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Number of Breast Milk Bacteria in Women with Normal Labor and Caesarean Delivery During Room Temperature Storage

Siti Noor Habibah, Ahmad Muhlisin, Leka Lutpiatina*, Aima Insana

Medical Laboratory Technology Poltekkes Kemenkes Banjarmasin
Mistar Cokrokusumo Street 4A Banjarbaru Indonesia.
*Email: leka.zns@gmail.com

Abstract: The presence of microbes in each breast milk sample can be different, one of which is influenced by the method of delivery. The research aimed to analyze the effect of storage time for breast milk from mothers giving birth normally and giving birth by Caesarean on the number of bacteria at room temperature. The independent variable in this study was the storage time for breast milk from mothers giving birth normally and giving birth by Caesarean for 0, 4, 8, and 12 hours at room temperature, while the dependent variable in this study was the number of bacteria in the breast milk. The research samples were breast milk from mothers who gave birth normally and Caesarean section which was treated with storage at room temperature for 0, 4, 8, and 12 hours. The results of the research were that the average number of bacteria in breast milk from mothers giving birth normally, stored at 0, 4, 6, 8, and 12 hours respectively, was 1.33 x 10^4 CFU/ml; 2.18 x 10^4 CFU/ml; 5.04 x 10^6 CFU/ml; 1.68 x 10^7 CFU/ml. The average number of bacteria in breast milk from mothers giving birth by Caesarean storage 0, 4, 6, 8, and 12 hours respectively 1.83 x 10^4 CFU/ml; 1.73 x 10^5 CFU/ml; 1.76 x 10^7 CFU/ml; 6.67 x 10^7 CFU/ml. There is a significant difference in the number of bacteria between breast milk from mothers who gave birth normally and breast milk from mothers who gave birth by Caesarean section. Storage time has a positive effect on the number of bacteria in breast milk from mothers giving birth normally and giving birth by Caesarean. It is recommended to store breast milk at room temperature for up to 4 hours for breast milk from women giving birth normally and for breast milk from mothers giving birth by Caesarean, storage for less than 4 hours by paying attention to the cleanliness of the breast milk storage area.
Keywords: Bacteria count; breast milk; cesarean delivery; normal birth.

INTRODUCTION

One of the differences in the shelf life of breast milk at various storage temperatures is caused by the presence of microbes. Breast milk from healthy women contains 10^3-10^5 CFU/ml of live bacteria[1,2]. The bacteria found in breast milk are good bacteria such as Bifidobacteria, opportunistic bacteria such as *Enterococcus* and *Streptococcus*, and pathogenic bacteria such as *Staphylococcus*[3].

The presence of microbes in each breast milk sample can be different, one of which is influenced by the method of delivery. Women can give birth through normal delivery or Caesarean section. Normal delivery is delivery through the vagina without the

Corresponding Author: Leka Lutpiatina
Medical Laboratory Technology Department,Poltekkes Kemenkes Banjarmasin,
Mistar Cokrokusumo Street 4a Banjarbaru Indonesia 70714.
Email: leka.zns@gmail.com

help of drugs and with only the mother's strength to push the baby[4]. Meanwhile, Caesarean delivery is a way of giving birth to a baby through surgery on the mother's stomach and is only carried out if a normal birth process is not possible[5]. According to Li et al, (2017), milk from mothers who have had a Caesarean section has a higher number of *Lactobacillus* and *Operational Taxonomic Units* (OTUs) than mothers who have had a normal delivery. These bacteria will grow depending on the temperature and storage time of breast milk[6].

Storing fresh breast milk at room temperature for up to 6-8 hours, in the refrigerator (4°C) for up to 5-8 days, and in the freezer (<-4°C) breast milk is safe to consume for up to 12 months[7]. Based on research conducted by Siahaya and Talarima (2017), the storage time for breast milk at a temperature of -15˚C influences protein, the total number of bacteria, the pH of breast milk as well as the color and odor quality of breast milk. The total number of microbes in breast milk storage for 0 and 4 days was 116 CFU/ml, then decreased to 22 CFU/ml at 8 days of storage, then increased at 12 days of storage, namely 133 CFU/ml[8]. This is confirmed by research conducted by Mustika et al, (2019), that the total bacteria in fresh breast milk is 20.3 x 10^3 CFU/ml, indicating a higher number compared to breast milk stored in pasteurized cooler bags, namely 18 x 10^3 CFU/ml[9]. The total number of bacteria in breast milk can determine the quality of the breast milk. The acceptable criteria for breast milk before pasteurization is a limit of bacterial counts ≤1x105 CFU/ml[10].

Research on the length of storage of breast milk at room temperature has been carried out by previous researchers, namely Mutaqin et al, (2018) who obtained the results that there was an effect of the storage time of breast milk for 0, 4, 6, and 8 hours at room temperature on reducing the quality of breast milk from total mesophyll results. aerobes, enterobacter, and fungi[11]. There is still limited research on germ numbers in breast milk samples from mothers who gave birth normally and mothers who gave birth by Caesarean with different storage times, so this research aims to analyze the effect of storage time for breast milk from mothers who gave birth normally and mothers who gave birth by Caesarean on the number of bacteria at room temperature.

MATERIALS AND METHODS

The research design used was a One Group Pretest-Posttest Design, namely measuring samples before storage and immediately measuring them (pretest) and measuring samples that were treated with storage for 4 hours, 8 hours, and 12 hours at room temperature (posttest).

The research sample used was breast milk from mothers who gave birth normally and mothers who gave birth by cesarean section which was released on the same day. Respondents had previously been given informed consent and this research was in accordance with the Declaration of Helsinki. Inclusion criteria are mothers who gave birth normally and cesarean section with a close delivery distance and have given birth within a maximum of one year; Willing to be a respondent; Do not smoke; Mother and baby are in good health and have no infectious diseases that require medical attention in the 2 weeks before breast milk collection. In this study, there were 8 treatments for each sample with 3 repetitions.

The tools used in this research were breast pumps, sample bottles of breast milk, alcohol, cotton, Petri dishes, oven, Erlenmeyer, analytical balance, hot plate, autoclave,

measuring cup, beaker glass, horn spoon, measuring pipette, bulb, test tube, tube rack, spirit lamp, incubator, and colony counter. The materials used in this research were distilled water, Nutrient Agar (Merck), and 0.9% NaCl.

The independent variable in this study was the storage time for breast milk from mothers giving birth normally and giving birth by cesarean section for 0 hours, 4 hours, 8 hours, and 12 hours at room temperature. The dependent variable in this research is the number of bacteria in breast milk.

Informed consent was given to respondents before agreeing to take part in the research. Sampling was carried out on both nipples as much as 15 ml on each nipple manually so that the amount of breast milk taken was 30 ml from each mother. Sample delivery is carried out in cold temperatures[6]

Breast milk samples for the 0-hour treatment are ready to be examined immediately. Meanwhile, breast milk samples with storage periods of 4 hours, 8 hours, and 12 hours were stored at room temperature (25ºC) in an incubator. The Total Plate Count (TPC) procedure was carried out by diluting the sample $10\text{-}10^5$ using 0.9% NaCl. Put 1 ml of sample at each dilution into a petri dish and add Nutrient Agar (NA). Incubate at 37ºC for 48 hours. Colonies that grow between 30-300 are counted using a colony counter. The TPC value (CFU/ml) is the number of colonies in the test petri dish minus the number of colonies in the control multiplied by the dilution divided by the calculated number of test Petri dishes.

RESULTS AND DISCUSSION

In this study, breast milk was taken from mothers who had given birth normally and Caesarean. Mothers who gave birth normally had a body mass index (BMI) of 24.9, while mothers who gave birth by Caesarean had a BMI of 24.8. The two respondents did not take antibiotics and had breastfed for 1 month so the respondents' breast milk was categorized as mature breast milk. The color of breast milk before and after being treated with long storage looks slightly different. Newly expressed breast milk has a white color with a typical breast milk aroma, but after storage, the color of the breast milk changes to milky white at the bottom and yellowish at the top so it needs to be homogenized, and has a slightly sour aroma. A more complete explanation of breast milk organoleptics is in Table 1.

Table 1. Organoleptic Table of Breast Milk

	Storage 0 hours	Storage >4 hours
Color	Milk white	Milky white at the bottom and yellowish at the top
Smell	Typical of breast milk	A bit sour
Texture	Merges	Layers are formed

The results of the research on the two breast milk samples were, that the average number of bacteria in breast milk samples from mothers giving birth normally at 0 hours of storage was 1.33×10^4 CFU/ml, 4 hours of storage was 2.18×10^4 CFU/ml, 8 hours of storage. hours of 5.04×10^6 CFU/ml, and 12 hours of storage of 1.68×10^7 CFU/ml. Meanwhile, the average number of bacteria in breast milk samples from mothers giving

birth by Caesar at 0 hours of storage was 1.83 x 10^4 CFU/ml, 4 hours of storage was 1.73 x 10^5 CFU/ml, 8 hours of storage was 1.76 x 10^7 CFU/ml, and 12-hour storage was 6.67 x 10^7 CFU/ml. From these data, it can be seen that there is an increase in the number of bacteria in breast milk from both mothers giving birth normally and Caesarean after being treated with the specified temperature and storage time. More complete numbers of germ numbers in breast milk samples are presented in Tables 2 and 3.

Table 2. The Number of Breast Milk Bacteria (CFU/ml) from Mothers Giving Birth Normally

Storage time	Replication			Average
	1	2	3	
0 hours	1,44 x 10^4	1,42 x 10^4	1,12 x 10^4	1,33 x 10^4
4 hours	2,20 x 10^4	2,01 x 10^4	2,33 x 10^4	2,18 x 10^4
8 hours	4,78 x 10^6	5,38 x 10^6	4,97 x 10^6	5,04 x 10^6
12 hours	1,76 x 10^7	1,67 x 10^7	1,60 x 10^7	1,68 x 10^7

Table 3. Number of Breast Milk Bacteria (CFU/ml) from Mothers Giving Birth by Caesarean

Storage time	Replication			Average
	1	2	3	
0 hours	1,75 x 10^4	1,82 x 10^4	1,91 x 10^4	1,83 x 10^4
4 hours	1,72 x 10^5	1,69 x 10^5	1,80 x 10^5	1,73 x 10^5
8 hours	1,70 x 10^7	1,75 x 10^7	1,83 x 10^7	1,76 x 10^7
12 hours	6,55 x 10^7	6,68 x 10^7	6,77 x 10^7	6,67 x 10^7

Breast milk samples from mothers who gave birth normally and from mothers who gave birth by Caesarean had the highest number of bacteria after being stored for 12 hours at room temperature, namely 1.68 x 10^7 CFU/ml in breast milk samples from mothers who gave birth normally and amounted to 6.67. x 10^7 CFU/ml for breast milk samples from mothers giving birth by Caesarean. Meanwhile, the lowest average number of bacteria was when breast milk was stored for 0 hours at room temperature, namely 1.33 x 10^4 CFU/ml for breast milk samples from mothers who gave birth normally and 1.83 x 10^4 CFU/ml for breast milk samples from mothers. gave birth to Caesar.

Before conducting analysis tests on research data, a normality test is carried out first. Based on the results of the Shapiro-Wilk normality test, the significance value obtained for the storage time of 0 hours, 4 hours, 8 hours, and 12 hours for both breast milk from mothers who gave birth normally and breast milk from mothers who gave birth by Caesarean was greater than 0.05, so the data was declared to be normally distributed. The results of the homogeneity test of research data obtained a significance value of more than 0.05, which means that the research data is homogeneous or has the same variance.

Statistical tests to determine the effect of storage time of 0 hours, 4 hours, 8 hours, and 12 hours on the average number of bacteria in breast milk using the One Way ANOVA test. Based on Table 4, the probability value is 0.000 for both breast milk data

from mothers giving birth normally and breast milk data from mothers giving birth by Caesarean section, because the significance value is less than 0.05, it can be concluded that there is an effect of storage time of 0 hours, 4 hours, 8 hours and 12 on the number of bacteria in breast milk.

Table 4. One-Way ANOVA Test on the Effect of Storage Time on the Number of Bacteria

One Way ANOVA Test					
		N	p value	Criteria Normal	Conclusion
Breast milk from mothers giving birth is normal	0 hours	3	0,000	P<0,05	There is a meaningful influence
	4 hours	3			
	8 hours	3			
	12 hours	3			
Breast Milk from Mothers Giving Birth by Caesarean	0 hours	3	0,000	P<0,05	There is a meaningful influence
	4 hours	3			
	8 hours	3			
	12 hours	3			

Because the data is normally distributed and has the same variance, to find out which treatments are different and which are not different, a Post Hoc LSD Test is carried out. The results of the Post Hoc LSD test are in Table 5 for each storage treatment for both breast milk from mothers who gave birth normally and breast milk from mothers Caesarean delivery had a significance value of <0.05, meaning that there was a significant difference in the number of bacteria in all treatment groups except for the 4 hours versus 0-hour treatment in breast milk samples from mothers who gave birth normally.

There was no difference in the number of bacteria with a significant value of >0.05. To determine whether or not there was a difference in the number of bacteria between the two samples, both breast milk from mothers who gave birth normally and breast milk from mothers who gave birth by Caesarean section, a One-way ANOVA test was carried out. Based on Table 6, it was found that the significance value was less than 0.05 for each storage length treatment, which means that there was a difference in the number of germ numbers between the two samples.

A regression test was carried out to determine how much influence storage time has on the number of breast milk bacteria. In Table 7 it can be concluded that storage time has a positive effect on the number of bacteria in breast milk from mothers giving birth normally by 89.9% and 10.1% is influenced by other factors. Meanwhile, for breast milk from mothers who gave birth by Caesarean section, storage time had a positive effect on the number of bacteria by 90.8%, and 9.2% was influenced by other factors. This positive influence means that the longer breast milk is stored, it will affect increasing the number of bacteria in breast milk.

Table 5. Post Hoc Analysis of Germ Numbers Between Groups

Post Hoc LSD Test				
		P value	Criteria Normal	Conclusion
Breast milk from mothers giving birth is normal	4 hours - 0 hours	0,522		There is no meaningful differences
	8 hours - 0 hours	0,000		There are significant differences
	12 hours - 0 hours	0,000		There are significant differences
	8 hours - 4 hours	0,000		There are significant differences
	12 hours - 4 hours	0,000		There are significant differences
	12 hours - 8 hours	0,000	P<0,05	There are significant differences
Breast Milk from Mothers Giving Birth by Caesarean	4 hours - 0 hours	0,000		There are significant differences
	8 hours - 0 hours	0,000		There are significant differences
	12 hours - 0 hours	0,000		There are significant differences
	8 hours - 4 hours	0,000		There are significant differences
	12 hours - 4 hours	0,000		There are significant differences
	12 hours - 8 hours	0,000		There are significant differences

The research results on breast milk from mothers giving birth normally at 4 hours of storage were 2.18×10^4 (Table 2) similar to research by Arslanoglu et al, 2010; Breast milk from mothers giving birth normally stored at room temperature still meets the limit for the number of microbes ($\leq 1 \times 10^5$) in unpasteurized expressed breast milk for 4 hours of storage[10]. However, this result is different from the results of research on breast milk from mothers who gave birth by Caesarean after 4 hours of storage, the number of bacteria in breast milk was 1.73×10^5.

The difference in the number of bacteria in the two breast milk was visible at 0 hours of storage. In breast milk from mothers who gave birth normally, the number of bacteria at 0 hours of storage was 1.33×10^4 CFU/ml, while in breast milk from mothers who gave birth by Caesarean section the number of bacteria at 0 hours of storage was 1.83×10^4 CFU/ml. This is by research conducted by Li, et al (2017) that the number of *Operational Taxonomic Units* (OTUs) in breast milk from mothers who gave birth by Caesarean is higher, namely 357 OTUs, compared to breast milk from mothers who gave birth normally, namely 136 OTUs[6].

Table 6 One Way ANOVA Test Results on the Number of Bacteria Between Breast Milk from Mothers with Normal Births and Caesarean Births

One Way ANOVA Test					
		N	P value	Criteria Normal	Conclusion
0 hours	Breast milk from mothers giving birth is normal	3	0,012	P<0,05	There are significant differences
	Breast milk from mothers giving birth by caesarean	3			
4 hours	Breast milk from mothers giving birth is normal	3	0,000		There are significant differences
	Breast milk from mothers giving birth by caesarean	3			
6 hours	Breast milk from mothers giving birth is normal	3	0,000		There are significant differences
	Breast milk from mothers giving birth by caesarean	3			
8 hours	Breast milk from mothers giving birth is normal	3	0,000		There are significant differences
	Breast milk from mothers giving birth by caesarean	3			

Table 7. Regression Test Results for the Number of Bacteria Between the Breast Milk from Mothers with Normal Births and Caesarean Births

	R Square	T value
Number of Breast Milk Bacteria from mothers giving birth normally	0,899	9,453
Number of Breast Milk Bacteria from Mothers Giving Birth by Caesarean	0,908	9,907

One of the reasons for this difference is that it is influenced by the variety of microbes present in each breast milk. Breast milk from healthy women contains 10^3-10^5 CFU/ml of live bacteria[1,2]. In general, the dominant microbes found in breast milk are *Streptococcus, Pseudomonas, Staphylococcus, Lactobacillus, Propionibacterium, Herbaspirillum, Rothia, Stenotrophomonas, Acinetobacter, Bacteroides, Halomonas, Veillonella, Sphingomonas, Delftia*, and *Corynebacterium*[6]. The variety of microbes in breast milk is influenced by several factors, namely Body Mass Index (BMI), body weight, weight gain during pregnancy, mode of delivery, stage of lactation, gestational age, maternal health during breastfeeding, and also antibiotics[12].

A mother's BMI and weight gain during pregnancy have an impact on the composition and diversity of microbes in breast milk. 1-month mature breast milk from obese mothers shows lower microbial diversity with higher amounts of Lactobacillus and *Staphylococcus* and lower *Bifidobacterium* than normal-weight breastfeeding mothers[12].

Therefore, the breast milk used as the research sample came from mothers who had a BMI and lactation period close to each other.

In mothers who gave birth normally, there was more *Leuconostocacease* and lower levels of *Carnobacteriaceae* and *Moraxellacease* than in breast milk from mothers who gave birth by Caesarean[12]. *Bifidobacterium* was found to be more abundant in breast milk from mothers who gave birth by cesarean section and *Staphylococcus* was found to be lower in breast milk from mothers who gave birth normally[13]. Apart from that, *Lactobacillus* is also more abundant in breast milk from mothers who give birth by cesarean section than in breast milk from mothers who give birth normally[6].

Regression test results (Table 7) show that storage time has a positive effect on the number of bacteria in breast milk from mothers giving birth normally by 89.9% and 10.1% is influenced by other factors. Meanwhile, for breast milk from mothers who gave birth by cesarean section, storage time had a positive effect on the number of bacteria by 90.8%, and 9.2% was influenced by other factors. This positive influence means that the longer breast milk is stored, it will affect increasing the number of bacteria in breast milk. Meanwhile, other factors that can influence the increase in the number of bacteria in breast milk are temperature fluctuations and environmental cleanliness[14].

The large difference in the effect of storage time on the number of bacteria between breast milk samples from mothers who gave birth normally and breast milk from mothers who gave birth by Caesarean section could be caused by differences in generation time. Generation time is the time required for division. Generation time varies between different microbes[15]. The microbes in each breast milk sample can be different depending on the method of delivery. The dominant microbes found in breast milk samples from mothers who gave birth normally were *Streptococcus* and *Pseudomonas* (>10%), while the dominant microbes in breast milk samples from mothers who gave birth by Caesarean were *Streptococcus, Pseudomonas*, and *Staphylococcus* (>10%)[6].

The storage time for breast milk depends on the storage conditions[3]. Various factors such as milk volume, room temperature when breast milk is expressed, temperature fluctuations, and environmental cleanliness can affect how long breast milk can be stored[14]. According to CDC guidelines (2019) at room temperature, breast milk can last up to 4 hours, and 6-8 hours is still acceptable if the breast milk storage area is very clean.

It is recommended to provide breast milk at room temperature for up to 4 hours so that babies avoid the risk of disease and get maximum nutrition from breast milk. According to Pranatami, 2020, breast milk is one of the main sources of commensal, mutualistic, and probiotic bacteria in the baby's intestines[16]. The limitation of this research is the small number of respondents used, so the entire population is not represented in this research.

CONCLUSION

The average number of bacteria in the storage time of 0, 4, 8, and 12 hours in the breast milk of mothers giving birth to normal mothers respectively: 1.33×10^4 CFU/ml, 2.18×10^4 CFU/ml, 5.04×10^6 CFU /ml 1.68×10^7 CFU/ml. In the breast milk of mothers who gave birth by Caesarean, it was found that 1.83×10^4 CFU/ml, 1.73×10^5 CFU/ml, 1.76×10^7 CFU/ml, and 6.67×10^7 CFU/ml. There is a significant difference in the number of bacteria between breast milk from mothers who gave birth normally and breast milk

from mothers who gave birth by Caesarean section. Storage time has a positive effect on the number of bacteria in breast milk from mothers giving birth normally by 89.9%. Meanwhile, for breast milk from mothers who gave birth by Caesarean, the storage time had a positive effect on the number of bacteria by 90.8%. It is recommended to store breast milk at room temperature for up to 4 hours for breast milk from women giving birth normally and for breast milk from mothers giving birth by Caesarean section for less than 4 hours, paying attention to the cleanliness of the breast milk storage area.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

REFERENCES

1. Martín, R., Langa, S., Reviriego, C., Jiménez, E., Marín, M. L., Xaus, J., Fernández, L., & Rodríguez, J. M. Human Milk Is A Source of Lactic Acid Bacteria For The Infant Gut. Journal of Pediatrics. 2003; 143(6):754–758
2. Perez PF, Doré J, Leclerc M, Levenez F, Benyacoub J, Serrant P, Segura-Roggero I, Schiffrin EJ, Donnet-Hughes A. Bacterial imprinting of the neonatal immune system: lessons from maternal cells? Pediatrics. 2007 Mar; 119(3):e724-32. doi: 10.1542/peds.2006-1649
3. Werdayanti, R. Bapak ASI dan Ibu Bekerja Menyusui. Familia: Jakarta, 2015
4. Siswosuharjo, S., & Chakrawati, F. Panduan Super Lengkap Hamil Sehat (1st ed.). Penebar Plus: Jakarta, 2010
5. Viandika N and Septiasari RM. Pengaruh continuity of care terhadap angka kejadian Sectio Cessarea. J. Qual. Women's Heal. 2020; 3(1):1-8 doi: 10.30994/jqwh.v3i1.41
6. Li, S. W., Watanabe, K., Hsu, C. C., Chao, S. H., Yang, Z. H., Lin, Y. J., Chen, C. C., Cao, Y. M., Huang, H. C., Chang, C. H., & Tsai, Y. C. Bacterial Composition And Diversity In Breast Milk Samples From Mothers Living In Taiwan And Mainland China. Frontiers in Microbiology. 2017; 8(MAY):1–15.
7. Eglash, A., & Simon, L. ABM Protocol. Breastfeeding Medicine. 2017; 12(7): 1– 6.
8. Siahaya, G. C., & Talarima, B. Pengaruh Lama Penyimpanan Air Susu Ibu (ASI) Pada Suhu -15^{o}C Terhadap Kualitas ASI. 2-Trik: Tunas-Tunas Riset Kesehatan. 2017; 7(2): 23-33
9. Mustika, D. N., Nurjanah, S., Noor, Y., Ulvie, S., & Semarang, U. M. Identifikasi Total Bakteri dan Keasaman Air Susu Ibu Perah (ASIP) Yang Disimpan Di Cooler Bag. Jurnal Gizi. 2019; 8(36):28–36.
10. Arslanoglu, S., Bertino, E., Tonetto, P., Nisi, G. D. E., Ambruzzi, A. M., Biasini, A., Profeti, C., Spreghini, M. R., Association, I., Banks, M., Italiana, A., Umano, L., Aiblud, D., Intensive, N., Unit, C., Intensive, N., Unit, C., Hospital, S. C., Melloni, M., & Hospital, M. Guidelines For The Establishment And Operation of A Donor Human

Milk Bank. The Journal of Maternal-Fetal and Neonatal Medicine, 2010; 23(September):1–20.
11. Mutaqin, L., Dlh, D., Husin, F., Melinda, H., Nataprawira, N., Kesehatan, D., Bima, K., Tenggara Barat, N., Ilmu, D., Anak, K., Kunci, K., Penyimpanan, D., & Asi, K. The Effect of The Duration of Storage of Room Association On Quality of Breastmilk. Jurnal Kesehatan Prima. 2018; 13(1):51–59.
12. Cabrera-Rubio, Raul, Collado, M. C., 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. American Journal of Clinical Nutrition. 2012; 96(3):544–551.
13. Cabrera-Rubio, R., Mira-Pascual, L., Mira, A., & Collado, M. C. 2016. Impact of Mode of Delivery On The Milk Microbiota Composition of Healthy Women. Journal of Developmental Origins of Health and Disease. 2016; 7(1):54–60.
14. CDC. Storage And Preparation Of Breast Milk. 1–2. 2019. https://www.cdc.gov/breastfeeding/recommendations/handling_breastmilk.htm, accessed on 29-02-2024
15. Murwani, S. Dasar-Dasar Mikrobiologi Veteriner. Universitas Brawijaya Press: Malang, 2015
16. Pranatami, D. A. Perbandingan Jumlah Total Bakteri pada Penggunaan Wadah Penyimpanan Air Susu Ibu (ASI) yang Berbeda. Al-Hayat: Journal of Biology and Applied Biology. 2020; 3(1):15–20.