

## Microbiological Status of Rehydrated Infant Formula Milk Powder Versus Expressed Breast Milk for Neonates

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### Abstract

Breast milk is considered the best food for infants, however in some cases the infant must stay away from his mother and must be supplied with milk by other means. In this case we have the choice between expressed breast milk and rehydrated infant formula and so, we applied this work to assess the microbiological status of both. The findings achieved in our study revealed that 27 (54%) out of the examined rehydrated infant formula milk samples were contaminated with different microbes with a mean count of  $6.8 \times 10^3 \pm 2 \times 10^2$  CFU/ml. In case of expressed breast milk samples, 78% were contaminated with an average count of  $3.3 \times 10^5$  CFU/ml. *Staphylococcus epidermidis* was the most prevalent micro-organism in both types of milk as it was present in 10% and 30% in infant formula and breast milk samples, respectively. Other microbes, including *Staphylococci*, *Enterobacteriaceae*, *Enterococci*, yeast and moulds were also detected with variable percentages. Although the higher contamination rate of expressed breast milk compared to the rehydrated infant formula, breast milk remains the best choice for feeding babies. Such finding is attributed to the immune protection normally provided through feeding on breast milk. However, strict hygienic measures during collection of breast milk should be followed to ensure minimal contamination.

**Keywords:** Infant formula, Breast milk, *Enterobacteriaceae*, *Enterococci*, *Staphylococci*, Yeast, Mould

### Introduction

Milk is considered a very important part of the daily diet, especially for both pregnant women and young children [1]. It is nearly sterile when secreted directly from the breast, however, it is easily to be contaminated with a wide range of microbes from different sources [2].

Human milk is the basic food for neonates, as it contains elements needed for healthy growth and increases the bond strength between the mother and child [3]. Furthermore, breast milk strengthens the immune system of neonates because development of the newborn immunity has not

yet been completed [4]. Therefore, the first six months of life for breast fed infants can be considered as the most healthy period [5]. Prematurity, low birth weight infants and immunocompromised conditions prevent the infant to suck the mother's breast effectively [6]. In this case, they must be supplied with milk by other means [7]. It is recommended to give these neonates expressed breast milk instead of breast feeding as the mother must stay away from the child for long period. Mothers are therefore encouraged to express breast and store milk in containers for a time [8].

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In case of breast feeding, the probability of milk contamination is very low as the infant suckles directly from the mother's breast. In case of expressed breast milk, it cannot be considered entirely sterile or free from bacterial contamination [9]. This variation could be attributed to the method of breast milk collection [10].

Manually expressed breast milk has been reported to be less contaminated than milk obtained with breast pumps and it is worth mentioning that manual expressing at home is a risk for contamination than that performed in a hospital due to the variation in personal hygiene [11]. Another possible reason for the contamination of expressed milk could be the storage temperature because the warm temperature of the environment, encourages bacterial growth [12].

Infant's formula (IF) is considered the most common breast-milk substitute during the first sensitive period of development [13]. It supplies infants with all the nutritional requirements during the first period of life until they are able to complete with breast feeding or complementary feeding [14]. The contamination of infant formula can occur during its preparation, reconstitution procedures or during their transportation and storage [15]. Neonates are considered to be a part of the high-risk group of individuals, as their immune systems may have not yet be fully developed and so they can be easily infected with microbes [16]. Consequently, it is reasonable that products used for infants should be of higher safety than foods for adults who have developed several mechanisms of defense against infection [17].

The current study aimed to assess the microbial profile of both expressed breast milk and rehydrated infant formula milk powder to conclude an advice about the most suitable and safe choice for neonates.

## **Material and Methods**

### ***Collection of samples***

A total of 100 samples of IF milk powder and expressed breast milk samples (50, each) were collected. Infant formula samples were collected from different markets and sent immediately to the laboratory for microbiological examination. Regarding expressed breast milk samples, they were collected from the neonatal intensive care unit at Benha Children Hospital, Benha, Egypt. Before examination, milk powder samples were reconstituted by following up reconstitution instruction on its original package, while expressed breast milk samples were thoroughly mixed.

### ***Microbial examination***

Eleven ml of each sample were added aseptically into sterile tube containing 99 ml of sterile saline solution. The latter was shaken well to have 1:10 dilution, followed by decimal serial dilution according to APHA [18]. Enumeration of the total bacterial count was performed using Standard Plate Count Agar (PCA) medium at  $32^{\circ}\text{C}\pm 1$  for  $48\pm 2$  hours [19]. Enumeration and identification of *Staphylococci* using Baird-Parker agar medium at  $35^{\circ}\text{C}\pm 1$  for  $48\pm 2$  hours [19], *Enterobacteriaceae* using Violet Red Bile Glucose (VRBG) agar medium at  $35^{\circ}\text{C}\pm 1$  for  $48\pm 2$  hours [18] and *Enterococci* using the ESD agar medium at  $35^{\circ}\text{C}\pm 1$  for  $48\pm 2$  hours [19] were carried out. Yeast and Mould count using Sabaroud Dextrose agar medium at  $22^{\circ}\text{C}$  for 7 days were performed according to APHA [20].

## **Results and Discussion**

The findings achieved in the present study revealed that 27 (54%) out of 50 examined rehydrated infant formula milk samples were contaminated with a mean value of  $6.8\times 10^3\pm 2\times 10^2$  CFU/ml (Tables 1 and 2). These results are nearly similar to those reported by Rajput *et al.* [21]. On the contrary, Toscano *et al.* [22]

found that all products analyzed during their study were free from bacterial contamination. Moreover, Abdullah Sani *et al.* [16] reported that only 19.6% of IF samples were contaminated with microorganisms within the range of  $10^3$ - $10^4$  CFU/ml, while Chap *et al.* [23] and Matug *et al.* [24] observed very high aerobic counts ( $>10^4$ ) in the examined samples. The results also showed that *Staphylococcus* spp. were detected in 12% of

the examined rehydrated IF milk samples with an average count of  $2 \times 10^2$  CFU/ml (Table 1).

The current results clarified that *S. epidermidis* isolation rate (10%) was higher than *S. aureus* (2%). Likewise, Wang *et al.* [25] detected *S. aureus* in 11.2% of powdered IF in China, while, Carneiro *et al.* [26] and Matug *et al.* [24] did not isolate *S. aureus* from any of the examined samples.

**Table 1: Microbial counts (CFU/ml) in the examined rehydrated infant formula and expressed breast milk samples (n=50, each)**

Microorganisms	Infant formula			Expressed breast milk		
	+ve samples (No)	%	Mean± S.E	+ve samples (No)	%	Mean± S.E
Total bacterial count	27	54%	$6.8 \times 10^3 \pm 2 \times 10^2$	39	78%	$3.3 \times 10^5 \pm 4.7 \times 10^4$
<i>Staphylococcus</i> count	6	12%	$2 \times 10^2 \pm 4.2 \times 10$	21	42%	$2.8 \times 10^3 \pm 6.8 \times 10^2$
<i>Enterobacteriaceae</i> count	12	24%	$6 \times 10 \pm 5.3$	14	28%	$9.5 \times 10 \pm 9.8$
<i>Enterococcus</i> count	6	12%	$7.9 \times 10 \pm 5.3$	9	18%	$3.5 \times 10^2 \pm 7.8 \times 10$
Yeast & Mould counts	7	14%	$6 \pm 0.9$	5	10%	$6.2 \pm 0.73$

n: Number of the examined samples.

In the current investigation, *Enterobacteriaceae* were identified in 12 (24%) of the examined infant formula samples (Table 1), including *E. agglumerans* (8%) followed by *E. Cloacae*, *C. Freundii*, *C. sakazaki* and *K. pneumoniae* with the isolation rates of 6%, 6%, 2% and 2%, respectively (Table 2). Abdullah Sani *et al.* [16] reported that *C. sakazaki* was not isolated from the examined samples, while, *Enterobacter* spp. and *Citrobacter* spp. (5.6%, each) followed by *Klebsiella* spp. (3.3%) were identified. Moreover, Iversen and Forsythe [27] isolated *Pantoea* spp., *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. from various infant milk samples.

In agreement with the present study Lai [28] and Leuschner *et al.* [29] clarified that infant milk powder is considered a vehicle of infection with different microbes including pathogenic *C. sakazaki*.

*Cronobacters* are generally incapable of surviving pasteurization [30], indicating that any contamination with them could be resulted

from contaminated additional ingredients, plant equipment or via asymptomatic diseased workers in the plant [31]. The potential growth of *Cronobacter* in reconstituted infant milk might be attributed to the inefficient temperature of water used in preparation or that of the room in which the milk was prepared and stored or reheated [32].

*Enterococci* and yeast and moulds were detected in 12% and 14% of the examined IF samples, respectively. Rajput *et al.* [21] detected yeast and moulds in various infant milk samples with a count less than 5 CFU/ml. While, Matug *et al.* [24] found that the total mould count in most of the examined samples was equal to or less than  $3.7 \log_{10}$  CFU/gm. In contrary, Tudela *et al.* [33] did not detect any pathogenic bacteria in 156 examined rehydrated milk formulas.

Infant milk contains highly nutritional substances that could support the growth of a wide range of bacteria as well as yeast and moulds [34]. Although IF is pasteurized during its manufacture, some microorganisms can be

detected especially those resist heat-treatment. Also, the presence of some microorganisms in the finished dried products could be attributed to contamination from the factory environment either during drying or packaging [35]. Moreover, the presence of pathogens in IF might be resulted from either improper handling such as inadequate cleaning of bottles and nipples or using contaminated water [36].

Multiple reheating, or improper rehydration procedures could also increase the number of harmful bacteria, therefore, reconstituted infant milk formula is considered a high-risk food causing serious illness. As a result, WHO [37] in 2007 released a guideline to the general public about safe milk handling to minimize possible contamination of IF when breast feeding is not possible [38]. One of these guidelines is cooling the reconstituted milk formula to 40-55°C because these temperatures are suitable for feeding infants. However, *Cronobacters* and other *Enterobacteriaceae* can grow at these temperatures [23]. Therefore, after cooling, IF should be given to the baby directly, to avoid the probability of contamination.

Concerning expressed breast milk samples, 39 (78%) samples were contaminated with a mean value of  $3.3 \times 10^5 \pm 4.7 \times 10^4$  CFU/ml (Table 1). This contamination varied between *Staphylococci*, *Enterobacteriaceae*, *Enterococci*, yeast and moulds with the percentages of 42%, 28%, 18% and 10%, respectively (Table 1).

In accordance, Deodhar and Joshi [39] and Serafini *et al.* [40] showed that 79.3% and 70.1% of their examined samples were contaminated, respectively. Higher contamination rate (85%) was reported by Karimi *et al.* [41], moreover, Israel-Ballard *et al.* [42], Collado *et al.* [43] and Hososaka *et al.* [4] found that all the examined breast milk samples were contaminated. In the present investigation, *S. epidermidis* (30%), *S. aureus* (12%), *K. pneumoniae* (12%), *E. faecium* (12%), *E. aerogens* (6%), *E. faecalis* (6%), *E. coli* (4%), *C. sakazaki* (2%), *E. agglumerans* (2%) and *E. cloaceae* (2%) were isolated (Table 2). While *Yeast* and *Mould* were detected in 10% of the examined samples (Table 1). Similarly, Rozolen *et al.* [9] and Karimi *et al.* [41] concluded that klebsiellae and coagulase negative *Staphylococci* were the most isolated microorganisms from the examined samples.

**Table 2: Isolation rates of different bacteria isolated from the examined rehydrated infant formula and expressed breast milk samples (n=50, each)**

Type of the organism	Infant formula		Expressed breast milk	
	No	%	No	%
<b><u>Staphylococcus spp.</u></b>				
<i>Staphylococcus epidermidis</i>	5	10	15	30
<i>Staphylococcus aureus</i>	1	2	6	12
<b><u>Enterobacteriaceae spp.</u></b>				
<i>Enterobacter agglumerans</i>	4	8	1	2
<i>Enterobacter cloacae</i>	3	6	1	2
<i>Enterobacter aerogens</i>	0	0	3	6
<i>Cronobacter sakazaki</i>	1	2	1	2
<i>Klebsiella pneumoniae</i>	1	2	6	12
<i>Citrobacter freundii</i>	3	6	0	0
<i>Escherichia coli</i>	0	0	2	4
<b><u>Enterococcus spp.</u></b>				
<i>Enterococcus faecium</i>	4	8	6	12
<i>Enterococcus faecalis</i>	2	4	3	6

n: Number of the examined samples

On the contrary, Deodhar and Joshi [39], Serafini *et al.* [40] and Israel-Ballard *et al.* [42] reported that *S. aureus* were the most frequent isolated strains, while Collado *et al.* [43] detected *Staphylococci* in all the examined samples. In addition, Karimi *et al.* [41] and Hososaka *et al.* [4] found that *E. coli* and *Klebsiellae* were predominated in the examined breast milk samples. The presence of enteric bacteria, such as *E. coli*, *Klebsiella* and *Citrobacter* is an indicator of contamination either from the body or clothes, therefore, babies are at risk of being infected with many diseases caused by enteropathogenic *E. coli* [45] while *klebsiella* spp. may lead to septicemia in neonates [46]. *S. aureus* forms part of the normal flora of skin, upper respiratory tract and intestinal tract, therefore, its presence in breast milk revealed the unsanitary condition of the breast nipples as well as the utensils employed in its manipulation [39]. Serafini *et al.* [40] detected yeast and moulds in 31.6% of the examined breast milk samples, while Collado *et al.* [43] detected *Enterococci* in 76% of the samples.

The aforementioned studies reported higher results than those obtained in the present study for yeast and moulds and even *Enterococci*. The presence of yeast and moulds is an indicator of an inadequate hygienic conditions due to contamination originating from the environment [47].

Bacteria can contaminate the breast milk during expression from the breast skin, hands, breast pump or other containers used for its collection [48]. The application of hygienic measures such as washing and disinfection of breast and hands as well as sterilization of all equipment used can decrease the contamination from the previously mentioned sources [49]. Also, temperature and the period of milk storage is of great importance, this was supported by Nwankwo *et al.* [50] who observed that storage of the expressed milk at warm ambient temperatures resulted in faster

growth rate of contaminating bacteria. Therefore, the storage of the expressed breast milk in the infants' ward, results in more chance for contamination posing risk of infection to infants [6].

## Conclusion

Although the expressed breast milk showed a higher contamination rate than that of rehydrated IF, breast milk remains the best choice for feeding babies at least for the first six months of life. Because formula-fed infants lack the immune protection provided normally by breast milk feeding. In addition, infant formula requires a high level of microbiological quality control during production, distribution and usage.

It is important to ensure that infant formulae are prepared using good hygienic practice, with rapid cooling and minimization of the time between preparation and consumption. In case of breast milk, it is essential to health-educate breast feeding mothers about personal hygiene to minimize bacteria adhering to their breast or containers and immediate storage after expression not for more than 2 hours by refrigeration.

## Conflict of interest

The authors declare no conflict of interest.

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### الملخص العربي

الحالة الميكروبيولوجية لوصفات حليب الأطفال المسحوقة المميّهة مقابل حليب الأم المعصر للأطفال حديثي الولادة

أسماء بدر مصطفى بدر طاحون ، إيمان نبيل عبدالفتاح

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يعد حليب الأم أفضل غذاء للرضع ولكن في بعض الحالات يجب أن يبقى الرضيع بعيدا عن والدته ويجب أن يمد بالحليب بوسائل أخرى وفي هذه الحالة علينا الاختيار بين حليب الأم المعصر وحليب الأطفال. ولهذا قامت هذه الدراسة لتقييم الحالة الميكروبيولوجية لكلا النوعين من الحليب. لقد أظهرت النتائج أن ٥٤٪ من عينات حليب الأطفال كانت ملوثة بمتوسط قدره  $6.8 \times 10^2 \pm 10^2$  خلية / مل، بينما بلغ نسبة تلوث عينات حليب الأم المعصر ٧٨٪ بمتوسط قدره  $3.3 \times 10^3 \pm 10^3$  خلية / مل. وقد تضمنت الملوثات المعزولة المكورات العنقودية، الميكروبات المعوية، المكورات المعوية والخميرة والعفن. كانت المكورات العنقودية من نوع اليبديرمس الأكثر انتشارا في كلا النوعين من الحليب بنسبة ١٠٪ في حليب الأطفال و ٣٠٪ في حليب الأم المعصر وعلى الرغم من أن عينات حليب الأم المعصر أظهرت تلوث أعلى من حليب الأطفال يبقى حليب الأم الخيار الأفضل لتغذية الرضع بسبب زيادة فرصة العدوى في الأطفال الذين يرضعون حليب الأطفال لأنه يفتقر إلى حماية الجهاز المناعي التي تمنح عادة عن طريق حليب الأم.