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ORIGINAL ARTICLE

Human milk as a source of next-generation probiotics: quantifying *Akkermansia muciniphila* and microbial contamination risks in donor milk

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ABSTRACT

Background and Objectives: Human milk provides nutrients, prebiotics, and probiotics that support infants' physical and mental growth. Human milk microbiota, as a potential source of probiotics and an indicator of the safety of donor milk, is of great importance. *Akkermansia muciniphila*, a core member of next-generation probiotics (NGPs), with the ability to degrade human milk oligosaccharides (HMOs), may be present in human milk. This study was carried out to assess the total bacterial count and presence of *A. muciniphila* in raw freshly expressed mothers' milk and pasteurized donor milk from human milk banks (HMBs).

Materials and Methods: 15 raw and 20 pasteurized milk samples were collected and analyzed using a real-time PCR technique with specific primers for *A. muciniphila* and universal 16S rRNA bacterial primers.

Results: Results showed that the average total bacterial count was 4.95 log CFU/ml, which is lower than the normal range reported for human milk. Samples with bacterial count over the standard range were related to the HMBs. Prevalence of *A. muciniphila* in human milk was 35% and was higher in raw milk samples than in pasteurized samples.

Conclusion: In conclusion, raw human milk can serve as a potential source for *A. muciniphila* isolation as a candidate for NGPs.

Keywords: *Akkermansia muciniphila*; Bacterial counts; Human milk; Microbiota; Pasteurization; Real-time polymerase chain reaction

INTRODUCTION

Breast milk is the first food consumed immediately after birth. It contains carbohydrates, protein, fat, minerals, vitamins, bioactive components, antibodies, and enzymes that support infants' short-term and long-term growth, immunity, and overall well-being. Breast-fed infants are at lower risk for infectious diarrhea and infant mortality. They also show normal weight gain and growth in early life, as well as a lower incidence of allergic diseases, metabolic diseases, and inflammatory bowel syndrome later in life (1-3).

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Before the 1920s, human milk was considered a sterile fluid, and any bacterial growth on culture medium was regarded as a result of contamination or mastitis (4). Since culture-independent methods and "microbiome" concept have emerged, human milk microbiota has received new attention. Human milk from a healthy mother who delivered a term infant contains 3-4 log CFU/ml bacteria dominated by 9 main genera as a "core" microbiome: *Staphylococcus, Streptococcus, Propionibacterium, Serratia, Pseudomonas, Ralstonia, Bradyrhizobium, Sphingomonas,* and *Corynebacterium* (5, 6). Additionally, some other genera more related to the probiotic category *Bifidobacterium*, Lactic acid bacteria, mainly *Lactobacillus*, have been found and isolated from human milk under anaerobic condition (7, 8). In this regard, breast-fed infants derive 4 to 7 log CFU/ml bacteria daily (4).

Recent studies demonstrated that microorganisms together with HMOs found in breast milk contribute to the formation of the gut microbiome in a healthy infant. Approximately 95% of HMOs pass through the intestinal tract intact, feeding only the members of the genera *Bifidobacterium, Bacteroides,* and *Akkermansia* (9). In addition, the breast milk microbiota plays a role in seeding the infant gut microbiome. In this regard, Shirvanian et al. (2023) isolated *Lactobacillus fermentum* with probiotic potential from human breast milk, which also exhibited antiproliferative and proapoptotic activities (10). In addition, Malekzadeh et al. (2019) studied the growth indices of breastfed infants until 6 months of age and formula-fed infants in southwest of Iran. Their findings suggest that formula feeding may help infants catch up in length and can lead to greater weight gain (11).

In some cases, premature or severely ill infants cannot be breastfed because their mothers are frequently unable to provide enough milk. For a baby in a Neonatal Intensive Care Unit (NICU), donated mothers' milk can be essential to life. For this reason, many human milk banks have been established worldwide. HMBs store safe and pasteurized donor milk and provide it to infants in NICUs (12). In Iran, 11 HMBs have been established so far. A comparison of neonatal outcomes showed that rates of feeding intolerance, sepsis, and mortality decreased, while the number of neonates achieving full enteral nutrition increased, following the launch of human milk banks in Iran (13).

In HMBs, milk is pasteurized to remove any harmful viruses and bacteria. While *Staphylococcus* and *Streptococcus* are two main members of human milk microbiota and are necessary for programming the immune system, some strains are pathogenic and have been reported to cause infectious diseases in infants (14). Therefore, pasteurization is required due to the vulnerable population served by the milk bank. The National Institute for Health and Clinical Excellence (NICE) recommends that donated milk be examined before pasteurization and be discarded if total microbial counts exceed 5 log CFU/ml. Furthermore, *Enterobacteriaceae* or *Staphylococcus aureus* counts should be below 4 log CFU/ml. Post-pasteurization, no bacterial growth in milk is permissible (15).

Pasteurization of milk samples at a temperature of 62.5°C for 30 min, followed by rapid cooling and freezing, also known as holder pasteurization (HoP), is the standard preservation method in milk banks worldwide (16). In regard to the damaging effect of HoP on bioactive components of mother's milk, an alternative thermal technique, HTST (High temperature short time), has emerged. This is particularly important since pasteurization kills not only pathogenic bacteria but also probiotic bacteria, which are sensitive to heat treatment (16).

Besides conventional probiotics (i.e., *Lactobacillus* and *Bifidobacterium*), human milk might be a source of next-generation probiotics. *Akkermansia muciniphila*, a core member of NGPs, was isolated from healthy human stool by Derrien et al in 2004 (17). Since then, many biochemical and molecular properties and health effects have been documented. The most impressive biochemical properties of *A. muciniphila* are the ability to use mucus as its main source of carbon and nitrogen. It is not able to degrade other typical carbon sources found in a standard culture medium, such as glucose, lactose, and fructose. Thus, the main oligosaccharides that can be used by *A. muciniphila* are HMOs. Therefore, human milk was considered a habitat for *A. muciniphila*, and this was first proven by Urbaniak et al. (18). They detected the gene for *A. muciniphila* in the microbiota of human breast tissue.

To the best of our knowledge, no study has evaluated the presence of *A. muciniphila* in human milk in Iran and microbial quality of human milk banks samples. We therefore conducted this first study in West Asia to analyze total bacterial count and the presence of *A. muciniphila* in pasteurized milk from HMBs compared to raw human milk.

MATERIALS AND METHODS

Subjects and sample collection. A total of 35 samples, consisting of 15 raw and 20 pasteurized human milk samples, were collected. Fifteen breastfeeding mothers who had delivered a full-term baby were selected. Written consent was obtained from the mothers as part of the clinical trial participants, and ethical approval was granted by the Golestan University of Medical Sciences Human Research Ethics Committee (IR.GOUMS.REC.1401.375). Before sample collection, the following data were recorded:

- Infant health status (e.g., occurrence of any illness, healthcare visits for sickness, antibiotic use, diarrhea, age, mode of delivery)
- Maternal health status (e.g., age, illnesses, antibiotic use).

To ensure the analysis focused on mature milk (not colostrum), mothers in the first week postpartum were excluded due to differences in chemical and microbial composition between colostrum and mature milk. Mothers who had taken antibiotics during lactation were also excluded.

Sample collection was done according to the protocol of Lackey et al. with some modifications (19). Before milk expression, hands were washed and sterilized with 70% alcohol, and nipples and areola were cleaned with sterile water and 70% alcohol. The first ten drops were discarded to ensure any exogenous contaminant in nipple ducts had been removed from the sample. Breast milk samples were collected in sterile tubes containing Cyctein-HCl (10%) (as a reducing agent to mitigate oxygen effects on *A. muciniphila* cells) either using gloved hands or via pump expression. In addition, 20 pasteurized samples were collected from two human milk banks (Tehran Hospital and Shahid Akbar Abadi Hospital, Tehran, Iran). The raw and pasteurized samples were transported to the laboratory in a cold box. Then milk fat was discarded by centrifugation (Hettich, Germany) (4000rpm/10min). Samples were aliquoted in 2ml tubes and quick-frozen with liquid nitrogen and stored at -80°C for further analysis.

DNA extraction and quantitative polymerase chain reaction (qPCR). Genomic DNA was isolated from milk samples using a tissue DNA extraction kit (cat. FATGM001, FAVORGEN, Taiwan) with some pretreatments: first, 5ml of milk was centrifuged at full speed for 1 min, then the remaining fat and whey were discarded with a pipettor and a cotton swab. The pellet was resuspended in 1 ml sterile ddH2O and transferred to a 1.5 ml tube. Subsequent steps were performed according to the manufacturer's instructions. The DNA concentration (ng/μL) and purity (A260/A280 and A260/A230) were determined using the Nano Drop spectrophotometer (Denovix, USA). The DNA samples were stored at -20°C until use in qPCR assays for *A. muciniphila* and total bacterial count.

To determine the abundance of *A. muciniphila*, qPCR was performed using the Add-Probe RT-PCR Kit (Add bio, Korea) and the StepOnePlus thermocycler (ABI, USA) with primers and a specific probe listed in Table 1. The reaction conditions were: 95°C for 2 min, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s.

16S bacterial DNA was amplified using SYBR Green master mix (Yekta Tajhiz Azma, Iran) on the StepOnePlus thermocycler (Applied Biosystems, USA) with the primers listed in Table 1. The amplification conditions were: 95°C for 5 min, followed by 35 cycles of 95°C for 20 s, 58°C for 30 s, and 72°C for 30 s.

Calibration curves. *A. muciniphila* abundance was quantified using a commercial probiotic supplement (Pendulum Life Co., San Francisco, California, USA) as a positive control to generate the standard curve. Using the manufacturer's data on the total *A. muciniphila* count per capsule, a tenfold serial dilution was performed to obtain a suspension ranging from 3 to 9 log CFU/ml. Then, DNA was extracted from 1ml aliquots of each dilution using the method described above.

Five genera of bacteria (*Staphylococcus, Lactobacillus, Acetobacter, Escherichia, Bacillus*) were used to prepare 8 log CFU/ml according to the McFarland standard method and verified by spectrophotometry (OD_{600}). Tenfold serial dilutions of this mixture, ranging from 3 to 8 log CFU/ml, were used to generate a calibration curve for calculating total bacterial count in human milk.

RESULTS

The total bacterial count and *A. muciniphila* abundance were determined in pasteurized milk from human milk banks and raw milk from breastfeeding

Table 1. primers and probes used for amplification of *A. muciniphila* and bacterial 16S rRNA

Bacteria	Primers	Amplicon Size	Reference
A. muciniphila	AM1: CAG CAC GTG AAG GTG GGG AC	329	(20)
	AM2: CCT TGC GGT TGG CTT CAG AT		
	Probe: CCT TGC GGT TGG CTT CAG AT		(21)
Bacterial 16S rRNA	F: CGG TGA ATA CGT TCC CGG	145	Generated by Primer3
	R: TAC GGC TAC CTT GTT ACG ACT T		program (version 4.1.0)

mothers (Fig. 1). The bacterial count in 35 samples ranged from 2.25 to 7.48 log CFU/ml with an average count of 4.95 log CFU/ml (Table 2). Fig. 2A shows that 20% of analyzed samples contained <4 log CFU/ml, approximately half of the samples contained 4-5.5 log CFU/ml and 8% of samples contained >7 log CFU/ml bacteria.

Results obtained by real-time PCR using specific primers for *A. muciniphila* showed that 35% (12/35) of all samples contained *A. muciniphila* (Table 2). *A. muciniphila* counts in positive samples ranged from 1 to 3.16 log CFU/ml with an average of 2.28 log CFU/ml. The distribution of *A. muciniphila* was as follows: in 65% of samples (23/35) *Akkermansia muciniphila* was not detected, 8% (3/35) had a range from 1-1.5, and 11% had 1.5-3 log CFU/ml and 11% had >3 log CFU/ml (Fig. 2B). Furthermore, the relative abundance of *A. muciniphila* in total bacterial count ranged

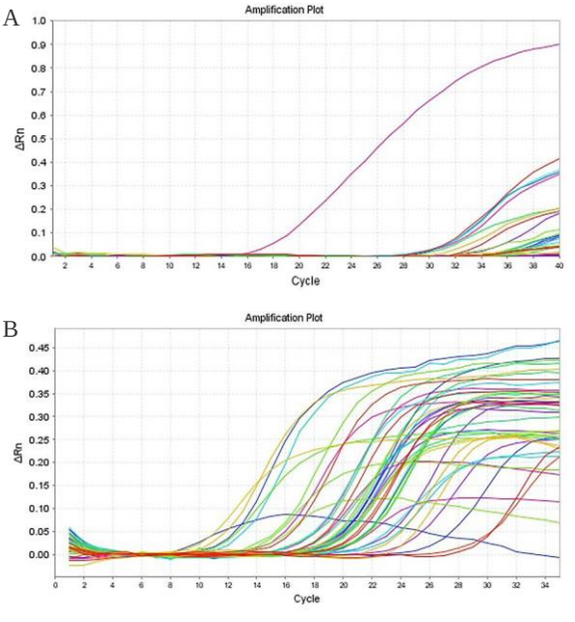

Fig. 1. Amplification plot of (A) *A. muciniphila* targeted gene and (B) bacterial 16S rRNA generated by Real-Time PCR technique

from 0.7% to 4.7%. In addition, our results showed that positive samples in the case of *A. muciniphila* were related to raw samples (8 out of 15) in comparison to pasteurized samples (4 out of 20). In other words, 40% of raw and 75% of pasteurized milks did not contain *A. muciniphila* (Table 2). In addition, the mean count of *A. muciniphila* in raw milk (2.75 log CFU/ml) was higher than in pasteurized milk (1.13 log CFU/ml).

DISCUSSION

Until recently, breast milk was known to be a sterile fluid (22); however, it has been now established that breast milk has its own microbiota consisting of probiotic and pathogenic bacteria. Consistent with this, our analysis found that the total microbial count in 78% of samples was <5 log CFU/ml. In 22% of samples, the bacteria level was >5 log CFU/ml. The normal range for microbial count in human milk has been reported to be <5 log CFU/ml (12) by culture-dependent techniques. Studies using culture-independent methods suggest that microbial count might reach 6 log CFU/ml due to the presence of some viable but non-culturable (VBNC) bacteria, extremely oxygen-sensitive species, and non-living in human milk, which are not detectable by culture-dependent methods, supporting our findings (23).

Microbial quality and safety of donated milk are primary concerns for human milk banks. NICE recommends that samples should be discarded if total microbial counts exceed 5 log CFU/ml (15). In this study, bacteria count with ≥7 log CFU/ml were found in 3 samples from human milk banks in which mothers expressed their milk at home. The high bacterial levels were likely due to exogenous contamination from practices such as using unsterile hands or pumps to express the milk and using unsterile containers (24). In addition, one of the main sources of

Table 2. Total bacterial and *A. muciniphila* count in human milk samples

Total bacterial count of human milk samples, log (CFU/ml)	Prevalence of *A. muciniphila* in all human milk samples, %	*A. muciniphila* abundance in all human milk samples, log (CFU/ml)	*A. muciniphila* Negative cases, %		*A. muciniphila* abundance in positive cases, log (CFU/ml)		Relative abundance of *A. muciniphila* in total bacterial count, %
			Raw milks	Pasteurized milks	Raw milks	Pasteurized milks	
2.25 - 7.48 Mean = 4.95	35	1.01 - 3.16 Mean = 2.28	40	75	1.78-3.16 Mean = 2.75	1.01-1.84 Mean = 1.31	0.7 - 4.7

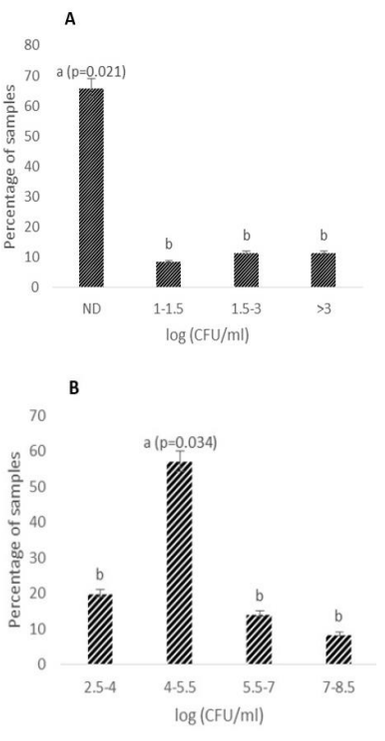

Fig. 2. Distribution of (A) *A. muciniphila* (B) Total bacterial count in 35 human milk samples

human milk microbiota is suggested to be the infant's mouth during sucking, through the backflow of milk into the mammary ducts (25). If the first few drops of milk were not discarded before the milk expression in a container, some genera of infant oral cavity, like *Streptococcus*, and of maternal skin, like *Staphylococcus*, present in nipple ducts will be transferred into milk samples. This also has been confirmed by Landers and Updegrove (2010), who cultured 810 individual samples from Mothers' Milk Bank of Austin in the USA and found that over 75% of samples exhibited some growth and *Staphylococcus* was the dominant strain in human milk (26). Expression of milk for donation introduces additional endogenous bacteria through collection, handling, and storage, and increases microbial count (27). Haiden et al. (2016), by comparison of microbial counts in expressed breast milk following standard or strict infection control regimens, reported that bacterial contamination of expressed milk was associated with the location of breast milk expression. They suggested that good hygiene in collection and storage equipment are the most important factors for microbial quality of expressed breast milk, which supports the finding of this study (28).

Like almost all microbiome sites, human milk microbiota and its microbial count vary significantly among different individuals in different countries (29). Besides exogenous contamination, maternal diet, medication, genetics, infant delivery mode, antibiotic administration, and geography are all factors that could influence the human milk microbiome (30-33). De Segura et al. (2012) in Spain, using culture techniques, reported that the viable bacteria in non-pasteurized samples ranged between 2.60 and 5.22 log CFU/ml in BHI medium (34). In a recent study, milk samples from 75 term and 135 preterm infants were analyzed. The results showed that the overall total bacterial concentrations were higher in milk from preterm infants than in term infants (preterm: 5.6 log CFU/ml; term: 2.6 log CFU/ml), suggesting that infant gestational age can influence the breast milk microbiome and microbial count (35). Moosavi et al. (2019) stated that composition and diversity of the human milk microbiota are influenced by maternal and early-life factors. They found mode of breastfeeding as a key determinant of milk microbiota composition. Providing pumped breast milk was specifically linked to an increase in potential pathogens and a decrease in *Bifidobacteria* (36).

The presence of the Verrucomicrobia phylum, to which *Akkermansia* belongs, has been demonstrated in a study on microbiota of human breast tissue from 43 women aged 18 to 90 years using 16S rRNA sequencing technique (18). Similarly, *A. muciniphila* in human milk and colostrum has been reported in other studies worldwide. Akko et al. (2017) analyzed eleven colostrum samples for microbiota composition by qPCR, and similar to our results they showed that *A. muciniphila* was present in the population at less than 1.5 log CFU/ml, with a median of 0.9. However, their detection rate was 63.6%, which is higher than our finding of 35% (37). This is due to the low detection rate of *A. muciniphila* in pasteurized milks. A previous study has shown that the integrity of DNA in milk could be affected by different heat treatments, such as pasteurization, boiling, and autoclaving (38). Therefore, the pasteurization employed in human milk banks may also result in DNA degradation and subsequently lead to a loss of the specific sequences needed for primer attachment. Akko et al. (2017) also observed a positive correlation between fucosylated HMOs and *A. muciniphila* abundance (37). In addition, structural similarities between mucin and HMOs explain why *Akkermansia*, which can degrade mucin as a sole carbon and nitrogen source, might be present in human milk (9). In contrast to its presence in the gut, where *A. muciniphila* is more abundant in lean individuals, its abundance in human milk has been reported to be higher in overweight mothers than in those of normal weight. Milk samples from overweight and normal-weight mothers were analyzed for microbiome composition immediately after delivery and at 1 and 6 months later (35). *A. muciniphila* levels were higher in overweight mothers' milk than in normal-weight mothers' milk. Moreover, the *A. muciniphila* count was higher in colostrum than in mature milk, with mean concentrations of 1.25 log gene copies/mL in colostrum, 1.09 at 1 month, and 1.20 at 6 months. Also, similar results to our findings were reported for *A. muciniphila* prevalence from 43% in colostrum to 29% in 6-month milk samples, indicating that *A. muciniphila* prevalence decreased as the infant aged. This might be of great importance and interest for further analysis to evaluate the influence of different maternal and infant-related factors on the next-generation probiotics population in human milk in Iran.

A. muciniphila begins to establish at birth, forming a barrier that prevents the colonization of pathogens and harmful substances, protects intestinal integrity, and promotes immune system development. Its colonization reaches levels comparable to those in adults (10^8 cells/g) within the first year of life but declines in the elderly. This increase may be linked to the development of a healthy intestinal tract and normal mucus production (21). In this regard, the risk of mortality may decrease in infants fed with human milk containing *A. muciniphila*. This is potentially due to enhanced gut barrier integrity against pathogenic bacteria, reduced adiposity, and improved glucose homeostasis in offspring during adulthood. Therefore, increasing the abundance of *A. muciniphila* may represent a promising strategy to promote infant health early in life (39).

CONCLUSION

The human milk microbiome is of great importance for infant short and long-term health as it contains probiotic bacteria, and is critical for human milk banks to provide microbiologically safe milk to infants in NICUs. Taken together, our results demonstrate that the mean microbial count of samples was within the standard range, but the microbial count of milk samples expressed at home and provided to milk banks was higher than that of freshly expressed samples. This highlights the importance of personal hygiene of donors during milk collection and the pasteurization process in milk banks to reduce microbial count and ensure the microbial quality of milk. In addition, the presence of *A. muciniphila* as the most important member of next-generation probiotics in human milk samples demonstrates that, other than stool, human milk could be a source of NGPs and emphasizes the importance of breastfeeding for seeding the infant's gut.

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REFERENCES

1. Barclay AR, Russell RK, Wilson ML, Gilmour WH, Satsangi J, Wilson DC. Systematic review: The role of breastfeeding in the development of pediatric inflammatory bowel disease. *J Pediatr* 2009; 155: 421-426.
2. Palou A, Picó C. Leptin intake during lactation prevents obesity and affects food intake and food preferences in later life. *Appetite* 2009; 52: 249-252.
3. Savilahti E, Saarinen KM. Early infant feeding and type 1 diabetes. *Eur J Nutr* 2009; 48: 243-249.
4. Jeurink PV, van Bergenhenegouwen J, Jiménez E, Knippels LM, Fernández L, Garssen J, et al. Human milk: A source of more life than we imagine. *Benef Microbes* 2013; 4: 17-30.
5. Gómez-Gallego C, García-Mantrana I, Salminen S, Collado MC. The human milk microbiome and factors influencing its composition and activity. *Semin Fetal Neonatal Med* 2016; 21: 400-405.
6. Martín R, Langa S, Reviriego C, Jimínez E, Marín ML, Xaus J, et al. Human milk is a source of lactic acid bacteria for the infant gut. *J Pediatr* 2003; 143: 754-758.
7. Martín R, Jiménez E, Olivares M, Marín ML, Fernández L, Xaus J, et al. Lactobacillus salivarius CECT 5713, a potential probiotic strain isolated from infant feces and breast milk of a mother-child pair. *Int J Food Microbiol* 2006; 112: 35-43.
8. Martín R, Jiménez E, Heilig H, Fernández L, Marín ML, Zoetendal EG, et al. Isolation of bifidobacteria from breast milk and assessment of the bifidobacterial population by PCR-denaturing gradient gel electrophoresis and quantitative real-time PCR. *Appl Environ Microbiol* 2009; 75: 965-969.
9. Marcobal A, Barboza M, Froehlich JW, Block DE, German JB, Lebrilla CB, et al. Consumption of human milk oligosaccharides by gut-related microbes. *J Agric Food Chem* 2010; 58: 5334-5340.
10. Shirvanian M, Azimian Zavareh V, Zamanzadeh Z, Janghorban M, Mohammadi E, Ahangarzadeh S. Probiotic potential, antiproliferative, and proapoptotic activities of *Lactobacillus fermentum* isolated from breast milk. *Adv Biomed Res* 2023; 12: 242.
11. Malekzadeh J, Synaii S, Ebrahimzadeh Koor B, Falsafian G, Nakhaie MR. Growth indices of exclusively breastfed until 6 months age and formula-fed infants in southwest of Iran. *Int J Prev Med* 2019; 10: 207.
12. Moro GE, Billeaud C, Rachel B, Calvo J, Cavallarin L, Christen L, et al. Processing of donor human milk: Update and recommendations from the European Milk Bank Association (EMBA). *Front Pediatr* 2019; 7: 49.
13. Hamidi N, Mousavi SS, Farahani LA, Khosravi A, Saboute M. Comparison of the short-term neonatal outcomes of preterm neonates before and after the launch of human milk bank in Iran: A retrospective descriptive study. *BMC Pediatr* 2025; 25: 202.
14. Fernández L, Langa S, Martín V, Maldonado A, Jiménez E, Martín R, et al. The human milk microbiota: Origin and potential roles in health and disease. *Pharmacol Res* 2013; 69: 1-10.
15. National Institute for Health and Clinical Excellence. Donor Breast Milk Banks: The Operation of Donor Milk Bank Services. London: NICE; 2010.
16. Conboy-Stephenson R, Ross RP, Kelly AL, Stanton C. Donor human milk: The influence of processing technologies on its nutritional and microbial composition. *Front Nutr* 2024; 11: 1468886.
17. Derrien M, Vaughan EE, Plugge CM, de Vos WM. *Akkermansia muciniphila* gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int J Syst Evol Microbiol* 2004; 54: 1469-1476.
18. Urbaniak C, Cummins J, Brackstone M, Macklaim JM, Gloor GB, Baban CK, et al. Microbiota of human breast tissue. *Appl Environ Microbiol* 2014; 80: 3007-3014.
19. Lackey KA, Williams JE, Meehan CL, Zachek JA, Benda ED, Price WJ, et al. What's normal? Microbiomes in human milk and infant feces are related to each other but vary geographically: The INSPIRE Study. *Front Nutr* 2019; 6: 45.
20. Krieg NR, Staley JT, Brown DR, Hedlund B, Paster B, Ward N, et al (2010). Bergey's Manual of Systematic Bacteriology, Volume 4: The Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes. https://link.springer.com/book/10.1007/978-0-387-68572-4
21. Collado MC, Derrien M, Isolauri E, de Vos WM, Salminen S. Intestinal integrity and *Akkermansia muciniphila*, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. *Appl Environ Microbiol* 2007; 73: 7767-7770.
22. Selma-Royo M, Calvo Lerma J, Cortés-Macías E, Collado MC. Human milk microbiome: From actual knowledge to future perspective. *Semin Perinatol* 2021; 45: 151450.
23. Lee S, Heo S, Park M-K, Sung M-H, Jeong D-W. Bacterial community of breast milk in breastfeeding women using culture-dependent and culture-independent approaches. *J Microbiol Biotechnol* 2024; 34: 2005-2011.
24. Gad S, Sheta MM, Al-Khalafawi AI, Abu El-Fadl HA, Anany M, Sahmoud S, et al. Expressed breast milk contamination in neonatal intensive care unit. *Pediatric Health Med Ther* 2021; 12: 307-313.
25. Ramsay DT, Kent JC, Owens RA, Hartmann PE. Ultrasound imaging of milk ejection in the breast of lactat-

ing women. *Pediatrics* 2004; 113: 361-367.
26. Landers S, Updegrove K. Bacteriological screening of donor human milk before and after Holder pasteurization. *Breastfeed Med* 2010; 5: 117-121.
27. Perrin MT, Fogleman AD, Davis DD, Wimer CH, Vogel KG, Palmquist AEL. A pilot study on nutrients, antimicrobial proteins, and bacteria in commerce-free models for exchanging expressed human milk in the USA. *Matern Child Nutr* 2018; 14 Suppl 6(Suppl 6): e12566.
28. Haiden N, Pimpel B, Assadian O, Binder C, Kreissl A, Repa A, et al. Comparison of bacterial counts in expressed breast milk following standard or strict infection control regimens in neonatal intensive care units: Compliance of mothers does matter. *J Hosp Infect* 2016; 92: 226-228.
29. Kumar H, du Toit E, Kulkarni A, Aakko J, Linderborg KM, Zhang Y, et al. Distinct patterns in human milk microbiota and fatty acid profiles across specific geographic locations. *Front Microbiol* 2016; 7: 1619.
30. 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: 544-551.
31. Hermansson H, Kumar H, Collado MC, Salminen S, Isolauri E, Rautava S. Breast milk microbiota is shaped by mode of delivery and intrapartum antibiotic exposure. *Front Nutr* 2019; 6: 4.
32. 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: 78-88.
33. Olivares M, Albrecht S, De Palma G, Ferrer MD, Castillejo G, Schols HA, et al. Human milk composition differs in healthy mothers and mothers with celiac disease. *Eur J Nutr* 2015; 54: 119-128.
34. de Segura AG, Escuder D, Montilla A, Bustos G, Pallás C, Fernández L, et al. Heating-induced bacteriological and biochemical modifications in human donor milk after Holder pasteurization. *J Pediatr Gastroenterol Nutr* 2012; 54: 197-203.
35. Miura K, Tanaka M, Date M, Ito M, Mizuno N, Mizuno K. Comparison of bacterial profiles in human milk from mothers of term and preterm infants. *Int Breastfeed J* 2023; 18: 29.
36. Moossavi S, Sepehri S, Robertson B, Bode L, Goruk S, Field CJ, et al. Composition and variation of the human milk microbiota are influenced by maternal and early-life factors. *Cell Host Microbe* 2019; 25: 324-335. e4.
37. Aakko J, Kumar H, Rautava S, Wise A, Autran C, Bode L, et al. Human milk oligosaccharide categories define the microbiota composition in human colostrum. *Benef Microbes* 2017; 8: 563-567.
38. Liao J, Liu Y, Ku T. Changes in physicochemical properties and DNA quality of milk as affected by different heat treatments. *Int J Dairy Technol* 2018; 71: 333-339.
39. Qi Y, Yu L, Tian F, Zhao J, Zhang H, Chen W, et al. *Akkermansia muciniphila* supplementation in mice during pregnancy and lactation affects the maternal intestinal microenvironment. *Nutrients* 2022; 14: 390.