under aseptic conditions, we would like to point out that we could not evaluate the contamination of the areola mammae and question the use of prebiotics, which is another factor that may affect the microbiota, and can be acquired from a very large group of nutrients.

## 5. CONCLUSION

As a result, the results of this study confirm that *Lactobacilli* are common members of the human milk microbiota in women who did not take antibiotics during pregnancy or breastfeeding. Therefore, the presence of such bacteria may be a marker of a healthy human milk microbiota without antibiotics, and this should be taken into account when defining a criteria standard for breast milk. Consequently, the administration of selected human milk lactobacilli to pregnant or breastfeeding women or their babies on antibiotics could create an attractive approach to restore the natural bacterial ecosystem found in breast milk. We think that long-lasting comprehensive studies should be conducted on the vital importance of the existing species, the development of the baby, and its various positive and negative effects.

### Funding

Research fund was granted from Gaziantep University, Gaziantep Turkey.

### Conflict of Interest

The authors have no conflicts of interest to declare.

## REFERENCES

[1] Rautava S. Early microbial contact, the breast milk microbiome and child health. J Dev Orig Health Dis 2016;7(1):5-14.

[2] Aslan NN. Yardımcı H. Anne Sütü ve Mikrobiyota. Turk Klin J Nutr Diet-Special Topics 2017;3(2):95-100. (Turkish)

[3] Arrieta MC, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The intestinal microbiome in early life: health and disease. Front Immunol 2014;5:427.

[4] Jeurink PV, Van Bergenhenegouwen J, Jimenez E, Knippels LM, Fernández L, Garssen J. Human milk: a source of more life than we imagine. Benef Microbes 2012;4(1):17-30.

[5] Sherman PM, Johnson-Henry KC, Yeong HP, Ngo PSC, Goulet J, Tompkins TA. Probiotics reduce enterohemorrhagic Escherichia coli O157:H7 – and enteropathogenic E. coli O127:H6-induced changes in polarized T84 epithelial cell monolayers by reducing bacterial adhesion and cytoskeletal rearrangements. Infect Immun 2005;73:5183-5188.

[6] Fernández L, Delgado S, Herrero H, Maldonado A, Rodríguez JM. The bacteriocin nisin, an effective agent for the treatment

of staphylococcal mastitis during lactation. J Hum Lact 2008;24(3):311-316.

[7] Lim YM, Barnes MB, Gras SL, McSweeney C, Lockett T, Augustin MA. Esterification of high amylose starch with short chain fatty acids modulates degradation by Bifidobacterium spp. J Funct Foods 2014;6:137-146.

[8] Bergmann H, Rodríguez JM, Salminen S, Szajewska H. Probiotics in human milk and probiotic supplementation in infant nutrition: a workshop report. Br J Nutr 2014;112(7),1119-1128.

[9] Jacobi SK, Odle J. Nutritional factors influencing intestinal health of the neonate. Adv Nutr 2012;3:687-696.

[10] Martín R, Langa S, Reviriego C, Jimínez E, Marín ML, Xaus J. Human milk is a source of lactic acid bacteria for the infant gut. J Pediatr 2003;143(6):754-8.

[11] Urbaniak C, Burton JP, Reid G. Breast, milk and microbes: A complex relationship that does not end with lactation. Womens Health (Lond.) 2012;8(4):385-398.

[12] Holzapfel WH, Haberer P, Snel J, Schillinger U, Huis in't Veld JH. Overview of gut flora and probiotics. Int J Food Microbiol 1998;41(2):85-101.

[13] Osmanagaoglu O, Kiran F, Ataoglu H. Evaluation of in vitro Probiotic Potential of Pediococcus pentosaceus OZF Isolated from Human Breast Milk. Probiotics Antimicrob Proteins 2010;2(3):162-174.

[14] Castellote C, Casillas R, Ramírez-Santana C, Pérez-Cano FJ, Castell M, Moretones MG. Premature delivery influences the immunological composition of colostrum and transitional and mature human milk. J Nutr 2011;141(6):1181-1187.

[15] Kwon HS, Yang EH, Yeon SW, Kang BH, Kim TY. Rapid identification of probiotic Lactobacillus species by multiplex PCR using species-specific primers based on the region extending from 16S rRNA through 23S rRNA. FEMS Microbiol Lett 2004;239(2):267-275.

[16] Gomez-Gallego C, Garcia-Mantrana I, Salminen S, Collado MC. The human milk microbiome and factors influencing its composition and activity. Semin Fetal Neonatal Med 2016;21(6):400-405.

[17] Fernández L, Langa S, Martín V, Maldonado A, Jiménez E, Martín R. The human milk microbiota: origin and potential roles in health and disease. Pharmacol Res 2013;69(1):1-10.

[18] Soto A, Martín V, Jiménez E, Mader I, Rodríguez JM, Fernández L. Lactobacilli and bifidobacteria in human breast milk: influence of antibiotherapy and other host and clinical factors. J Pediatr Gastroenterol Nutr 2014;59(1):78-88.

[19] Bode L, McGuire M, Rodriguez JM, Geddes DT, Hassiotou F, Hartmann PE. It's alive: microbes and cells in human milk and their potential benefits to mother and infant. Adv Nutr 2014;5(5):571-573.

[20] Murphy K, Curley D, O'Callaghan TF, O'Shea CA, Dempsey EM, O'Toole PW. The Composition of human milk and infant faecal microbiota over the first three months of life: A pilot study. Sci Rep 2017;7:40597.

[21] Boix-Amorós A, Collado MC, Mira A. Relationship between milk microbiota, bacterial load, macronutrients, and human cells during lactation. Front Microbiol 2016;7:492.

[22] Khodayar-Pardo P, Mira-Pascual L, Collado MC, Martínez-Costa C. Impact of lactation stage, gestational age and mode of delivery on breast milk microbiota. J Perinatol 2014;34(8):599-605.

[23] Latuga MS, Stuebe A, Seed PC. A review of the source and function of microbiota in breast milk. Semin Reprod Med 2014;32(1):68-73.

[24] Isolauri E. Development of healthy gut microbiota early in life. J Paediatr Child Health 2012;48 Suppl 3:1-6.

[25] Martín V, Maldonado-Barragán A, Moles L, Rodriguez-Baños M, Campo RD, Fernández L. Sharing of bacterial strains between breast milk and infant feces. J Hum Lact 2012;28(1):36-44.

[26] Schanche M, Avershina E, Dotterud C, Øien T, Storrø O, Johnsen R. High-resolution analyses of overlap in the microbiota between mothers and their children. Curr Microbiol 2015;71(2):283-290.

[27] Martin R, Heilig GH, Zoetendal EG, Smidt H, Rodríguez JM. Diversity of the Lactobacillus group in breast milk and vagina of healthy women and potential role in the colonization of the infant gut. J Appl Microbiol 2007;103(6):2638-2644.

[28] Goldsmith F, O'Sullivan A, Smilowitz JT, Freeman SL. Lactation and Intestinal Microbiota: How Early Diet Shapes the Infant Gut. J Mammary Gland Biol Neoplasia 2015;20(3-4):149-158.

[29] Mueller NT, Bakacs E, Combellick J, Grigoryan Z, Dominguez-Bello MG. The infant microbiome development: mom matters. Trends Mol Med 2015;21(2):109-117.

[30] Guaraldi F, Salvatori G. Effect of breast and formula feeding on gut microbiota shaping in newborns. Front Cell Infect Microbiol 2012;2:94.

[31] Coppa GV, Bruni S, Morelli L, Soldi S, Gabrielli O. The first prebiotics in humans: human milk oligosaccharides. J Clin Gastroenterol 2004;38(6 Suppl):S80-S83.

[32] Cabrera-Rubio R, Collado MC, Laitinen K, Salminen S, Isolauri E, Mira A. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. Am J Clin Nutr 2012;96(3):544-551.

[33] Collado MC, Laitinen K, Salminen S, Isolauri E. Maternal weight and excessive weight gain during pregnancy modify the immunomodulatory potential of breast milk. Pediatr Res 2012;72(1):77-85.

[34] Laitinen K, Collado MC, Isolauri E. Early nutritional environment: focus on health effects of microbiota and probiotics. Benef Microbes 2010;1(4):383-90.

[35] Bai DL, Wu KM, Tarrant M. Association between intrapartum interventions and breastfeeding duration. J Midwifery Womens Health 2013;58(1):25-32.

[36] Neu J, Rushing J. Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. Clin Perinatol 2011;38(2):321-331.

[37] Urbaniak C, Angelini M, Gloor GB, Reid G. Human milk microbiota profiles in relation to birthing method, gestation and infant gender. Microbiome 2016; 4:1.

**How to cite this article:** Daif A, Zer Y, Erinmez M. *Lactobacillus* Species in Breast Milk: Do They Get Affected by Birth Style?. Clin Exp Health Sci 2022; 12: 390-395. DOI: 10.33808/clinexphealthsci.915721