Bacterial factors of mastitis in lactating women and its effect on the physical properties and chemical composition of breast milk

Xu Shuyang, Yu Qiang*

Department of Breast Surgery, Changzhou Second People's Hospital, Nanjing Medical University, Changzhou 213000, Jiangsu, China

*Correspondence to: yuqiang202103@163.com
Received July 24, 2021; Accepted September 27, 2021; Published November 22, 2021

Doi: http://dx.doi.org/10.14715/cmb/2021.67.3.27

Abstract: Mastitis is a complication seen in some breastfeeding mothers and is the most common inflammatory lesion of the breast in breastfeeding mothers. In this complication, breast milk undergoes chemical and physical changes. It can lead to a drop in breastfeeding, weight loss, and, consequently, stunted growth of infants. Bacteria are the main cause of breast inflammation. Therefore, in this study, bacterial factors of mastitis were evaluated in lactating women. Also, their effects were considered on the physical properties and chemical composition of mothers’ breast milk. For this purpose, 210 breastfeeding mothers referred to health centers were randomly selected, and their milk samples were collected. In addition to collecting mothers’ demographic information by a questionnaire, the chemical composition (sugar, protein, and fat) and the physical properties (pH, density, and freezing temperature) of milk were measured. Bacterial evaluations were performed on the milk of these mothers by catalase test, coagulase test, and mannitol salt agar. Data were analyzed by SPSS software, Chi-square, Mann-Whitney U test, and T-test. The results showed that 56 mothers had mastitis, and Staphylococcus aureus and coagulase-negative staphylococci were the main bacteria in the milk of these mastitis mothers. These bacteria caused physical and chemical changes in breast milk so that mothers with Staphylococcus aureus mastitis had less sugar in their milk, and mothers with coagulase-negative staphylococci had less protein in their milk. Therefore, Staphylococcus aureus may reduce milk sugar by consuming milk sugar, and coagulase-negative staphylococci may also target milk protein. But to confirm these results, a larger population of mothers with mastitis is needed. Further studies are also needed to prove this result.

Key words: Bacterial factors; Breast milk; Lactating women; Mastitis.

Introduction

Mastitis is an inflammation of the breast that usually occurs during breastfeeding and is therefore commonly referred to as lactational mastitis (1). Mastitis usually occurs in the second or third week after delivery. Most reports indicate that 74 to 95% of mastitis cases occur in the first 12 weeks after delivery and are rarely seen after the twelfth week. Mastitis is relatively common and affects 33-35% of women during breastfeeding (2). The main causes of mastitis are milk stasis and infection. Milk stasis occurs when the milk does not come out completely during breastfeeding (3). Inadequate position of the baby during breastfeeding, the insufficient ability of the baby to suckle, limited frequency and duration of breastfeeding and obstruction of the urethra and ducts can lead to milk stasis. As a result of milk accumulation, suitable conditions are provided for the growth of bacteria (4).

The most common organisms found in mastitis are Staphylococcus aureus and coagulase-negative staphylococci. Also, sometimes, gram-negative bacilli (including Escherichia coli) are found in mastitis (5). But rare species of Salmonella, Mycobacterium, Candida and Cryptococcus have also been found. Ways of entering the organism include the entry of the organism through the milk-carrying ducts to the lobe, through the fissures and cracks of the nipple, and then spread through the blood (6).

The diagnosis of mastitis is usually based on clinical signs. Usually, one of the breasts is red, painful, swollen and hard, and there are signs of local inflammation and a decrease in milk. General symptoms may include 38.5°C or higher fever, lethargy, and chills (6). However, it is not possible to distinguish infectious mastitis from non-infectious ones through clinical signs. If possible, the culture of milk samples is recommended to determine infectious agents. A sample with more than 10⁶ leukocytes and more than 10³ bacteria per milliliter of milk indicates infectious mastitis (7).

Mastitis is important for several reasons. First, it reduces milk production, and about a quarter of mothers avoid breastfeeding their babies because of mastitis, and weaning will affect the baby's health (8). Second, mastitis increases the risk of mother-to-child transmission. In the case of retroviruses, the risk of transmission increases by 2 to 4 times (9). Also, because the disease affects the alveoli of the breast, breast milk undergoes chemical and physical changes. For example, the ratio of sodium to potassium in breast milk increases, and in some cases the baby does not suckle from the inflamed breast. This may be due to a change in the taste of breast milk (10). If the infant is exclusively breastfed, this can lead to reduced breastfeeding and weight loss, resulting
in impaired growth (11).

Due to the importance of mastitis in the health of infants, it is necessary for women with symptoms of mastitis during breastfeeding to be identified and treated immediately (12). In cases of infectious mastitis, the best way for treatment is to culture the milk sample for recognizing the exact bacteria to determine the appropriate antibiotic (13). This study aimed to evaluate mastitis in lactating women and to diagnose the associated bacterial infection and the effect of these bacterial agents on the physical properties and chemical composition of breast milk.

Materials and Methods

Demographic characteristics

The present study was a cross-sectional descriptive study. The study population consisted of all breastfeeding mothers who were less than 6 months after delivery, and they were referred to health centers. 210 mothers were randomly selected and their milk samples were collected. The data collection tool was a questionnaire. The questionnaire included 41 questions related to demographic factors, physical and chemical characteristics, bacterial factors that cause mastitis and physical characteristics of breast milk. After recording the demographic information of the mothers, they were asked to pour a sample of their milk into disposable sterile containers after washing their hands with soap and water without contacting the nipple with the sampling container. Then, the milk samples were stored in the refrigerator. At the end of the same day, the samples collected in the vicinity of the ice were transferred to the laboratory for microbiological tests and determination of chemical and physical properties.

Physical properties and chemical composition

The physical characteristics of breast milk in this study include milk pH, milk density, milk freezing point, the presence of blood and coagulation clots, and milk color. The chemical properties of milk in this study refer to the amount of fat, protein, and sugar.

Microbial assessments

For microbial culture, the samples were inoculated into a blood agar medium and incubated at 37°C for 24 hours. After the growth of bacteria on the culture medium, a direct slide was prepared from the colonies and the following differential tests were performed to detect the bacteria.

A. Catalase test: The catalase test is an important diagnostic key for identifying and differentiating bacteria. For example, streptococci (negative catalase) can be differentiated from staphylococci and micrococi (positive catalase). It is used to differentiate Clostridium (catalase-negative) from Bacillus (catalase-positive) and Listeria monocytogenes (catalase-positive) from beta-hemolytic streptococci (catalase-negative) (14). The protocol for performing this test is using 3% hydrogen peroxide as a substrate. With a wooden applicator, some microbial samples were transferred from the center of a colony to the surface of a glass slide. A few drops of 3% hydrogen peroxide were added to it and mixed. The positive reaction of catalase was characterized by the production of oxygen gas bubbles at the slide surface.

B. Coagulase test: The coagulase test is used to differentiate Staphylococcus aureus (coagulase-positive) from coagulase-negative staphylococci. Coagulase is an enzyme produced by Staphylococcus aureus that converts fibrinogen (soluble) in plasma to fibrin (insoluble) (15). For this purpose, one-quarter (1ml of plasma, 4ml of sterile saline) was prepared from rabbit serum plasma coagulate. Then a thick suspension of bacteria was prepared and placed in an incubator at 37°C for 2 to 4 hours. In the case of coagulation, a positive result was considered.

C. Mannitol salt agar: High salt concentration (7.5%) inhibits the growth of most gram-negative and gram-positive strains except Staphylococcus aureus. Staphylococcus can ferment mannitol (mannitol is the only carbohydrate in the culture) and produce acid. This leads to a decrease in pH and discoloration of phenol to yellow (16). In this experiment, staphylococcal colonies are distinctly yellow and surrounded by a yellowish halo, but other staphylococci and micrococci do not have the ability to ferment mannitol. They create red colonies with a purple-red halo by breaking down the peptone in the environment.

To assess bacterial susceptibility to antibiotics, 0.5 McFarland bacterial suspension was prepared. The bacteria were then cultured in a Mueller-Hinton agar medium. Finally, antibiotic discs were placed at appropriate intervals on the culture. Bacterial susceptibility was measured to the antibiotics amoxicillin, tetracycline, erythromycin, azithromycin, flucloxacillin, dicloxacillin, cloxacillin, cephalothin, and co-trimoxazole.

Statistical Analysis

All obtained information was calculated by SPSS 16 statistical software. Chi-square, Mann-Whitney U test, and t-tests were also used for correlation and the results were reported at a significant level of 5%.

Results

Demographic characteristics

Out of 210 samples, 56 mothers (26.66%) had mastitis. The mean age of healthy mothers was 28.71±4.6 years and the mean age of mothers with mastitis was 32.02±3.2 years. There was a significant difference between the mean age of mothers in the two groups (P <0.05). Mothers with and without mastitis were evaluated and compared in terms of the number of children, the number of deliveries, and the duration of previous breastfeeding (Table 1). The results showed that there was a significant relationship between the previous history of breastfeeding and mastitis (P <0.05).

About 38.7% of mothers with mastitis fed their children with other foods and fluids in addition to breast milk. In relation to maternal breast problems, all mothers with mastitis had clinical signs of mastitis in the form of pain, swelling, and redness. The method of delivery in 75.8% of mothers with mastitis was a cesarean section. Also, in relation to contraception, 31.2% of mothers with mastitis used the IUD method, 45.2% of mothers used natural methods of contraception and the rest of the mothers used other methods of contraception.
There was no significant relationship between contraceptive use and maternal mastitis. In addition, none of the mothers with mastitis had an underlying disease (diabetes, hypertension, and heart disease). In addition, 51 (91.07%) of mothers with mastitis did not receive breastfeeding training during pregnancy or after delivery. The gender of the child in 51.78% of mothers with mastitis was male, which was 53.7% for non-infected mothers. There was no significant relationship between child gender and mastitis.

According to the central indices related to height, head circumference, birth weight, and current weight of children of mothers with mastitis and healthy mothers, it was observed that there was a significant difference between the average height and head circumference of children in the two groups (p < 0.05) and in other cases no significant difference was observed (Table 2).

### Physical properties and chemical composition

The chemical properties of milk in this study include milk fat, protein, and sugar (Table 3). There was a significant difference between the mean amount of sugar and protein in the two groups (P = 0.003).

Table 4 compared the physical properties of milk, including pH, density, and freezing point of milk for affected and non-affected mothers. As can be seen, there was a significant difference between the mean pH and density between the two groups. Regarding the appearance of breast milk, 83.9% of mothers with mastitis had clots in their milk, while no clots were found in any of the samples of healthy breast milk. There is a significant relationship between mastitis and clots in milk (p <0.001).

### Microbial assessments

Out of 210 patients, 56 (26.66%) were positive cultures and 154 samples (73.34%) were negative cultures. Of 56 culture-positive samples, 51 samples (91.1%) were coagulase-negative for staphylococci and 5 samples (8.9%) were positive for Staphylococcus aureus. The number of Staphylococcus aureus bacteria isolated was more than 10³ per ml of milk. In the case of coagulase-negative staphylococci, out of 51 positive culture samples, 21 samples had more than 103 colonies per ml of milk, and in 30 samples, the number of colonies was less than 10³ per ml.

The susceptibility and resistance of Staphylococcus aureus isolated from 5 samples to different antibiotics are shown in Table 5. Staphylococcus aureus isolated from all five samples was sensitive to the antibiotics flucloxacinillin, dicolaxcinill, and cloxacinill. Of the 5 samples, 2 samples were sensitive to amoxicillin, 3 samples to tetracyline, 3 samples to erythromycin, 3 samples to azithromycin, 2 samples to cephalothin, and 1 sample to co-trimoxazole.

Coagulase-negative staphylococci isolated from all 21 samples were sensitive to the antibiotics flucloxacinillin, dicolaxcinill, and cloxacinill. Of the 21 samples, 15 samples were sensitive to amoxicillin, 16 samples to tetracyline, 17 samples to azithromycin, 18 samples to cephalothin, and 13 samples to co-trimoxazole. Table 6 shows the frequency distribution of isolated coagulase-negative staphylococci in terms of resistance and sensitivity to various antibiotics.

Milk samples of mothers with Staphylococcus aureus mastitis and mothers with coagulase staphylococci were compared in terms of physical characteristics and chemical composition (Table 7). The results showed...
Table 5. Sensitivity and resistance of 5 positive samples of *Staphylococcus aureus* isolated from patients to different antibiotics.

| Sample | Amoxicillin | Tetracycline | Erythromycin | Azithromycin | Flucloxacillin | Dicloxacillin | Cloxacillin | Cephalothin | Co-Trimoxazole |
|--------|-------------|--------------|--------------|--------------|---------------|--------------|-------------|-------------|---------------|
| 1      | Resistant   | Sensitive    | Sensitive    | Sensitive    | Sensitive     | Sensitive    | Sensitive   | Sensitive   | Resistant     |
| 2      | Resistant   | Sensitive    | Resistant    | Resistant    | Sensitive     | Sensitive    | Sensitive   | Resistant   | Resistant     |
| 3      | Sensitive   | Resistant    | Sensitive    | Sensitive    | Sensitive     | Sensitive    | Resistant   | Resistant   | Sensitive     |
| 4      | Resistant   | Sensitive    | Resistant    | Resistant    | Sensitive     | Sensitive    | Sensitive   | Resistant   | Resistant     |
| 5      | Sensitive   | Resistant    | Sensitive    | Sensitive    | Sensitive     | Sensitive    | Resistant   | Resistant   | Resistant     |

Table 6. Absolute and relative frequency distribution of coagulase-negative staphylococci isolated in terms of resistance and sensitivity to different antibiotics.

|          | Resistant | Sensitive | Total |
|----------|-----------|-----------|-------|
| Number   | Percent   | Number    | Percent | Number   | Percent |
| Amoxicillin | 6       | 28.6      | 15     | 71.4     | 21     | 100     |
| Tetracycline | 5      | 23.8      | 16     | 76.2     | 21     | 100     |
| Erythromycin | 4      | 19        | 17     | 81       | 21     | 100     |
| Azithromycin | 4    | 19        | 17     | 81       | 21     | 100     |
| Flucloxacillin | 0    | 0         | 21     | 100      | 21     | 100     |
| Dicloxacillin | 0    | 0         | 21     | 100      | 21     | 100     |
| Cloxacillin | 0     | 0         | 21     | 100      | 21     | 100     |
| Cephalothin | 3     | 14.3      | 18     | 85.7     | 21     | 100     |
| Co-Trimoxazole | 8    | 38.1      | 13     | 61.9     | 21     | 100     |

Table 7. Comparison of milk samples of mothers with *Staphylococcus aureus* mastitis and mothers with coagulase negative staphylococci in terms of physical characteristics and chemical composition.

| Staphylococcus aureus | coagulase-negative staphylococci | P-value |
|-----------------------|----------------------------------|---------|
| Fat (%)               | 3.5±0.7                          | 3.7±0.8 | 0.22    |
| Protein (%)           | 1.5±0.12                         | 1.1±0.32| 0.004   |
| Sugar (%)             | 5.9±0.07                         | 6.7±0.29| 0.002   |
| pH                    | 6.71±0.23                        | 6.79±0.39| 0.6     |
| Density (µg/ml)       | 1036±0.9                         | 1036±1.9| 0.91    |
| Freezing point (°C)   | -0.054±0.05                      | -0.056±0.08| 0.21    |

that there was significantly less sugar in the milk of mothers with *Staphylococcus aureus* mastitis, and mothers with coagulase-negative staphylococci had less protein in their milk.

**Discussion**

Mastitis is an inflammation of the breast and usually occurs during breastfeeding. The prevalence of mastitis in breastfeeding mothers is 9.5-10% in the first year after delivery (6). In the current study, out of 210 samples, 56 mothers (26.66%) had mastitis. The mean age of healthy mothers was 28.71±4.6 years and the mean age of mothers with mastitis was 32.02±3.2 years. There was a significant difference between the mean age of mothers in the two groups (P <0.05). In this regard, Hartmann (17) cited the causes of mastitis as primiparous mothers over 30 years of age. Another study showed the same results. According to this study, out of 56 mothers with mastitis, 38 (67.85%) were primiparous. In some studies, the elements of breast milk from mothers with mastitis are different compared to healthy mothers. There was a significant difference between the mean height and head circumference of children in the two groups. The results of Kasonka et al. research also reflected the same findings.

Another factor that affects the growth of children of mothers with mastitis is the change in the amount of sodium to potassium and other elements of milk that are not completely drained from the breast and milk stasis in the breast. Also, 38.7% of mothers used other foods and fluids with their milk to feed the baby, which can lead to changes in the taste of milk and as a result, inadequate nutrition of the child (3). In some studies, the elements of breast milk from mastitis-infected and healthy mothers were compared. The results showed that the amount of sodium to potassium in the milk of mothers with mastitis increased and the amount of glucose in the milk of these mothers decreased. The amount of these elements in the milk of the two groups of mothers showed a statistically significant difference. In the present study, there was a significant difference between the mean amount of sugar...
and protein in the two groups. In addition, there was a significant difference between the two groups regarding the physical properties of breast milk, i.e. pH and milk density.

In several reports, researchers have named *Staphylococcus aureus* and coagulase-negative staphylococci as the most common causes of mastitis (26). In the present study, out of 56 culture samples, 5 samples were *Staphylococcus aureus* and 51 samples were coagulase-negative staphylococci. Bacteria can be isolated from breast milk samples without the clinical signs of mastitis, but the range of these isolated bacteria is usually very similar to the bacteria present on the surface of the skin. Therefore, contamination of samples with skin surface bacteria should be avoided as much as possible. In this study, we tried to provide suitable conditions during sampling.

Also, in the present study, it was found that *Staphylococcus aureus* and coagulase-negative staphylococci played an important role in the development of infectious mastitis. These bacteria caused physical and chemical changes in breast milk so that mothers with *Staphylococcus aureus* mastitis had less sugar in their milk, and mothers with coagulase-negative staphylococci had less protein in their milk. Therefore, *Staphylococcus aureus* may reduce milk sugar by consuming milk sugar, and coagulase-negative staphylococci may also target milk protein. But to confirm these results, a larger population of mothers with mastitis is needed. Further studies are also needed to prove this result.

In general, mastitis is common in different populations and breastfeeding is important for the baby and therefore the health of the baby. Proper breastfeeding methods (such as close contact between mother and baby, the proper way of hugging the baby while breastfeeding, and proper frequency and duration of breastfeeding) are good ways to prevent the flow of milk and cause infection.

In this study, mastitis in lactating women and the associated bacterial infection were investigated. The effect of these bacterial agents was also investigated on the chemical composition and physical properties of breast milk. The result showed that *Staphylococcus aureus* and coagulase-negative staphylococci played an important role in the development of infectious mastitis. These bacteria caused physical and chemical changes in breast milk so that mothers with *Staphylococcus aureus* mastitis had less sugar in their milk, and mothers with coagulase-negative staphylococci had less protein in their milk. Therefore, *Staphylococcus aureus* may reduce milk sugar by consuming milk sugar, and coagulase-negative staphylococci may also target milk protein. But to confirm these results, a larger population of mothers with mastitis is needed. Further studies are also needed to prove this result.

References

1. Jiménez E, Manzano S, Schlembach D et al. Ligilactobacillus salivarius PS2 Supplementation during Pregnancy and Lactation Prevents Mastitis: A Randomised Controlled Trial. Microorganisms 2021; 9(9): 1933.

2. Dagla M, Dagla C, Mrvoljak-Theodoropoulo I et al. Do Maternal Stress and Depressive Symptoms in Perinatal Period Predict the Lactation Mastitis Occurrence? A Retrospective Longitudinal Study in Greek Women. Diagnostics 2021; 11(9): 1524.

3. Lai B-Y, Yu B-W, Chu A-J et al. Risk factors for lactation mastitis in China: A systematic review and meta-analysis. PloS one 2021; 16(5): e0251182.

4. Toktas O, Toprak N. Treatment Results of Intraleisional Steroid Injection and Topical Steroid Administration in Pregnant Women with Idiopathic Granulomatous Mastitis. Eur j breast health 2021; 17(3): 283.

5. Bo XR, Zhang H. Study on the Mechanism of Lactobacillus Rhamnosus Probio-M9 Combined With Antibiotics on Staphylococcus Aureus Mastitis in Rat. 2021.

6. Angelopoulou A, Field D, Ryan CA, Stanton C, Hill C, Ross RP. The microbiology and treatment of human mastitis. Med Microbiol Immunol 2018; 207(2): 83-94.

7. De Andres J, Jiménez E, Espinosa-Martos I, Rodriguez JM, García-Conesa M-T. An exploratory search for potential molecular targets responsive to the probiotic Lactobacillus salivarius PS2 in women with mastitis: gene expression profiling vs. interindividual variability. Front microbiol 2018; 9: 2166.

8. Pevzner M, Dahan A. Mastitis While Breastfeeding: Prevention, the Importance of Proper Treatment, and Potential Complications. Multidisciplinary Digital Publishing Institute; 2020.

9. Wilson E, Wood SL, Benova L. Incidence of and risk factors for lactational mastitis: A systematic review. J Hum Lact 2020; 36(4): 673-686.

10. Bond DM, Morris JM, Nassar N. Study protocol: evaluation of the probiotic Lactobacillus Fermentum CECT5716 for the prevention of mastitis in breastfeeding women: a randomised controlled trial. BMC Pregnancy Childbirth 2017; 17(1): 1-8.

11. Deng Y, Huang Y, Ning P, Ma S-G, He P-Y, Wang Y. Maternal Risk Factors for Lactation Mastitis: A Meta-analysis. West J Nurs Res 2020: 019345920967674.

12. Barker D, Adelson P, Peters MD, Steen M. Probiotics and human lactational mastitis: A scoping review. Women Birth 2020.

13. Santos EMS, Almeida AC, Santos HO et al. Mechanism of Brascia oleracea performance in bovine infectious mastitis by bioinformatic analysis. Microb Pathog 2019; 129: 19-29.

14. Raţu RN, Usturoi M, Usturoi A, Radu-Rusu R. Research on the treatment of mastitis in lactating women: gene expression profiling vs. interindividual variability. Microb Pathog 2019; 129: 19-29.

15. Hiko A. Isolation, DNase-cross-Coagulase test and antimicrobial resistance test on Staphylococcus along beef abattoir line in Addis Ababa Ethiopia. Ethiop vet j 2019; 23(1): 90-110.

16. Levine D, Spratt H, Rowin M. Effectiveness of a Cleaning Protocol on Environmental Contamination in a Pediatric Intensive Care Unit. Am J Infect Control 2019; 47(6): S3.

17. Hartmann PE. The lactating breast: an overview from down under. Breastfeeding Medicine 2007; 2(1): 3-9.

18. Kasonka L, Makasa M, Marshall T et al. Risk factors for subclinical mastitis among HIV-infected and uninfected women in Lusaka, Zambia. Paediatr Perinat Epidemiol 2006; 20(5): 379-391.

19. Heidari Z, Keshvari M, Kohan S. Breastfeeding promotion, challenges and barriers: a qualitative research. Int J Pediatr 2016; 4(5): 1687-1695.

20. Ndugubam OC, Ndu IK, Bisi-Onyemaechi A et al. Assessment of breastfeeding techniques in Enugu, South-East Nigeria. Ann Afr Med 2021; 20(2): 98.

21. Hoban R, Poeliniz CM, Somerset E et al. Mother’s own milk biomarkers predict coming to volume in pump-dependent mothers of preterm infants. J Pediatr 2021; 228: 44-52. e43.

22. Tourang M, Fang L, Zhong Y, Suthar R. Association between Human Endogenous Retrovirus K gene expression and breast cancer. Cell Mol Biomed Rep 2021; 1(1): 7-13.
23. Murase M, Wagner EA, Chantry CJ, Dewey KG, Nommsen-Rivers LA. The relation between breast milk sodium to potassium ratio and maternal report of a milk supply concern. J Pediatr 2017; 181: 294-297. e293.

24. Aziziaram, Z., Bilal, I., Zhong, Y., Mahmod, A., Roshandel, M. Protective effects of curcumin against naproxen-induced mitochondrial dysfunction in rat kidney tissue. Cell Mol Biomed Rep 2021; 1(1): 23-32.

25. Codo CRB, Caldas JPdS, Peixoto RRA et al. Electrolyte and mineral composition of term donor human milk before and after pasteurization and of raw milk of preterm mothers. Revista Paulista de Pediatria 2018; 36: 141-147.

26. Algharib SA, Dawood A, Xie S. Nanoparticles for treatment of bovine Staphylococcus aureus mastitis. Drug Deliv 2020; 27(1): 292-308.