



Evaluation of Microbial Quality and Hygienic Process of Powdered Infant Formulas in the Omani Market

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Abstract

Substitution of breast milk with powdered infant formula (PIF) for infants is a common practice worldwide. However, since PIF is not sterile, microbial contamination from processing lines and environment is quite possible. The objective of the study was to evaluate the microbial quality and status of hygienic process of PIF in the Omani market. In total 25 PIF samples from five different brands were evaluated microbiology by total bacterial and Enterobacteriaceae counts as well as measuring water activity. The results showed that the total bacterial counts were lower than the maximum limit set by CODEX Standards; 3 log CFU/g, while, Enterobacteriaceae were not detected. Water activity ranged from 0.1-0.3. In conclusion, PIF were found at good microbial quality and processed under good hygienic conditions.

Keywords: Powdered Infant Formula; Total Bacteria; Enterobacteriaceae

Abbreviations

PIF: Powdered Infant Formula; TAB: Total Aerobic Bacteria; MRD: Maximum Recovery Diluent; VRBG: Violet Red Bile Glucose.

Introduction

Worldwide, substituting of breastfeeding with powdered infant formula (PIF) for infants up to 12 months old is common practice; nevertheless, this product is not sterile. Therefore, it is important to closely monitor the microbiological quality of powdered infant formula (PIF) [1]. According to Codex Alimentarius Commission (2008), Infant formula is a specially made alternative for breast milk that is intended to meet an infant's nutritional needs on its own during the first months of life until proper complementary feeding is introduced [2]. Due to infant susceptibility to bacterial infection, the infant formula processing requires

extremely high levels of microbiological quality compliance with national and international microbiological guidelines. In 2004–2006, a collaborative FAO/WHO consultation group determined the principal bacteria that could contaminate PIF [3]. Moreover, the opportunistic pathogens such as *Cronobacter sakazakii*, *Salmonella sp.* and *Escherichia coli* of Enterobacteriaceae family were the most frequent bacterial contaminants found in powdered infant formula [4,5].

The majority of Enterobacteriaceae grow best at water activity (aw) levels greater than 0.95, with a minimum value of roughly 0.94, yet organisms such as *Salmonella sp.* and *Cronobacter sp.* still can survive for years in such an environment [6]. In fact, outbreaks associated to powdered milk with low-moisture (aw < 0.85) was reported [6].

The post-processing environment could serve as a good source of contamination as it was found in some outbreaks [7]. For instance, an aerobic plate counts and Enterobacteriaceae



of 3.2 log CFU g⁻¹, and 1.5 log CFU g⁻¹ respectively were found in some formulas [8]. Similarly, Heperkan D, et al. [9] found 1.7 log CFU g⁻¹ of mesophilic aerobic bacteria and <0.48 log CFU g⁻¹ of Enterobacteriaceae respectively in PIF. Despite the microbial contamination chance of PIF, information on PIF bacterial count of PIF in Omani markets is limited. Therefore, this study aimed to evaluate the microbial quality and the status of hygienic process through total bacterial and Enterobacteriaceae counts as well as measuring the water activity of PIF.

Materials and Methods

Samples

In this study, 25 of PIF samples of 5 commonly found brands with a category of 1–6 months, were collected from Muscat Governorate markets. Each sample was run in duplicate in microbial analyses.

Enumeration of Total Aerobic Bacteria

The total aerobic bacteria (TAB) was enumerated on tryptone soya agar medium (Oxoid, UK). Briefly, 25g of sample was mixed with 225 ml of maximum recovery diluent (MRD) (Oxoid, UK) and blended by stomacher for 1 min. After serial dilutions, 0.1 ml was plated tryptone soya agar. The plates were incubated at 35± 2°C for 72 hours [10]. The total aerobic bacteria (TAB) were expressed as the colony forming units (CFU/g) of microorganisms in the samples.

Enumeration of Enterobacteriaceae

Enterobacteriaceae were enumerated in violet red bile glucose agar medium (VRBG) (Oxoid, UK). Briefly, 10 g of sample was mixed with 90 ml of MRD (Oxoid, UK) and blended by stomacher for 1 min. After serial dilutions, 1 mL of each dilution sample was mixed with 15 mL from VRBG and the plates were incubated for 24 h at 37 ± 2°C [11]. The typical purple–pink colonies were counted.

Measurement of Water Activity (aw)

The aw of the powdered infant formulas was measured with an AQUALAB 4TE Water Activity Meter (METER Group, Inc., U.S.). A portion of the sample was taken into a disposable cup, then, placed in the water activity meter. All the measurements were done in 5 replicates.

Results and Discussion

Total Bacterial Count

Table 1 shows the average values of total aerobic bacterial count and Enterobacteriaceae counts for five

different PIF brands. Total aerobic bacterial count may serve as an indicator for the microbiological quality of PIF in the Omani market and the hygienic process. Brand 3 showed the highest total bacterial count; 1.7±0.07 log CFU/g, whereas brands 4 and 5 showed the lowest total microbial count (< 1 log CFU/g). All brands were lower than the maximum limit set by Codex, 2008; 3 log CFU/g. This indicates that the PIF in the markets have a good microbial quality, the products were processed under good hygienic conditions and the products complied with Codex Alimentarius Commission [2] (Table 2). As compared to other studies, the total bacterial count of PIF in the current study was lower than that found in Iraq study by Al-Atrash MK [1] and AL-Timimi S, et al. [12] and agreed with what was found by Sezer C, et al. [13,5] and Iversen C, et al. [14]. Differences in results between studies are likely to be attributed to initial raw materials, processing conditions and handling. 78 % the contaminations can be from processing area, 12% from ingredients and 10% from final product [15].

Formula Name	TAB, log CFU/g	ENT, log CFU/g
Brand 1	1.6 ±0.28	ND
Brand 2	1.6 ±0.07	ND
Brand 3	1.7 ±0.07	ND
Brand 4	< 1	ND
Brand 5	< 1	ND

Table 1: Infant formulas total aerobic bacterial (TAB) and Enterobacteriaceae (ENT) count.

ND: Not Detected, n = 2

Formal Name	Compliance	Non-compliance
Brand 1	√	
Brand 2	√	
Brand 3	√	
Brand 4	√	
Brand 5	√	

Table 2: Infant formulas compliance with Codex Alimentarius Standard (CAC/RCP 66 – 2008).

Enterobacteriaceae

The Enterobacteriaceae count is considered as an indicator of hygienic practices status during food process and handling, thus its count indicates poor hygienic conditions, inadequate treatments (especially heat treatment), and post-treatment contamination [16]. According to Codex Alimentarius Commission (2008), the infant formula must be free from Enterobacteriaceae [2]. Considering these criteria, it is clear from Table 1 that Enterobacteriaceae were absent from all brands which eliminated the chance of PIF

contamination from sanitary source, PIF were processed at high hygienic conditions and PIF producers compliance with Codex Alimentarius Commission [2] (Table 2). The possible sources of contamination with Enterobacteriaceae include post pasteurization environment, equipment used in the process, soil, water, plants, and the gastrointestinal tract of animals and humans [17]. In comparison with other studies, our results agreed with Al-Atrash [1] and AL-Timimi S, et al. [12]. However, the current study results did not agree with Sezer, et al. [13] who found a higher count than what was found in the current study. High Enterobacteriaceae associated with the presence of different opportunistic microorganisms and pathogens linked to infant illness such as *Salmonella sp* and *Cronobacter sakazakii* [18]. Moreover, the low total bacterial count and absence of Enterobacteriaceae in the current study can be attributed further with low water activity (Table 3).

Formula Name	a_w
Brand 1	0.2 ±0.031
Brand 2	0.2 ±0.044
Brand 3	0.2 ±0.027
Brand 4	0.3 ±0.026
Brand 5	0.1 ±0.020

n=5

Table 3: Infant formula water activity.

Conclusion

The results showed that the total bacterial count were within the limit of the Codex Alimentarius Commission, whereas, Enterobacteriaceae was not detected. All PIF were found to have a good microbial quality and the products were processed under good hygienic conditions complying with the Codex Alimentarius requirements which include good hygienic animal husbandry practices, good processing and packaging practices by using facilities that deny access of pathogenic bacteria, time/temperature recorder is checked and maintained, good personal hygiene policy is implemented, microbial hazards in processing environment are controlled, cross-contamination are avoided, cleaning and sanitation requirements are met and products are stored at good conditions. This study recommends increasing the sampling size and including more microbial parameters to evaluate the microbial safety of PIF.

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