RESEARCH ARTICLE

Investigation of Microbial Contamination of Powdered Infant Formula During Different Storage Periods after Opening

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ABSTRACT

The present study was carried out to knowing effect different storage periods of the microbial quality for the powdered infant formula (PIF) after opening the tin and ensuring from the safety note (after opening, use within 3 weeks). Thirty (30) samples of (PIF) from category 1–6 months in five different types are collected from pharmacies and local markets in Baquba city/ Iraq, which are used as substitutes for breast milk during the first day of opening the tin powders such as total viable count, total coliform count, *Salmonella* count and yeast and molds (YM) count. These experiments repeated at each week of same samples within 5 weeks. Results were obtained at opening the tin, total viable count (< 0.05 1.0 x 10^3 ±1.5x10 CFU/g) were significantly higher than total coliform count (< 0.05 ± 0.3 x 10 CFU/g) and total salmonella count (< 0.05 0 x 10 CFU/g) and Yeasts and Molds (< 0.05 ± 0.3 x 10 CFU/g). while results obtained at fifth week were (< 0.05 8.8 x 10^3 ± 5.5x10^2 CFU/g), (< 0.05 0.9 x 10^2 ± 0.4x10^1 CFU/g), (< 0.05 0 x 10 CFU/g), (< 0.05 9.5 x 10 ± 1.2x10^1 CFU /g) respectively. All samples of (PIF) having a non-significant difference. These results compared to Iraqi Quality Standards (IQS), all the results from the opening samples to 5th week were within the range of IQS and USA Environmental Protection Agency (USEPA) and as indicates the hygienic condition of (PIF) without risk level for human health, also observed an increase in microbial contamination in each week because increase the moisture content for powdered milk. It can be used more than 3 weeks after opening if stored in good conditions with good hygienic practices during milk preparation.

Keywords: Microbial quality, Molds count, Powdered infant formula, Total viable count, Yeasts.

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INTRODUCTION

Basically, infants are the most sensitive category in the community. Thus, the study of microbial quality for powdered infant formula (PIF) is very significant.[1] PIF is a product that supplies a good environment for the growth of many harmful bacteria species. Even if existing in powdered formula at very little levels, unsuitable preparation and handling of milk provide typical conditions for the growth of microorganisms, which helps to increase the risk of infection. However, These risks can be controlled if PIF is prepared and handled correctly. Presently, the new industrial techniques cannot attain the production of sterile PIF. Contamination might occur at any stage of manufacturing (e.g., from the manufacturing environment, or raw ingredients). Many factors are effects in the type and number of microorganisms that contaminate of the dry milk after opening. The ambient environment condition (such as temperature and humidity), storage methods, and safety preparation are important factors to ensure microbial quality for the product. World health organization (WHO) recommends that breastfeeding should be exclusive during the first 6 months from age infants and is the best source to infant feeding because PIF may be cause several serious diseases if was contamination.[2-5] Salmonellosis, typhoid fever, dysentery, diphtheria, scarlet fever, and diarrhea are milk borne diseases.[6] Pathogenic microorganisms in dried milk such as *Proteus, Streptococcus, Staphylococcus, E. coli, Salmonella*, Yeast, and Molds, are considered to occurs contamination in one of the manufacturing stages.[7] Good ventilation is an important factor in controlling microbial contamination in the ambient environment for the sample, particularly after opening. Ventilation will prevent the moisture, which allows to growth of bacteria and molds. In addition to storage, the temperature is 30^{o}C as maximum. The packaging is a critical step to the success of the milk production process.[8] Food and agriculture organization (FAO)/WHO recommends that *Salmonella* should be not present in a product, especially *S. enterica* because maybe results in severe diseases and sometimes fatal to infants. Poor hygiene and stored incorrectly are increases severity. Therefore, preparation (PIF) should be within guideline to WHO, include preparation of milk by using hot water (no lower than 70^{o}C), consume directly after prepared, during prepared should use the soap and boiling water, in addition, to make sure from hygiene during (PIF) preparation.[3,9,10]

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MATERIALS AND METHODS

Collection of samples and preservation conditions

In this study, thirty (30) samples of (PIF) from category 1–6 months in five different types are collected from pharmacies and local markets in Baquba city/Iraq, transferring to the microbiology Lab., Department of Pathological Analysis, Baquba Technical Institute, Middle Technical University for microbiological examination. Milk samples were preservation after each experiment in a dry and cool place, away from light and contamination at 18–25 ºC and relative humidity (50%–65%). Also, ensure from hygienic practices daily after each use.

Preparation of test samples

The samples were prepared according to FDA.[11] The PIF (10 gram) was diluted in warm (45ºC) sterile diluents peptone water solution (90 mL) to make a primary solution (10^{-1}). Then a transferring (1 mL) from primary solution to test tube including sterile diluents (9 mL) to get a secondary solution (10^{-2}) and the repeating the way to obtain series dilution, 10^{-3}, 10^{-4}, and 10^{-5}.

Method of the total viable count

The total viable count was used plate count agar (PCA) medium according to the method International Organization for Standardization (ISO).[12] The plates of PCA were inoculated by transferred (1 mL) from test sample (10^{-2}, 10^{-3}, and/or 10^{-4} dilutions) using a sterile pipette and warm (45 ± 1ºC) sterile (PCA) medium (15 mL) was mixed with inoculum, then allowed to solidify. The plates were incubated at (30 ± 1ºC) for 3 days (72 ± 3 hours). The plates containing 30–300 colonies were selected and counted. The total viable count (TVC) was represented as the number of microbes of colony forming units per mL (CFU/mL) of samples.

Method of the Total Coliform count

The total Coliform count was used violet red bile lactose agar medium (VRBL) according to the method of ISO.[13] Transferred (1 mL) of each dilution sample by sterile pipette into sterile perti dishes by pour–plate technique. Prepared pour plates containing 1 mL of the milk sample and (15 mL) from VRBL, after allowing the mixture to solidify, incubated for (18–24 h) at (35–37 ºC). The typical purple–pink colonies was counted.

Enumeration of *Salmonella*

The medium used to the enrichment of milk sample was Tetrathionate Brilliant Green Broth (TBG). Added 1 g from the sample to the broth and mix then incubate at (35ºC) for (18–24 hour) and subculture to xylose–lysine deoxycholate agar (XLD) medium. (XLD) medium were the medium used for isolation and enumeration of *Salmonella* according to the method of ISO.[14] Transferred 1 mL of inoculum by sterile pipette into XLD and incubated for a further (24 hours) at (35–37ºC). Red colonies with a black center are the character of *Salmonella*.

Method of the Yeasts and Molds count

Chloramphenicol Yeast Glucose Agar (CYG) was the medium used for yeasts and molds count according to the method of ISO.[15] Transferred (1 ml) from inoculum by sterile pipette to sterile petri plate by pour–plate technique. Incubate the petri plates at (25 ±1ºC) counting of the yeast, and molds are done after 3–5 days incubation. Depending on their morphological characterization, the colonies' yeasts and molds are distinguished.

Total viable count

All examples within each week was estimated, and the results are presented in Figure 1. No important variation was observed in TV count in all samples within each week were examined in the present study. TV count after opening the tin, ranged between 8 $x10^2$ to $12x10^2$ CFU/g and averaged 1.0 $x10^3$±1.5x10 cfu/g. While in 5th week, the TV counts were observed in between $5.9x10^3$ to $9.5x10^3$ CFU/g with a mean value of $8.8x10^3$±$5.5x10^2$ CFU/g. Further, Statistical analysis results (AOV) showed no significant difference ($p > 0.05$), in TV counts after opening and in the fifth week. These results compared to that of IQS, 2013 i.e., ≤ $1.0x10^4$ cfu/g (Table 1).

Total Coliform count

All examples within each week was estimated and the results are shown in Figure 2. No wide variation was observed in TC

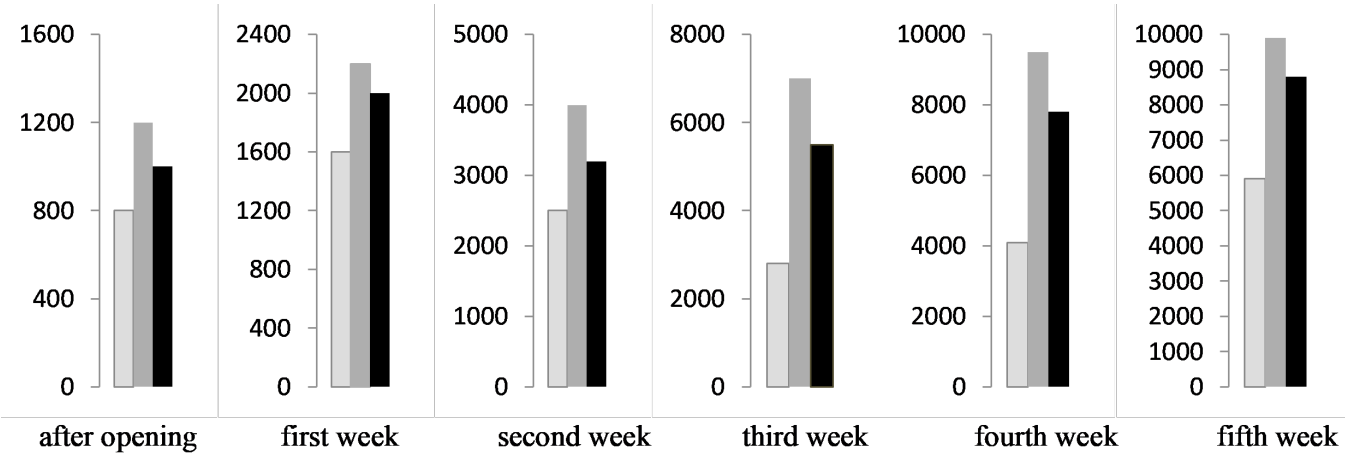

Figure 1: Graph shows minimum, maximum and mean values of total viable counts (CFU/g) in (PIF).

Table 1: Total viable counts (CFU/g) in different infant formula samples compared to IQS.

	Total Viable Count (TVC) CFU/g	
	Observed	*Deviation in folds from IQS*
Sample	(a)	(b) = (x) ÷ (a)
After opening	1.0×10^3	– 10 < 0.05
Fifth week	8.8×10^3	– 1.13 < 0.05

a = Observed values

x = (Standard value of IQS = ≤ 10000 cfu/g)

count in all samples within each week. TC count after opening the tin, ranged between (0.15×10^1) to (0.4×10^1 CFU/g) and averaged (0.3 x10 CFU/g). While in fifth week, the TC counts were observed in between (0.6×10^1) to (0.98×10^1 CFU/g) with mean value of ($0.9 \times 10^2 \pm 0.4 \times 10^1$ CFU/g). It was further observed that TC count of at opening and after 5th week was no significantly different ($p > 0.05$) in all samples. These results compared to that of (IQS, 2013) i-e ≤ 1.0×10^2 CFU/g (Table 2).

Yeasts and molds count

All examples within each week were examined, and the results are shown in Figure 3. It was observed that YM count in all samples within each week did not show wide variation. YM count after opening the tin, ranged between 0 $\times 10^1$ to 0.5×10^1 CFU/g and averaged 0.3 x10 CFU/g. While in the 5th week were observed in between 8×10^1 to 1.0×10^2 CFU/g with mean value of $9.5 \times 10^1 \pm 1.2 \times 10^1$ CFU/g. It was further observed that YM count from opening the tin to the 5th week was not significantly different ($p > 0.05$) in all samples. These results compared to that of (IQS, 2000) i-e ≤ 1.0×10^2 CFU/g (Table 3).

Analysis of Variance (ANOVA) for TV counts, TC count, and YM count showed a significant difference ($p < 0.05$) between microbial quality in the first day and fifth week.

RESULTS AND DISCUSSION

The present study was conducted to assess the general hygienic quality of (PIF) and its microbial content. The spoilage rate can be influenced by factors such as moisture content and temperature of storage.[16-19]

In this study, the total viable count ($1.0 \times 10^3 \pm 1.5$x10 cfu/g) at

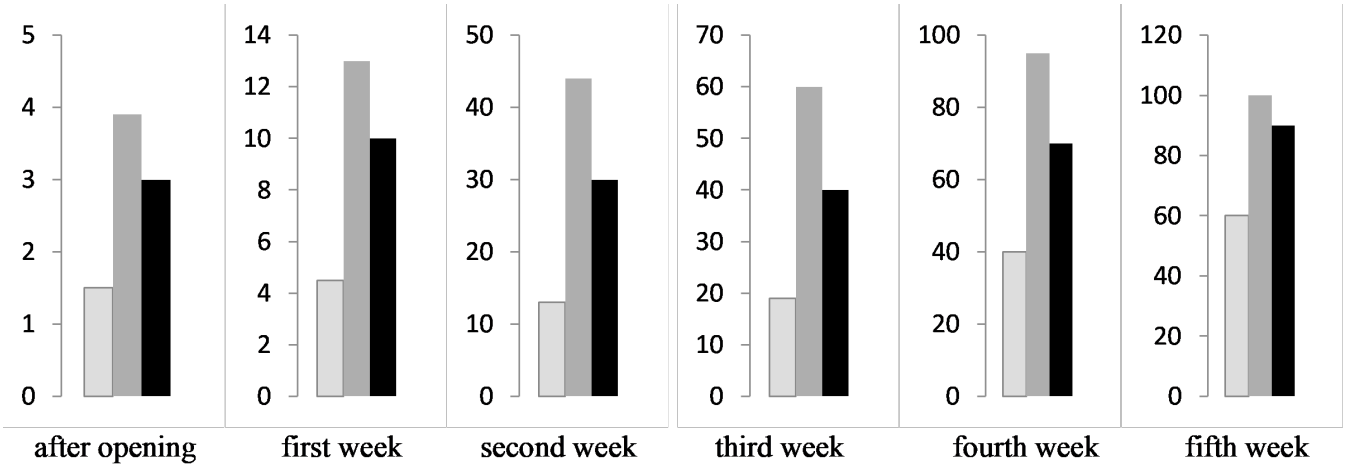

Figure 2: Graph shows minimum, maximum and mean values of total Coliform counts (CFU/g) in (PIF).

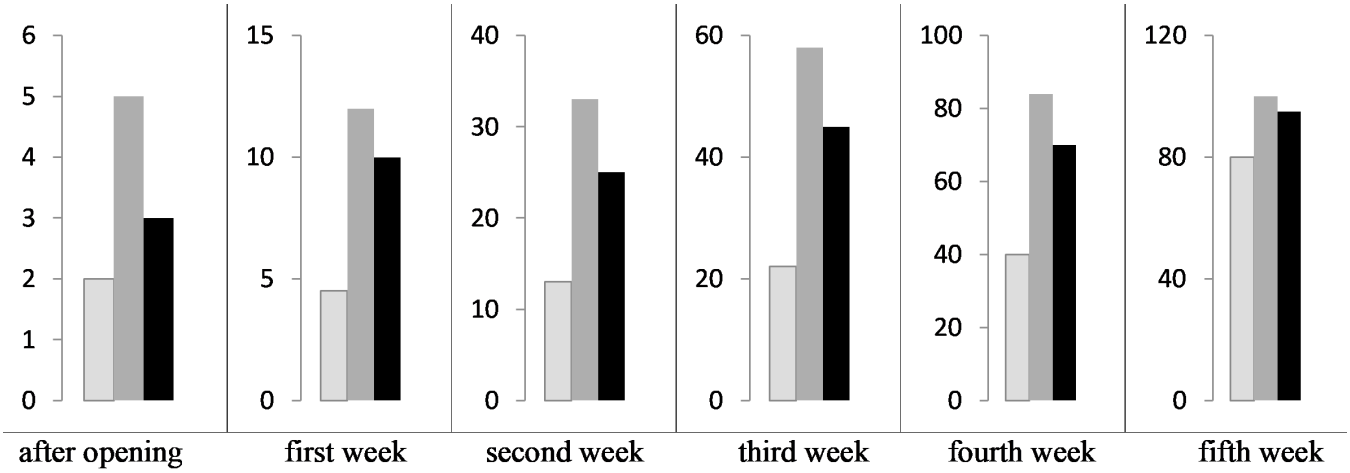

Figure 3: Graph shows minimum, maximum, and mean values of total YM counts (CFU/g) in (PIF).

Table 2: Total coliform counts (CFU/g) in different infant formula samples compared to IQS.

	Total Coliform Count (TC) cfu/g	
	Observed	Deviation in folds from IQS
Sample	(a)	(b) = (x) ÷ (a)
After opening	0.3×10^1	- 33.3 < 0.05
Fifth week	0.9×10^2	- 1.1 < 0.05

a = Observed values
x = (Standard value of IQS = ≤ 100 cfu/g)

Table 3: Total yeast and molds counts (CFU/g) in different infant formula samples compared to IQS.

	Total yeast and molds count (YMC) cfu/g	
	Observed	Deviation in folds from IQS
Sample	(a)	(b) = (x) ÷ (a)
After opening	0.3×10^1	- 33.3 < 0.05
Fifth week	0.9×10^2	- 1.1 < 0.05

a = Observed Values
x = (Standard Value of IQS = ≤ 100 cfu/g)

opening the tin, While in fifth week ($8.8x10^3 \pm 5.5x10^2$ cfu/g) was not significantly ($p > 0.05$). It is of interest to point out these results is similar than reported by[2,5,20] (5.3×10^3–2.2×10^4 CFU/g), (1.2×10^2–3.5×10^3CFU/g), (5.0×10^2–3.4×10^3 CFU/g) respectively. Often, bacterial cells can be killed at 80°C for 10 min. But in this study, the microbes were observed in (PIF) because of their great ability to adhere to surfaces and form biofilms. The reason increased of microbial growth in the 5th week is powder's moisture content increased; if the moisture content in the powdered milk above 15%, it then becomes liable to contamination and microbial growth and should not be consumed.[21]

Total coliform bacteria count obtained in present study at opening the tin ($<0.05 \pm 0.3 \times 10^1$), While in fifth week ($0.9 x10^2 \pm 0.4x10^1$ CFU/g), Is lower than reported by[7] (1.0×10^2 CFU/g). Few numbers of coliform bacteria are usually found in milk; these bacteria are easily killed by heating. maybe the reason in the present is raw milk, can be reduced the risk by hygienic practices at preparation and control of the samples' preservation conditions.[3]

Salmonella was not isolated from any samples of powdered infant formula; this result different from reported by[7] (1.0 x 1.0 CFU/g). *Salmonella* is rarely found in PIF, *Salmonella* are the pathogens of most worry in PIF according to The FAO/WHO.[3,22]

Total YM count ($<0.05 \pm 0.3 \times 10^1$) at opening the tin, While in fifth week ($<0.05 \pm 0.9 \times 10^2$). The results obtained is lower than reported by[7,8] (1.0 x10 cfu/g), ($<5 \pm 1.0$ CFU/g), respectively. Contamination of PIF with yeast and molds can occur from outer sources or intrinsically from raw ingredients).[2,3,13]

All the results from the opening samples to the 5th week were within the range of IQS and USA Environmental Protection Agency (USEPA) and as indicates the hygienic condition of PIF without risk level for human health.

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