

# Bacterial profile of infant feces associated with lactation infectious breasts

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**Background:** Mastitis is a common complication in lactating women. However, the diversity of intestinal bacteria in infant exclusively fed infectious milk remains uncharacterized. Our colleagues recently established a method based on 16S and 23S rRNA-targeted reverse transcription–quantitative PCR (RT-qPCR) for detecting bacteria.

**Materials and methods:** In the present study, the bacteria present in 14 samples of milk and infant feces were characterized using the RT-qPCR method, and concentrations of fecal organic acids were measured during the period of breast massage using HPLC.

**Results:** *Streptococcus agalactiae* and *Str. parasanguinis* were detected in milk from mastitis patients, whereas *Str. salivarius* and *Str. thermophilus* were the predominant bacteria in milk from engorged breasts. In feces of breastfed infants, *Str. salivarius*, *Str. thermophiles*, and *Str. parasanguinis* were isolated. Levels of lactate were high in fecal samples, whereas the pH of infant feces stabilized during breast massage. The bacterial diversity of milk from lactation infectious breasts was similar to that in feces of infant fed milk from lactation infectious breasts.

*Streptococcus* species isolated from the feces of breastfed infants are related to oral cavity health.

**Conclusion:** These results suggest that *Streptococcus* species, which are part of the healthy oral microflora, may play an important role in preserving the intestinal bacterial flora in infants fed infectious milk.

**Keywords:** infant feces, infectious breast milk, intestinal bacteria flora, *Streptococcus* species, fecal organic acids

## Introduction

Breast milk provides nutrition and exerts anti-infectious effects in infants. Although human breast milk is traditionally considered sterile, recent studies have demonstrated that milk of healthy women has a normal microbiota. Species of *Staphylococcus*, *Streptococcus*, *Lactobacillus*, and *Bifidobacterium* are prevalent in breast milk.<sup>1,2</sup> Breast milk affects the establishment of the microbiota of the mouth and the gut of infants.<sup>3,4</sup> Mastitis is an inflammatory disease that commonly occurs during lactation and causes a reduction in the milk supply. Breast massage is a technique widely performed to resolve breastfeeding complications associated with engorgement, plugged ducts, and mastitis. Generally, it is recommended that a mother continue breastfeeding, even if she has signs of mastitis, because it is believed that this does not pose a risk to the infant.<sup>5</sup> Species of the genus *Staphylococcus* were found to be the predominant bacteria in human mastitis. Increases in *S. aureus* in milk can cause acute mastitis, whereas high numbers of *S. epidermidis* are associated with subacute mastitis.<sup>6,7</sup> Both *S. aureus* and *Streptococcus agalactiae* are etiologic organisms in infections occurring during the early neonatal period.<sup>8,9</sup>

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The recent development of culture-independent molecular techniques, particularly those based on 16S rRNA genes, has allowed a more complete assessment of the biodiversity of milk and the intestinal microbiota.<sup>6,10</sup> In a previous study using subgroup- or species-specific primer sets targeting 16S or 23S rRNA genes from *Enterococcus*, *Streptococcus*, and *Lactobacillus* species, we were able to quantify the target populations with detection limits of  $10^3$ – $10^4$  cells per gram of feces, a sensitivity more than 100 times greater than that of qPCR analyses. This rRNA-targeted reverse transcription–quantitative PCR (RT-qPCR) method enabled the accurate and sensitive differentiation of a diverse array of bacterial groups.<sup>11</sup>

The aim of this study was to compare the species isolated from breast milk of women with lactation infections with species in the feces of their breastfeeding infants using our 16S/23S rRNA RT-qPCR method. In addition, organic acids derived from infant feces were analyzed using HPLC, and the pH of feces of infants fed milk from women treated with breast massage was also measured.

## Materials and methods

### Study design

This prospective study was conducted between July 2013 and March 2015 and was approved by the ethics committee of Kameda Medical Center (10-065). All mothers of participants gave written informed consent. Fourteen breastfeeding women with mastitis and their infants were enrolled. The median age of the mothers was 32 years (range, 20–40 years). The women had symptoms of acute inflammation, such as breast pain, erythema, warmth, and induration. They were diagnosed by the attending lactation nurses at Oketani Breast Management Research Institute, and therapeutic breast massage was performed by the nurses in the breast care center during the lactation period. Women with bilateral mastitis or mammary abscesses and women taking antibiotics were excluded from the study. Only infants exclusively fed breast milk were included. Clinical data for women and their infants were collected at enrollment. Breast massage was employed once each day, with a massage duration of at least 10 minutes.

For each woman, samples of breast milk were collected from sites of both mastitis and engorgement. Milk was sampled before the first and the final massage, as follows. Samples (5–10 mL) were obtained following cleaning of the nipple and areola using a sterile swab after wiping with sterile water by breast care nurses wearing sterile gloves. Fecal samples were collected by both the mothers and the breast care nurses. A spoonful of fecal sample (approximately 0.5 g)

was placed into each collection tube immediately after the child had defecated. All samples were collected in the breast care center and kept at 4°C in a cooling box with refrigerants during shipment to the Kameda Medical Center. The samples were then labeled and stored at –20°C in a refrigerator. Each sample with a case number was kept at 4°C in a cooling box and sent to the Yakult Central Institute. All data were collected and managed at the Kameda Medical Center. This study was carried out in accordance with the principles of the Declaration of Helsinki.

### Primary treatment of samples

Milk samples were centrifuged at  $12,000 \times g$  for 5 minutes at 4°C, and the supernatant was decanted and discarded. The pellet was then stored at –80°C until RNA extraction. Fecal samples were weighed, diluted tenfold by addition of RNAlater (v/w), and then homogenized using a Shake Master Auto Ver2.0 (Bio Medical Science Co., Tokyo, Japan). Next, 40  $\mu$ L of the fecal homogenate was transferred into a 2 mL screw-cap microtube and centrifuged at  $12,000 \times g$  for 5 minutes at 4°C, after which the supernatant was decanted and discarded. The pellet was stored at –80°C until RNA extraction. Primary sample treatment for the quantification of fecal organic acids was performed as follows: fecal samples were weighed, homogenized in 4 volumes of 0.15  $\mu$ mol/L perchloric acid, and allowed to stand at 4°C for 12 hours.

### RNA extraction and RT-qPCR

To quantify the bacteria present in milk and feces samples, total RNA was extracted using a previously described method.<sup>3,12,13</sup> The microbiota composition was determined using 16S rRNA-targeted RT-qPCR with a Yakult Intestinal Flora-SCAN system (YIF-SCAN<sup>®</sup>). Three serial dilutions of the extracted RNA samples were used for bacterial rRNA-targeted RT-qPCR analyses,<sup>3,12,13</sup> and the threshold cycle values in the linear range of the assay were applied to the standard curve to obtain the corresponding bacterial cell count for each nucleic acid sample. Those data were then used to determine the number of bacteria per sample.

### Sequencing of RT-PCR-amplified rRNA genes

The sequence homology of the rRNA genes among bacteria isolated from the samples was determined using 16S and 23S rRNA gene fragments. RT-qPCR products amplified using the g-Str-F/g-Str-R primer set were purified using a High-Pure PCR Product Purification Kit (Hoffman-La Roche Ltd., Basel, Switzerland). The sequences were automatically

analyzed on an ABI PRISM 3130 genetic analyzer (Thermo Fisher Scientific, Waltham, MA, USA). The resulting rRNA gene sequences were analyzed using the BLAST program of the DNA Data Bank of Japan (<http://blast.ddbj.nig.ac.jp/>) to assign each strain to a particular species.

## Identification and quantification of fecal organic acids

The fecal suspension was centrifuged at  $20,400 \times g$  at  $4^{\circ}\text{C}$  for 10 minutes. The supernatant was then filtered through a membrane filter with a pore size of  $0.45 \mu\text{m}$  (EMD Millipore, Billerica, MA, USA). Organic acids were analyzed by HPLC using a Waters system (Waters 432 conductivity detector; Waters, Milford, MA, USA) equipped with two columns (Shodex RSpack KC-811, column size:  $8 \times 300 \text{ mm}$ ; Showa Denko Co. Ltd., Tokyo, Japan). The concentrations of organic acids were normalized against those of an external standard mixture composed of succinic acid, lactic acid, formic acid, acetic acid, propionic acid, butyric acid, iso-valeric acid, and valeric acid. The total concentration of organic acids was determined as the sum of the concentrations of the individual acids.

## Fecal pH measurement

The pH of each experimental feces specimen was measured by directly inserting the glass electrode of a D-51 pH meter (Horiba Seisakusho Co., Ltd., Tokyo, Japan) into the homogenized feces.

## Statistical analyses

Results are expressed as mean  $\pm$  SD, unless otherwise stated. The log-transformed bacterial counts of milk and feces samples were used for the statistical analyses. Logarithmic transformation was not applied to fecal pH and organic acid concentration data. Differences in the bacterial counts between the milk obtained from engorgement and mastitis sites were assessed using the Mann–Whitney U-test. Differences in the bacterial counts of milk and feces and differences in the levels of organic acids and fecal pH during breast massage were analyzed using the Wilcoxon signed-rank sum test. Statistical analyses were performed using SPSS 11.0 software (SPSS Inc., Chicago, IL, USA).  $P < 0.05$  was considered indicative of statistical significance.

## Results

### Characteristics of the subjects

Milk samples were collected from 14 women with mastitis. The breast opposite the breast with mastitis was engorged

in all enrolled women. The median age of the mothers was 32 years (range, 20–40 years). The median axillary temperature before the first massage of the mastitis site was  $38.2^{\circ}\text{C}$  (range,  $37.1^{\circ}\text{C}$ – $39.7^{\circ}\text{C}$ ), and the median axillary temperature of the engorgement site was  $38.0^{\circ}\text{C}$  (range,  $35.9^{\circ}\text{C}$ – $39.0^{\circ}\text{C}$ ). All bilateral axillary temperatures before the final breast massage decreased to below  $37.0^{\circ}\text{C}$ . The median frequency of breast massage was 3.5 times (range, 1–10), and the median duration of massage was 30 minutes (range, 10–60 minutes). Milk was sampled before the first and final massages, with a median interval of 7.5 days (range, 5–20 days). Fecal samples were collected from 14 infants exclusively fed breast milk. The median age of the infants was 2.5 months (range, 1–5 months). Eleven of the infants were male, and three infants were female. The fecal consistency was soft, muddy, and liquid in nine, three, and two infants, respectively. That consistency did not change during breast massage (Table 1).

## Identification of milk bacteria

The bacteria identified in the breast milk are shown in Table 2. The prevalence of bacteria in milk obtained from either breast site before and after massage was high for *Streptococcus* and *Staphylococcus*. *Clostridium perfringens*, *Enterococcus*, and *Pseudomonas* were not detected in any of the milk samples. The counts of *Streptococcus* in the samples obtained from engorgement and mastitis sites before massage were significantly higher than those of *Staphylococcus* ( $P=0.001$  and  $<0.0001$ , respectively). After massage, the counts of *Streptococcus* in the samples

**Table 1** Clinical characteristics of lactation mastitis mothers and their infants

Variables	Subjects
Mothers (n=14)	
Age (years)	32 (20–40)
Axillary temperature ( $^{\circ}\text{C}$ )	
Mastitis site	38.2 (37.1–39.7)
Engorgement site	38.0 (35.9–39.0)
Frequency of breast massage (times)	3.5 (1–10)
Interval of milk sampling (days)	7.5 (5–20)
Infants (n=14)	
Age (months)	2.5 (1–5)
Gender	
Male	11
Female	3
Fecal consistency	
Soft	9
Muddy	3
Liquid	2

**Note:** Data are expressed as median and range.

**Table 2** Change in counts of bacteria in milk during breast massage

	Before massage				After massage			
	Engorgement site		Mastitis site		Engorgement site		Mastitis site	
	log <sub>10</sub> cells/mL	Prevalence	log <sub>10</sub> cells/mL	Prevalence	log <sub>10</sub> cells/mL	Prevalence	log <sub>10</sub> cells/mL	Prevalence
<i>Clostridium coccooides</i> group	ND		ND		ND		0.3	1/14
<i>C. leptum</i> subgroup	ND		0.8	1/14	1	1/14	0.5	
<i>Bacteroides fragilis</i> group	ND		ND	1/14	ND		ND	
<i>Bifidobacterium</i>	1.6	2/14	2.2±0.5 <sup>a</sup>	3/14	1.3	2/14	2.2±0.3	3/14
<i>Atopobium</i> cluster	1.5	2/14	3.1	1/14	2.3	1/14	0.7	2/14
<i>Prevotella</i>	2.3	1/14	2.5	1/14	ND		0.7	2/14
<i>C. perfringens</i>	ND		ND		ND		ND	
Total <i>Lactobacillus</i>	0.9±0.3	4/14	0.6	2/14	1.5±0.9	3/14	0.8	2/14
<i>Enterobacteriaceae</i>	0.3	1/14	0.5	1/14	ND		0.6	1/14
<i>Enterococcus</i>	ND		ND		ND		ND	
<i>Streptococcus</i>	2.7±1.0*	14/14	3.3±1.4*	14/14	2.5±0.9**/**	14/14	2.7±0.6*	14/14
<i>Staphylococcus</i>	1.5±0.7	13/14	1.6±1.0	14/14	1.3±0.8	12/14	1.6±0.7	10/14
<i>Pseudomonas</i>	ND		ND		ND		ND	

**Notes:** <sup>a</sup>Data are expressed as mean and SD. \* $P < 0.01$ , as obtained by comparison with *Staphylococcus* using the Mann–Whitney U-test; \*\* $P = 0.056$ , as obtained by comparison with the sample before massage on engorgement site using the Wilcoxon signed-rank test.

**Abbreviation:** ND, not detected.

from the engorgement and mastitis sites were also significantly higher than those of *Staphylococcus* ( $P < 0.0001$ ). Regarding the effect of breast massage on bacterial levels, the counts of *Streptococcus* in milk from the engorgement site decreased after massage, but the difference was not significant ( $P = 0.056$ ). Similarly, the counts of *Streptococcus* in milk from the mastitis site did not differ before and after massage ( $P = 0.198$ ).

### Identification of intestinal bacteria

The intestinal bacteria in infant feces samples are shown in Table 3. The total bacterial count before massage was  $10 \log_{10}$  cells/g, and that did not change after massage. In pre-massage samples, the counts of *Streptococcus* were significantly higher than those of *Staphylococcus* ( $P = 0.019$ ). In the post-massage samples, the counts of *Streptococcus* were also significantly higher than those of *Staphylococcus* ( $P < 0.0001$ ). *Bifidobacterium*, *Atopobium* cluster, total *Lactobacillus*, *Enterobacteriaceae*, *Enterococcus*, *Streptococcus*, and *Staphylococcus* were detected at high prevalence before massage, whereas the *Clostridium coccooides* group, *C. leptum* subgroup, *B. fragilis* group, *Prevotella*, and *C. perfringens* were detected at low prevalence before massage. These distributions in the feces did not change after massage. *Pseudomonas* was not detected in any fecal samples. The counts of *Enterococcus* in the feces decreased significantly after massage ( $P = 0.033$ ), whereas the counts of other bacteria in the feces did not change significantly after massage.

### Distributions of *Streptococcus* species isolated from milk and infant fecal samples

A variety of *Streptococcus* species were isolated from milk samples. *Str. agalactiae* and *Str. dysgalactiae* sub sp. in the pyogenic group were identified in five samples and one sample, respectively. *Str. parasanguinis*, *Str. oralis*, *Str. dentisani*, and *Str. pneumoniae* in the mitis group were isolated from 4, 13, 4, and 4 samples, respectively. *Str. salivarius* and *Str. thermophilus* in the salivarius group were isolated from seven and five samples, respectively. *Str. agalactiae* and *Str. parasanguinis* were detected prevalently in the mastitis site. *Str. salivarius* and *Str. thermophilus* were the predominant bacteria in the engorgement site (Figure 1A). Various *Streptococcus* species were isolated from infant feces. *Str. salivarius* and *Str. thermophilus* were detected in fecal samples before and after massage, at about  $8.0 \log_{10}$  cells/g, *Str. parasanguinis* was also detected in fecal samples, and the prevalence of this species was higher after massage compared to before massage. *Str. oralis* was only detected in one fecal sample after massage (Figure 1B).

### Fecal organic acids and the pH of infant feces

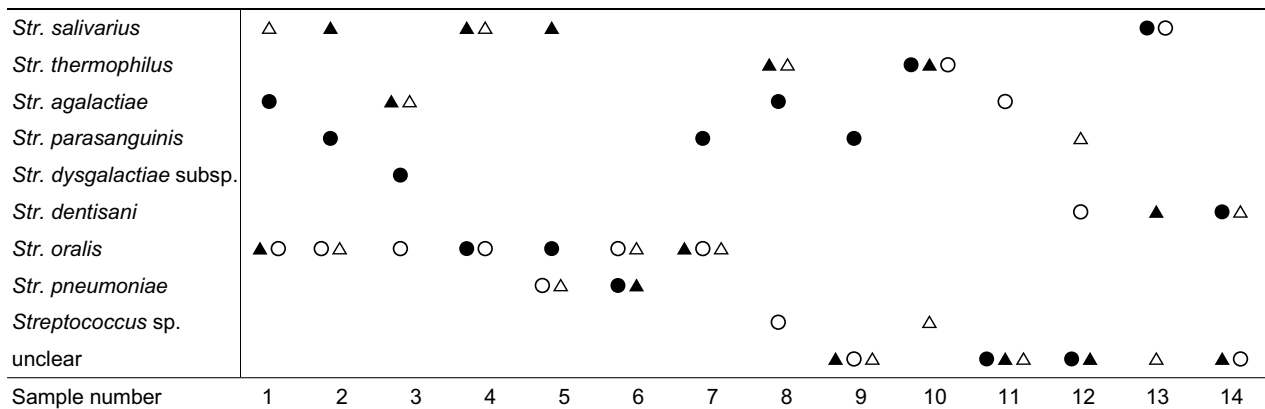
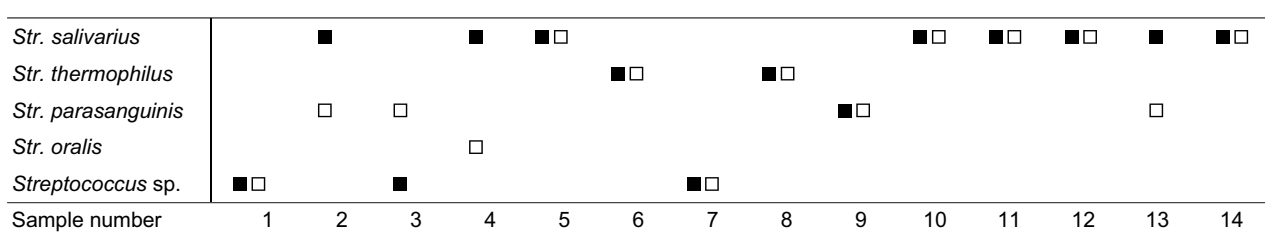
The concentration of total organic acids before and after massage was  $81.8 \pm 55.5$  and  $102.3 \pm 77.6 \mu\text{mol/g}$ , respectively. The predominant acids detected before massage were lactate and acetate, at concentrations of  $34.7 \pm 30.6$  and  $37.8 \pm 25.6 \mu\text{mol/g}$ , respectively. The distribution of fecal organic acids after massage was similar to that before massage. No significant

**Table 3** Change in counts of intestinal bacteria in infant feces during breast massage

	Before massage		After massage	
	log <sub>10</sub> cells/g	Prevalence	log <sub>10</sub> cells/g	Prevalence
Total bacteria	10.0±0.6 <sup>a</sup>	14/14	9.9±0.9	14/14
<i>Clostridium coccoides</i> group	7.7±1.2	4/14	8.0±0.6	4/14
<i>C. leptum</i> subgroup	5.7	2/14	6.3	2/14
<i>Bacteroides fragilis</i> group	9.2±0.6	3/14	9.2±0.9	3/14
<i>Bifidobacterium</i>	9.7±1.2	12/14	9.4±1.7	10/14
<i>Atopobium</i> cluster	8.1±1.3	8/14	7.6±0.8	8/14
<i>Prevotella</i>	5.9±0.5	5/14	5.8±0.5	7/14
<i>C. perfringens</i>	6.6±2.9	3/14	6.1±2.9	3/14
Total <i>Lactobacillus</i>	5.6±1.6	9/14	6.0±1.9	8/14
<i>Enterobacteriaceae</i>	8.1±1.1	12/14	7.9±1.1	12/14
<i>Enterococcus</i>	7.8±1.0	12/14	7.2±1.3**	12/14
<i>Streptococcus</i>	8.1±1.2*	14/14	7.6±0.8*	14/14
<i>Staphylococcus</i>	6.9±1.1	14/14	6.5±0.9	14/14
<i>Pseudomonas</i>	ND		ND	

**Notes:** <sup>a</sup>Data are expressed as mean and SD. \*P<0.01, as obtained by comparison with *Staphylococcus* using the Mann–Whitney U-test; \*\*P=0.033, as obtained by comparison with the sample before massage using the Wilcoxon signed-rank test.

**Abbreviation:** ND, not detected.

**A****B**

**Figure 1 (A)** Distribution of *Streptococcus* species isolated from the milk samples at mastitis site (before massage, ●; after massage, ○) and at engorgement site (before massage, ▲; after massage, △). **(B)** Distribution of *Streptococcus* species isolated from the infant's fecal samples (before massage, ■; after massage, □).

changes in the concentrations of fecal organic acids were observed during massage. The fecal pH was about 5.3 both before and after massage (Table 4).

## Discussion

The etiology of human lactation mastitis involves an increase in the number of bacteria caused by breast milk stasis.<sup>6,14</sup> The diversity of bacterial communities in milk of engorged

breast has not been reported. In this study, milk samples were collected from not only mastitis-affected breasts but also engorged breasts in the same women. Bacteria representing 11 genera and species were identified. The counts of *Streptococcus* in milk samples from the engorgement sites were significantly higher than those of *Staphylococcus*, similar to the mastitis sites. After alleviating the infection by breast massage, *Streptococcus* remained the dominant

**Table 4** Change in fecal organic acids and pH in infant feces during breast massage

	Before massage		After massage		P-value <sup>a</sup>
	μmol/g	Prevalence	μmol/g	Prevalence	
Total organic acid	81.8±55.5 <sup>b</sup>	14/14	102.3±77.6	14/14	0.55
Succinic acid	9.5±9.6	12/14	10.4±14.1	14/14	0.22
Lactic acid	34.7±30.6	13/14	49.3±48.6	10/14	0.86
Formic acid	6.1±6.8	7/14	5.7±3.5	9/14	0.39
Acetic acid	37.8±25.6	14/14	41.9±35.8	14/14	0.83
Propionic acid	1.2±1.2	6/14	0.7±0.7	5/14	0.21
Butyric acid	ND		5.5	2/14	
Isovaleric acid	ND		ND		
Valeric acid	ND		ND		
pH	5.3±0.7	14/14	5.3±1.0	13/14	0.59

**Notes:** <sup>a</sup>Wilcoxon signed-rank test was used for the difference in changes of fecal organic acids and pH during breast massage. <sup>b</sup>Data are expressed as mean and SD.  
**Abbreviation:** ND, not detected.

bacteria in milk from both engorged and mastitis-affected sites. *Staphylococcus* is reportedly the predominant bacteria in human lactation mastitis, with *Streptococcus* the second most abundant group.<sup>6</sup> In our series, the median axillary temperature of the engorgement and mastitis sites was <38.5°C before massage and <37.0°C after massage, which suggests that *Streptococcus* can predominate in milk stasis without causing a severe infection.

Based on 16S rRNA gene sequences, *Streptococcus* species can be categorized into six groups as follows: pyogenic, anginosus, mutans, mitis, salivarius, and bovis. *Str. agalactiae*, in the pyogenic group, was first described as a cause of bovine mastitis and is the most common cause of neonatal sepsis. Recently, an association between *Str. agalactiae* in infectious breast milk and late-onset neonatal infections was reported.<sup>15</sup> However, signs of infection were not observed in our infants, who were fed milk containing *Str. agalactiae*. This suggests that *Str. agalactiae* in milk of mastitis-affected breasts does not necessarily cause infections in healthy breastfed infants. *Str. parasanguinis*, in the mitis group, is one of the major early colonizers of dental surfaces in humans, and its presence in the oral cavity is associated with development of a healthy microflora by the formation of dental plaque,<sup>16</sup> whereas although *Str. parasanguinis* was reportedly isolated from milk of human lactation mastitis, the organism was not significantly associated with the mastitis.<sup>6</sup> In this study, *Str. parasanguinis* was detected in milk samples from engorgement sites at counts higher than *Staphylococcus*. *Str. salivarius* is known to be a pioneer colonizer of infants, who typically acquire it from their mother shortly after birth.<sup>17</sup> This species is a prominent member of the oral microbiota in healthy humans and is closely related to *Str. thermophilus*,<sup>18</sup> a species that is considered safer than other *Streptococcus*

species and is generally used in the production of yogurt. *Str. salivarius* and *Str. thermophilus* were identified in this study as the predominant bacteria in milk from engorgement sites, which suggests that these species may increase in number during the early stage of milk stasis.

Facultative anaerobes such as *Enterococcus*, *Streptococcus*, *Staphylococcus*, *Enterobacter*, and *Escherichia* are considered to be the first colonizers of the meconium.<sup>19</sup> The sources of bacteria transmitted to the infant during the period of microbial variation include the mother's vaginal, gut, and oral microbiota, the skin of caretakers and siblings, and breast milk and other foods. Microbial colonization of the gut is more influenced by early feeding practices than the mode of delivery.<sup>3,20</sup> Regarding the effect of breast milk on the intestinal microbial flora, several studies have reported that the counts of *Bifidobacterium* are higher and the counts of *Enterobacteriaceae* and *Bacteroides* lower in neonates fed breast milk compared to those fed formula milk.<sup>21,22</sup> *Bifidobacterium* is a major member of the normal infant microbiota and is believed to provide health-promoting benefits.<sup>23</sup> In this study, neither the counts nor the prevalence of *Bifidobacterium* in the feces of infants fed breast milk by women with mastitis or engorgement that changed significantly after massage were similar to infants fed healthy breast milk in a previous study.<sup>3</sup> Conversely, in the feces of infants in our study, *Enterococcus* numbers decreased after breast massage for mastitis. *Enterococcus* is a member of the normal human intestinal flora and has both an intrinsic and acquired resistance to antibiotics, making it an important nosocomial pathogen. The changes in *Bifidobacterium* and *Enterococcus* numbers suggest that homeostasis of the infants' gut bacterial flora was maintained while the clinical symptoms of mastitis were treated with breast massage.

Breast milk provides nutrition for the infant and is a source of oral bacteria. A previous study demonstrated that qPCR-based detection of *Lactobacillus* in the oral cavity of infants is consistent with detection of *Lactobacillus* in breast milk.<sup>24</sup> Holgerson et al reported that viable *Lactobacilli* detected in saliva from breastfed infants has an inhibitory effect on oral *Streptococcus*.<sup>4</sup> In this study, many of the streptococci detected in infant feces during breast massage, such as *Str. salivarius*, *Str. thermophiles*, and *Str. parasanguinis*, play a role in maintaining oral cavity health. This suggests that *Lactobacillus* in the saliva of breastfed infants may inhibit the pathogenic streptococci in milk affected by engorgement and mastitis and maintain the healthy bacterial flora of the intestine.

Variations in the microbiota composition of breast milk are known to affect nutrient absorption and energy metabolism in infants, but few studies have examined the association between fecal microbiota and fecal organic acids in infants fed mastitis milk. Under normal conditions, acetate, propionate, and butyrate are the principal organic acids, and lactate is present at low concentrations in the feces of healthy infants.<sup>3</sup> A previous study showed that the concentration of acetate in infant feces is related to the *Bifidobacterium* population in the intestine.<sup>25</sup> Since acetate is the major product of *Bifidobacterium* fermentation, an increase in acetate in the feces appears to reflect the establishment of *Bifidobacterium* as the predominant microbiota in infants.<sup>3</sup> In this study, the concentration of acetate in the feces of infants fed infectious breast milk (37  $\mu\text{mol/g}$ ) was lower than that in healthy infant feces in the previous report (50  $\mu\text{mol/g}$ ),<sup>3</sup> whereas the counts of *Bifidobacterium* (9.7  $\log_{10}$  cells/g) were similar to that in healthy infant feces (9.8  $\log_{10}$  cells/g).<sup>3</sup> *Lactobacillus* reportedly produces D- or DL-lactate, which contributes to fecal acidosis.<sup>26</sup> In our infants, who were fed milk from their mothers with engorgement or mastitis, the counts of *Lactobacillus* in the feces (5.6  $\log_{10}$  cells/g) were similar to those in the feces of infants fed milk from healthy women (5.8  $\log_{10}$  cells/g), whereas the concentration of lactate in our study (34  $\mu\text{mol/g}$ ) was higher than that in healthy infant feces (16  $\mu\text{mol/g}$ ).<sup>3</sup> *Str. salivarius* can modulate the intestinal pH through the hydrolysis of urea, which is upregulated by induction of urease gene expression by acidic pH.<sup>27</sup> In our infant feces, *Str. salivarius* was the predominant species during breast massage, suggesting that the fecal pH of our samples (5.3 $\pm$ 0.7) was within the normal range of healthy infant feces (5.9 $\pm$ 0.7).<sup>3</sup> These results suggest that *Bifidobacterium*, *Lactobacillus*, and *Str. salivarius* may play an important role in modulating the metabolic environment in the intestine of breastfed infants, thereby preserving the metabolic balance.

## Limitations

Our study has several limitations. First, only a small group (14 cases) was evaluated. Second, we did not examine the feces of infants after the women had recovered from mastitis. Third, pathogenic *Streptococcus* species remained in the milk after the breast massage, and trends in the number of pathogenic bacteria over time in infected breasts were not evaluated. To prove the safety of breastfeeding, a further study is necessary, which examines the relationship between intestinal bacterial flora and fecal organic acids in feces of infants fed infectious breast milk in large number group.

## Conclusion

This study demonstrates that the bacterial diversity of milk from engorgement sites was similar to that from mastitis sites in the same women, and the *Streptococcus* species isolated from the feces of breastfed infants and detected in milk from infectious breasts are related to oral cavity health. Therefore, our results suggest that an increase in the numbers of *Streptococcus* species may initiate infection, and the intestinal microbiota of breastfed infants may adjust to the environment through organisms derived from the saliva. As *Streptococcus* species are major constituents of the healthy oral microflora, *Str. salivarius* may play an important role in preserving the intestinal bacterial flora and the metabolic balance in infants fed infectious milk. As this study has several limitations, further studies are necessary to support this hypothesis.

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## Disclosure

The authors report no conflicts of interest in this work.

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