

ORIGINAL ARTICLE

# Isolation of Enterococci and Streptococci Species from Human Breast Milk

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## ABSTRACT

**Key words:**

**Breast milk,  
Enterococci,  
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**Background:** Bacterial colonization of the infant gut is a gradual process that exerts a strong influence on the health status of the host. The source of bacterial diversity in breast fed babies remains unclear. For many decades, breast milk has been regarded as a sterile body fluid which exerts its influence on the infant's microbiota environment via presenting only some growth factors and optimal conditions for helping the growth of bacteria. However, in recent years, breast milk has been hypothesized to be a source of commensal bacteria for the infant gut. **Objective:** This study aimed at searching for bacteria in breast milk to assess the role of breast milk as their probable source. **Methodology:** Samples of breast milk were obtained from 50 lactating women and were tested for the presence of different bacteria, using specific media and specific biochemical reactions. **Results:** Culture of the 50 breast milk specimens showed growth of different species of enterococci and streptococci in 60% and 84% of the specimens; respectively. **Conclusion:** breast milk can be a source of enterococci and streptococci for the infants.

## INTRODUCTION

Bacterial colonization of the infant gut is a gradual process that exerts a strong influence on the health status of the host, since the members of the gut microbiota may contribute to the barrier effect against pathogens and to the maturation of the intestinal immune system<sup>1</sup>. Traditionally, it has been considered that facultative anaerobic bacterial groups, such as streptococci, staphylococci, enterococci and lactobacilli together with some strictly anaerobic ones, especially bifidobacteria, are among the first colonizers in breast-fed infants<sup>2</sup>. These bacterial groups may play an important role in the reduction of the incidence and severity of infections in the breast-fed infant as they have the ability to inhibit the growth of a wide spectrum of pathogenic bacteria<sup>3</sup>.

The source of bacterial diversity in breast fed babies remains unclear. It has been suggested that neonates acquire them by oral contamination with vaginal strains during delivery; However, the findings confirm, at the molecular level that the bacterial composition of breast milk and infant feces is not related to the delivery method<sup>4</sup>. The breast skin may be claimed to be the source; however this does not agree with the strict anaerobic nature of some bacteria like bifidobacteria for example<sup>5</sup>.

For many decades, breast milk has been regarded as a sterile body fluid which exerts its influence on the infant's microbiota environment via presenting only some growth factors and optimum conditions for helping the growth of these important groups of bacteria<sup>6</sup>.

However, in recent years, breast milk has been hypothesized to be a continuous source of commensal bacteria for the infant gut<sup>7,8</sup>.

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This study aimed at searching for bacteria in breast milk to assess the role of breast milk as their probable source.

## METHODOLOGY

### Subjects:

50 Egyptian women attending the Breast Feeding Clinic at The Center of Social and Preventive Medicine (CSPM), Cairo University Hospitals, who were breastfeeding their infants, participated in the study.

Subjects were excluded from the study if there was:

- (1) Infant and/or maternal perinatal problems,
- (2) History of antibiotic intake in the previous month or
- (3) breast inflammatory condition like mastitis or breast abscess.

The study was approved by the Ethical Committee of the Faculty of Medicine, Cairo University and prior to their enrollment in the study, the mothers were informed of the investigational character of the study and they gave their informed consent.

### A. Samples :

#### 1. Samples Collection:

Samples of breast milk were obtained from the lactating women:

- The milk samples were obtained by manual expression using sterile gloves, and collected in sterile containers; one of which contained 0.5 ml of thioglycolate transport medium.
- Initially, the nipples and mammary areola were cleaned with soap and water, dried with sterile gauze then wiped with 70% alcohol. The first drops (~500 µl) were discarded<sup>9</sup>. Two parts of the specimen including the one collected on the transport medium were subjected to culture immediately after being delivered to the laboratory.
- Skin sampling was performed as a control; the areola, after treatment with alcohol was gently rubbed using sterile cotton swabs then plated on blood agar media. It yielded no bacterial growth.

#### 2. Isolation and identification of bacteria:

Pour plate technique was used to isolate the organisms<sup>10</sup>. One ml of the milk was inoculated into nine ml of M17 broth (Conda Laboratories, Madrid, Spain), well shaken and then they were serially ten folds diluted. One ml aliquot of the samples and dilutions

were plated into M17 agar medium (Conda Laboratories, Madrid, Spain) for culture of lactic acid producing streptococci<sup>11</sup>.

Inoculated M17 plates, were incubated aerobically at 37°C for 24 h<sup>11</sup>.

Colonies of different morphologies and sizes growing on M17 agar were subcultured into M17 broth aerobically for 24 h.

Bacterial isolates were characterized on the basis of their colony morphology, microscopic appearance after Gram staining and Slide Catalase Test was done to verify the bacteria as Catalase negative which were already identified as Gram positive cocci<sup>12</sup>.

#### 3. Preservation of isolates:

The different isolates were preserved in M17 broth medium containing glycerol and stored at -80 °C until further testing. The glycerol stocks of samples were prepared by adding 0.5 ml of active cultures to 0.5 ml M17 medium containing 40% sterile glycerol and thioglycolate plus 0.5ml reconstituted autoclaved skimmed milk<sup>5</sup>.

#### 4. Further identification of the isolated organisms:

This was done by using the API identification strips (bioMérieux, Marcy l'Etoile, France).

API<sup>®</sup> 20 Strep was used for the identification of streptococci and enterococci following the manufacturer protocol and then, by referring to the Analytical Profile Index and by using the identification software (Apiweb), the isolates were identified to the genus and sometimes to the species level.

#### The statistical analysis of the results:

Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate.

## RESULTS

Culture of the breast milk specimens showed growth of enterococci in the milk of 30 mothers (60% of the study population) and streptococci in the milk of 42 mothers (84% of the study population) .

Each genus showed growth of one to three different species per mother's milk specimen as shown in table 1.

**Table 1: Number of species of each genus per breast milk sample:**

Genus	Number of species per specimen				Total breast milk samples
	0	1	2	3	
<i>Enterococcus</i>	20 (40%)	24(48%)	6 (12%)	0	50 (100%)
<i>Streptococcus and other related genera</i>	8 (16%)	28 (56%)	13 (26%)	1(2%)	50 (100%)

The total number of cocci revealed from breast milk were **93** as follows:

- 36 enterococci and 57 streptococci .

- The distribution and types of the 36 enterococci isolates among the 30 positive milk specimens are shown in table 2.

**Table 2: Distribution of enterococci isolates in enterococci positive breast milk specimens.**

Genus	Culture positive milk specimens	Species	No.	Percent
<i>Enterococcus</i>	30	<i>E. faecium</i>	26	86.7 %
		<i>E. avium</i>	4	13.3 %
		<i>E. durans</i>	2	6.7 %
		unidentified	4	13.3 %

The majority of the enterococci-positive milk specimens revealed the species *E. faecium*; 26/30 (86.7%) The species of four enterococci isolates could not be identified by the API.

The distribution and types of the 57 streptococci isolates (and other related genera) in the streptococci positive milk specimens are shown in table 3.

**Table 3: Distribution of streptococci (and other related genera) isolates in streptococci positive milk specimens.**

Genus	Culture positive milk specimens	Species	No.	Percent
<i>Streptococcus</i> (and other related genera)	42	<i>Aerococcus viridans</i>	15	35.7 %
		<i>Lactococcus lactis ssp lactis</i>	14	33.3%
		<i>Streptococcus uberis</i>	8	19 %
		<i>Globicatella sanguinis</i>	5	11.9 %
		<i>Streptococcus salivarius</i>	4	9.5 %
		unidentified	11	26.2%

*Aerococcus viridans* and *Lactococcus lactis ssp lactis* were the most revealed species from the streptococci culture positive specimens (15/42 and 14/42; 35.7% and 33.3% respectively). The species of 11 streptococci isolates could not be identified by API.

## DISCUSSION

Bacterial colonization of the infant gut is a gradual process that exerts a strong influence on the health status of the host, since the members of the gut microbiota may contribute to the barrier effect against pathogens and to the maturation of the intestinal immune system<sup>1</sup>. Traditionally, it has been considered that facultative anaerobic bacterial groups, such as streptococci, staphylococci, enterococci and lactobacilli together with some strictly anaerobic ones, especially bifidobacteria, are among the first colonizers in breast-fed infants<sup>2</sup>. In concert, they create the condition required for the proliferation of anaerobic bacteria, which becomes predominant after weaning<sup>1</sup>. These bacterial groups may also play an important role in the reduction of the incidence and severity of infections in the breast-fed infant as they have the ability to inhibit the growth of a wide spectrum of pathogenic bacteria either by competitive exclusion and/or through the production of antimicrobial compounds, such as bacteriocins, organic acids, or hydrogen peroxide<sup>3</sup>.

The source of bacterial diversity in breast fed babies remains unclear. It has been suggested that neonates acquire bacteria by oral contamination with vaginal strains during delivery. However, findings confirm that the bacterial composition of infant faeces is not related to the delivery method<sup>4</sup>.

For many decades, breast milk has been regarded as a sterile body fluid which exerts its influence on the infant's microbiota environment via presenting only some growth factors and optimum conditions for helping the growth of these important groups of bacteria<sup>6</sup>.

However, in recent years, breast milk has been hypothesized to be a continuous source of bacteria for the infant gut<sup>7,8</sup>.

The present study aimed at searching for bacteria in breast milk, to assess the role of breast milk as their probable source to the infant.

The study was conducted on 50 lactating Egyptian women who were attending the Breast Feeding Clinic at The Center of Social and Preventive Medicine (CSPM), Cairo University Hospitals, in the period from March 2011 till May 2012. They participated in this study by providing samples of breast milk in absence of all exclusion criteria.

The included mothers had a mean age of 28 ±5.09 years (range from 19 to 37 years).

In the present study, culture of the 50 breast milk specimens showed growth of streptococci and enterococci in 84% and 60% of the specimens; respectively.

This is in partial agreement with Heikkilä and Saris<sup>7</sup> in Finland who recovered streptococci in 29 out of 40 breast milk samples (72.5%) and enterococci in 3/40 (7.5%) .

Also, in their study, Mehanna *et al.*<sup>13</sup> recovered cocci representing 44.8% of the total isolated bacteria from 30 breast milk specimens in the form of *Enterococcus*, *Lactococcus* and *Streptococcus*.

In the present study, the majority of the enterococci-positive milk specimens revealed the species *E. faecium*; 26/30 (86.7%), followed by *E. avium* then *E. durans*.

This is in partial agreement with Mehanna *et al.*<sup>13</sup> who reported that the majority of enterococci isolated from breast milk belonged to *E. faecium*; 46 out of 78 (59%), followed by *E. faecalis* (41%). While our results are in agreement with Martín *et al.*<sup>8</sup> in Spain, who isolated *E. faecium* from all the eight breast-milk samples and they didn't report the isolation of *E. faecalis*.

In the present study, concerning streptococci and other related genera, *Aerococcus viridans* and *Lactococcus lactis ssp lactis* were the most recovered species from the streptococci culture positive milk specimens (15/42 and 14/42; 35.7% and 33.3% respectively). *Streptococcus uberis*, *Globicatella sanguinis*, *Streptococcus salivarius* were also detected.

Our results are in agreement with Beasley and Saris<sup>14</sup> who found *Lactococcus lactis* in 6 out of 20 (30%) human milk samples. Also, Mehanna *et al.*<sup>13</sup> identified *L. lactis ssp. lactis* as the predominant species.

However our results seem to be in partial agreement with Heikkilä and Saris<sup>7</sup> who found *S. salivarius* in 18 out of 40 breast milk samples (45%) and *L. lactis* in one sample.

Most of the specimens revealed growth of a single species of the corresponding genus. However, some samples showed the growth of more than one species (up to three different species in the same specimen), making the total number of isolates retrieved from breast milk to equal to 93 (36 enterococci and 57 streptococci).

The elucidation of the origin of the bacteria present in breast milk has been an attractive research target<sup>9</sup>.

Traditionally, it was considered that they are acquired by skin contamination during sampling of breast milk. This was excluded in our study by simultaneous culture of skin samples after alcohol cleaning which yielded no growth on control blood agar plates. Also, this source seems very unlikely to strictly anaerobic bacteria like bifidobacteria for example<sup>5</sup>. Moreover, it has been reported that lactobacilli and enterococcal isolates present in human milk are genotypically different from those isolated in the skin, within the same bacterial species and the same host<sup>15</sup>.

Another theory is that inhabitants of the oral cavity transfer from the infant's mouth to the milk ducts and consequently to breast-milk samples before analysis. However, it has been shown that breast-milk contains similar bacteria both before and after breastfeeding<sup>16</sup>.

The suggestions that the origin of the live bacteria found in breast milk could be the maternal gut and that the bacteria arrive at the mammary gland through an endogenous route (the so-called entero-mammary

pathway) involving maternal dendritic cells and macrophages has been confirmed<sup>12</sup>. Dendritic cells can penetrate the gut epithelium and take up commensal bacteria directly from the gut lumen<sup>17</sup>. With pregnancy, the breast-milk lymphocytes express intestinal-homing receptors as many as 20 times compared with the blood-derived lymphocytes<sup>18</sup>.

## CONCLUSION

Human breast milk has been found to have different species of enterococci and streptococci and can be a source of such bacteria to the infant's gut.

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