

ORIGINAL ARTICLE

The Comparison of Breast milk from Mother with Preterm Infant at 4°C Temperature in 0, 24 and 48 Hours on Bacterial Growth

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ABSTRACT

Introduction: Preterm and fullterm breast milk has different leukocyte and bioactive concentration, which affect it's bactericidal activity and storage duration. The preterm breast milk potentially has longer storage duration than fullterm breast milk. The research and guideline about the acceptable of storage duration of preterm breast milk at 4°C was limited. The objective of this study was to assess the correlation of storage duration of preterm breast milk preterm baby at 4°C temperature on bacterial growth.

Methods: The study design was experimental using pre-post control groups design. We used 9 samples of breast milk which met the inclusion criteria divided into 3 groups. The first, second and third groups was storage in 4°C for 0, 24 and 48 hours respectively before the inoculation to the culture media.

Results: After the samples were kept at 4°C temperature for 0, 24 and 48 hours, the mean bacterial colony forming unit for each group were 92 ($\pm SD$ 112), 79 ($\pm SD$ 97) and 63 ($\pm SD$ 73) CFU respectively. Compared to the first group, the second and third group has lower bacterial colony forming unit but no significant differences statistically ($P=0.214$ and $P=1.39$ respectively). Likewise there was no significant bacterial colony forming unit between second and third group ($P=0.086$).

Conclusion: Storage of preterm breast milk in 4°C for 24 and 48 hours show no change in total bacterial colony count (TBCC).

Introduction

Every year about 15 million preterm births occur throughout the world, this number are expected to increase.¹ Preterm infant have lower immunity and physiological endurance than fullterm infant.^{2,3} Potentially life-threatening complications include necrotizing enterocolitis, respiratory distress syndrome, sepsis and others.⁴ Breast feeding is the most effective way to reduce potential complications and mortality of preterm babies.^{1,5} Special care for preterm baby in neonatal intensive care unit would delay the breast feeding process. Breast milk storage in refrigerator is the most common way to preserve it.⁶ At present, various recommendations and research on the duration of breast milk storage from mothers of full-term infants. However, research and recommendations on the duration of storage of breast milk from mothers of preterm infants

are still limited.

One of the most important factor affecting the length of breast milk storage is viable bacterial concentration.⁷ The limit of breast milk viable bacterial concentration 105 per ml.⁵ Intrinsic factor in breast milk such as bactericidal capacity affects the bacterial growth in breast milk.⁸ Extrinsic factors such as temperature affect the number of bacterial growth directly, or indirectly by affecting the availability of bactericidal capacity component of breast milk.^{9, 10, 11}

The composition of preterm breast milk adapting to the needs of preterm infant.¹² Preterm breast milk have higher levels of leukocytes and antimicrobial substances such as lactoferrin, lysozyme and defensin than breast milk from mothers of full-term infants.^{13, 14} Higher content of antimicrobial peptide and leukocyte provide higher bactericidal capacity of preterm breast milk compared to fullterm breast milk.^{15, 16} It's higher bacterial capacity



potentially inhibit bacterial growth, thus preterm breast milk probably has longer storage duration than fullterm breast milk. This study was aims to determine the recommendation storage duration of preterm breast milk in refrigerator based on viable bacterial concentration.

Methods

Milk sampling, processing and storage

The procedures in this study were based on the Declaration of Helsinki which involves human subjects. This research was approved by the ethic committee of Dr. Soetomo General Hospital. Sample were taken from mother whose baby were treated in Neonatal Intensive Care Unit of Dr. Soetomo General Hospital. The inclusion criteria of sample were mother who birth premature baby, produce breast milk not more than 1 month. The exclusion criteria were taking antibiotics in the last 5 days, severe sepsis and mastitis. Information for consent was explained by researcher and assistant prior to breast pumping. Patient consent was written in available document.

Breast pumping was performed in lactation room of Dr. Soetomo General Hospital by a female researcher assistant. Nipple and breast were cleaned with 70% alcohol. Breast pumping was done by sterile breast pump kit, about 5-10 mL of breast milk sample were taken and stored in sterile breast milk bottle. Breast milk were temporarily stored in ice box with 4°C temperature the transported to the microbiology laboratory of the Faculty of Medicine, Universitas Airlangga in less than 30 minutes. The sample were immediately separated into 3 sterile reaction tubes and stored in the refrigerator with a standard temperature of 4 degree Celsius.

Breast milk culture and bacterial counting

We used nutrient agar to culture breast milk. After breast milk was divided into 3 sterile tubes, we labelled 0, 24 and 48 hours. Tube with 0 hour label was immediately cultured at the media, while tubes with 24 and 48 hours label were stored at refrigerator temperature. 500 microliter breast milk diluted in 4.5 ml sterile normal saline three times, the final dilution factor is 103. 100 microliter of the final solution were cultured by spread plate technique on agar nutrient. The culture were incubated in incubator at 36.6°C for 24 hours. After 24 hours total colonies were calculated by colony counter. The tubes with labels 24 and 48 hours also performed the same procedure but adjusted to the hours on the label counted as 0 hour labelled tube

Statistical method

The data of total colony counted were abnormally distributed by Sapiro-Wilk test. The Wilcoxon test was used to determine the significance of differences in the number of colonies between each groups with confidence level 0.95. We uses SPSS 1.6 application.

Results

Characteristic of mothers and baby

The characteristic of mothers from preterm baby was obtained by interview prior the patient consent. It was summarized in Table 1.

Table 1. Characteristic of mothers and baby

| Variable | Information |
|---------------------|-------------|
| Mother age | |
| 19-30 years | 3 |
| 31-40 years | 4 |
| 41-50 years | 1 |
| >50 years | 1 |
| Gestational week | |
| 28-31 weeks | 4 |
| 32-37 weeks | 5 |
| Number of pregnancy | |
| First | 4 |
| Second | 3 |
| Third | 1 |
| Fourth | 1 |
| Number of birth | |
| First | 4 |
| Second | 3 |
| Third | 1 |
| Fourth | 1 |
| Occupation | |
| Work | 5 |
| Housewife | 4 |
| Last education | |
| Kindergarten | 1 |
| Elementary school | 1 |
| Senior high school | 2 |
| Bachelor/Master | 5 |

Preterm breast milk cultures

Total colony forming units observed in 9 samples of each group at the 0, 24 and 48 hours are in Table 2 and the characteristics of the colony number in each treatment group are summarized in Table 3.

Table 2. Total colony forming unit in 0, 24 and 48 hours groups.

| Groups | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|----------|-----|----|----|-----|----|-----|----|-----|----|
| 0 hour | 281 | 9 | 12 | 110 | 7 | 270 | 24 | 102 | 11 |
| 24 hours | 255 | 27 | 11 | 48 | 16 | 235 | 22 | 80 | 16 |
| 48 hours | 166 | 53 | 8 | 39 | 9 | 206 | 8 | 64 | 16 |

Table 3. Total colony number characteristic in 0, 24 and 48 hours groups.

| Groups | Min | Max | Mean ± SD | Median |
|----------|-----|-----|-----------|--------|
| 0 hour | 7 | 281 | 92 ± 112 | 24 |
| 24 hours | 16 | 255 | 79 ± 96 | 27 |
| 48 hours | 8 | 206 | 63 ± 73 | 39 |

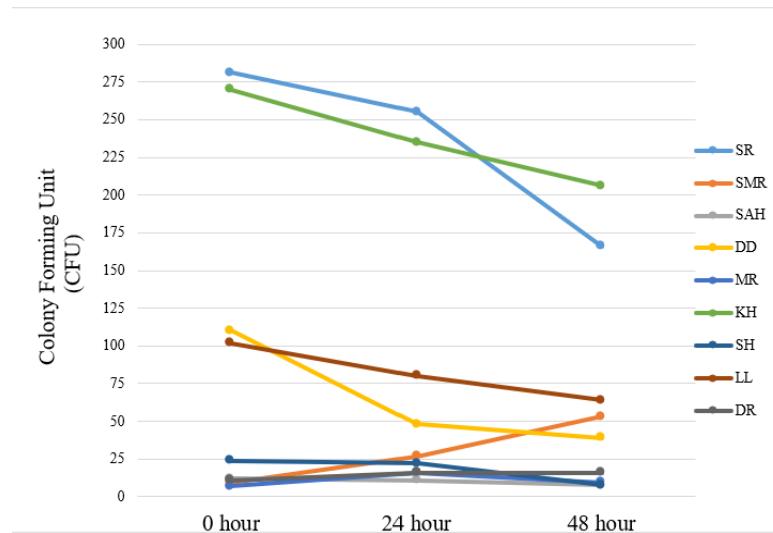


Figure 1. Curve of Colony Forming Unit (Y) and time (X) in each sample

Discussion

The results of breast milk culture shown in 0, 24 and 48 hours (Table 1) show high variation of total bacterial colonies from each sample and it occurred in all groups. We found there were 2 upper extreme values and lower extreme values of total bacterial colonies at in all groups, with upper extreme value were 270 and 281 CFU and lower extreme values were 7 and 9 CFU. Variation of total bacterial colonies at 24 and 48 hours group were probably the manifestations of variation initial bacterial concentration. Total bacterial are affected by factors such as humidity, temperature, hygiene, socioeconomic status and geographic condition which different in one area to other area.¹⁷ Surabaya located at tropical region with high humidity, temperature, and relatively lower socioeconomic and hygiene compare to western country. This made the variation of total (28–33°C) bacterial between the mothers will be higher. The research conducted by Alba using qPCR method show high variations with lower extreme values 6×10^4 per ml and upper extreme values 6×10^7 , whereas study conducted by Roberto using culture method show high variations with lower extreme value 103 per ml and upper extreme value 1.4×10^5 per ml.^{18, 19} Whereas the research conducted by Slutzah (2010), the number ranged from 1.6 to 2.9×10^5 bacteria per ml.¹³ There were high variation of bacterial concentration per ml breast milk from one mother to others, even breast milk from the same mother if it was taken at different timescales.¹⁸ Basically there were a high variation of bacterial concentration of breast milk sample and it was normal.

The pattern of bacterial concentration of preterm breast milk to the storage duration at 4°C of each sample can be seen in Figure 1. High bactericidal capacity of preterm breast milk decrease bacterial colony forming unit after 24 and 48 hours storage compared to initial bacterial colony forming unit (0 hour group). Preterm breast milk has a higher antimicrobial peptide concentration and leukocytes than fullterm breast milk, including H β D1, sCD14, LZ, LF, TGF- β 2 and sIgA.^{13, 14, 15} Refrigerator temperature (about 4°C) directly inhibit bacterial growth in breast milk.¹¹ It's also reserve the availability of antimicrobial peptide from

degradation without inhibit the lipolysis so it maintain antibacterial, antiprotozoal and antiviral fatty acid of breast milk. Which indirectly inhibit bacterial growth.^{13, 16}

Mean bacterial colony forming unit for group 0, 24 and 48 hours was 92, 79 and 63 CFU respectively. Mean bacterial concentration continuing decrease proportionally to storage duration at 4°C. The 0, 24 and 48 hours bacterial colony forming unit of 92, 79 and 63 CFU with amplification was 103. High bactericidal capacity in the first 48 hours is one of the main factors that causes a decrease in the number of bacteria after 24 and 48 hours storage, besides the influence of temperature in inhibiting bacterial growth. Bactericidal capacity of fullterm breast milk will be greatly reduced after 48 hours after the breast milk was pumped.²⁰ The phenomena could not be seen here because longest storage duration was 48 hours calculated after taking breast milk, possibly if breast milk culture continued at 72 hours (counted 72 hours after sampling) or afterwards there would be an increase in the number of colonies compared to 48 hours group.

According to the National Institute of Health and Clinical Excellence the limit for breast milk consumption was 105 bacteria per ml, the results showed preterm breast milk stored at 4°C were still proper for infant consumption based on bacterial concentration criteria.⁶ Even there was decline in total bacterial colony proportionally to storage duration (24 and 48 hours) the statistic (Wilcoxon test) show no significant difference between 0 hour and 24 hours groups ($P : 0.214$), 0 hour and 24 hours groups ($P : 0.139$) and 24 and 48 groups ($P : 0.086$). (Table 4) The result show 48 hours storage duration of preterm breast milk at 4°C didn't significantly change bacterial concentration of breast milk. This was consistent with research conducted by Slutzah , Pardou, and Roberto for fullterm breast milk.^{11, 13, 19} According to bacterial concentration parameter, preterm breast milk can be store in refrigerator temperature for at least 48 hours.

There was an unique discovery that researchers observed, initial bacterial colony in 0 hour group in working mothers is relatively higher than non-working mothers. The number of bacterial colony in 0 hour group working mothers was

281, 270, 110, 102 and 7 CFU while the number of bacteria in non-working mothers (Housewives) was 24, 12, 11 and 9. In the study conducted by Hamosh, there was no significant difference between the number of breast milk bacteria in working mothers and non-working mothers.¹⁶ Another study from Boo show no differences of total breast milk bacteria by manual and electrical pumping method of preterm breast milk at the hospital, but breast milk bacteria pumped using electric pump was higher than the manual pump when it's done at home.²¹ When compared with Hamosh and Boo's research, it is possible there are other factors that affect the higher number of bacteria in working mothers than non-working mothers especially with electric pumping method, which in this study those factors were not recorded as data by researchers.

Conclusion

There was no significant differences of pre-term breast milk bacterial colony count between 0, 24, and 48 hours duration of storage at 4°C. Mean bacterial colony count declines proportionally to the duration of storage (0, 24, and 48 hours). Breast milk from mother with pre-term infant may be stored at refrigerator with 4°C for 48 hours without compromising its bacterial colony count. As other researches show the decrease of bactericidal capacity in full-term breast milk after 48 hours of storage at 4°C, more researches are needed to investigate the advisability of pre-term breast milk after 48 hours of storage at 4°C.

Conflict of Interest

The author stated there is no conflict of interest

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