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The Microbiological Quality of Infant Milk and Follow - on Formula

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SUMMARY

This study was conducted for the purpose of evaluating the microbiological quality of commercially available infant formulas in terms of public health. A total of fifty infant milk and follow-on formulas (in jars or powder form) sold in markets in the province of Kars from five different international companies were analysed. Microbiological quality of samples were analysed with conventional culture method. According to the microbiological criteria specified in the Turkish Food Codex Regulation for Baby Food-Baby Formulas and Baby-İnfant Supplements, one sample (2%) was found to be in noncompliance with regard to total aerobic mesophilic organisms, 11 samples (22%) for the coliform bacteria count, eight samples (16%) for total yeast and mold counts and five samples (10%) for the *B. cereus* count. Furthermore, *L. monocytogenes* was identified in three samples (6%), *Salmonella* spp in two samples (4%), *B. cereus* in five samples (10%) and *E. coli* in seven samples (14%). In conclusion, thirteen (26%) of the fifty samples analyzed in this study failed to comply with the regulation, and they were found to contain pathogens that could cause serious health problems in babies. It has been demonstrated that baby foods and follow-on formulas can be a potential source of food poisoning with infants and pose a significant risk for babies.

Key Words: *Infant milk, Follow-on formula, Microbiological quality*

ÖZET

Bebek Sütü ve Devam Formüllerinin Mikrobiyolojik Kalitelerinin Araştırılması

Bu araştırma bebek sütü ve devam formüllerinin mikrobiyolojik kalitelerinin belirlenmesi ve halk sağlığı açısından değerlendirilmesi amacıyla yapılmıştır. Kars ilinde satışa sunulan 5 farklı firmaya ait toplam 50 adet bebek sütü ve devam formülü (kavanoz veya toz formda/doğumdan itibaren-6 aylık) alınmış ve mikrobiyolojik kaliteleri klasik kültür tekniği ile belirlenmiştir. Türk Gıda Kodeksi Bebek Mamaları-Bebek Formülleri ve Bebek-Küçük Çocuk Ek Gıdaları Tebliği'nde verilen mikrobiyolojik kriterlere göre 1 örnek (%2) toplam aerobik mezofilik canlı sayısı, 11 örnek (%22) koliform bakteri sayısı, 8 örnek (%16) toplam maya ve küf sayısı ve 5 örnek (%10) *B. cereus* sayısı bakımından uygun bulunmamıştır. Ayrıca 3 örnekte (%6) *L. monocytogenes*, 2 örnekte (%4) *Salmonella* spp, 5 örnekte (%10) *B. cereus,* 7 örnekte (%14) *E. coli* tespit edilmiştir. Bu durumda analiz edilen 50 örneğin 13 (%26) adedinin Tebliğ'e uygun olmadığı bebeklerde ciddi sağlık sorunlarına neden olabilecek patojen mikroorganizma içerdikleri belirlenmiştir. Bebek mamaları ve devam formüllerinin özellikle bebeklerde gıda kaynaklı zehirlenmeler açısından potansiyel bir kaynak olabilecekleri ve bebekler için büyük bir risk oluşturdukları görülmüştür.

Anahtar Kelimeler: *Bebek sütü, Devam formülü, Mikrobiyolojik kalite*

INTRODUCTION

Ready to use infant formulas can be consumed directly and require no processing other than adding water; these products meet the special nutritional needs of babies for the first few months of life, up until the time they are introduced to suitable supplemental food. Infant milk is defined as infant formulas produced exclusively from the protein in cow's milk (Anon 2009a)

Newborn babies are extremely sensitive to infections, particularly those originating from food. Therefore, maintaining the microbiological safety is quite important during the production and preparation of the products considering that the quality of raw ingredients and hygienic conditions of production plant are the major factors affecting the quality of the final product. When it comes to infant formulas, these quality control measures are exceptionally critical (Iversen and Forsythe 2003).

However, some of the studies investigating the microbiological condition of the infant foods showed that they could threaten the consumer's health by having poor hygienic quality. The researchers indicated that *S. aureus, E. coli, Cronobacter sakazakii* and *Bacillus cereus* were the most frequent microorganisms existing in these kinds of products (Ergün and Ergün 1994; Ergün et al. 2002; Chap

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et al. 2009; Wang et al. 2012). The microorganisms that might be found in ready to use powdered infant foods have been evaluated according to the risk they pose and 3 basic risk groups have been identified. As a result of epidemiological and microbiological research, Salmonella and *C. sakazakii* have been assigned to group A found in infant foods and have been demonstrated to cause infection. The other members of the Enterobacteriaceae family (*Pantoea agglomerans, Escherichia vulneris, Hafnia alvei*, *Klebsiella pneumoniae*, *Citrobacter koseri*, *C. freundii*, *Klebsiella oxytoca* and *Enterobacter cloacae*) cause diseases in babies, but they were placed in group B because there is not sufficient epidemiological and microbiological evidence to indicate that infant foods are the source or medium for these diseases. *Clostridium botulinum, S. aureus, L. monocytogenes* and *B. cereus* are found in group C (Anon 2007).

The purpose of this study was to evaluate the microbiological quality of commercially available infant formulas in terms of public health.

MATERIALS and METHODS

In this study, fifty samples of infant milk and follow - on formulas (in jars or powder form) produced by five different international companies were purchased from markets in the province of Kars and brought to the laboratory being kept in a cold chain at 4 °C. The characteristics of the samples are provided in Table 1.

Table 1. Sample distribution

Sample	Number of different product examined
İnfant milk (powder) / From birth	5
İnfant formula (powder) / From birth	5
Rice flour with milk (powder) / Starting in the 4th month	5
Fruit and grain (jar) / Starting in the 4th month	5
Mixed vegetable puree (jar) / Starting in the 4th month	5
Fruit pudding (jar) / Starting in the 4th month	5
Blend of milk and grain (powder) / Starting in the 5th month	5
Follow - on formula (powder) / Starting in the 6th month	5
Follow - on milk (powder) / Starting in the 6th month	5
Garden vegetable puree (jar) / Starting in the 6th month	5

Microbiological Analyses

Twenty five grams were weighed from the samples and homogenized in 225 ml of sterile saline (0.9%). After preparing decimal dilutions, the plates were inoculated by spread or pour plate techniques and incubated under the following conditions: Plate Count Agar (Oxoid CM 325) 30 °C/48 hours for Total Aerobic Mesophilic Colony Count; Violet Red Bile Lactose Agar (Oxoid CM 107) 37 °C/24 hours for Coliform Group Bacteria; Violet Red Bile Lactose Agar (Oxoid CM 107) 44.5 °C / 24–48 hours for *Escherichia coli*; Potato Dextrose Agar (Difco B 13) 22 °C/5–10 days for Yeast-Mold; Perfringens Agar (Oxoid, CM0543) 35 °C/18–24 hours (Anaerobic) for *Clostridium perfringens*; *Bacillus cereus* Agar (with Polymixin B) (Oxoid, CM 617) 30 °C/24–48 hours for *Bacillus cereus* and de Man Rogosa Sharpe Agar (Oxoid, CM 361) 30 °C/48–72 hours for lactic acid bacteria. Typical colonies of *C. perfringens, B. cereus* ve *S. aureus* were selected and stocked in slant agar. Biochemical tests (Gram reaction, catalase, oxidase, hemolysis, IMVIC test, reduction of nitrate, lecithinase activity, motility, hydrolysis of aesculin, produce acid from glucose anaerobically, carbohydrate fermentation test, etc.) were performed for identification (Kandler and Weiss 1986; Harrigan 1998; Halkman 2005).

Isolation of *Salmonella* spp

Twenty-five grams were weighed from the sample, homogenized in 225 ml of buffered peptone water and incubated for 24 hours at 37 °C. At the end of the incubation period, 0.1 ml was removed and inoculated in Rappaport Vassiliadis Broth (Oxoid, CM 669), incubated for 18 - 24 hours at 42 °C and inoculated using the spread plate technique on, Brilliant Green Agar (Oxoid, CM 263), Hektoen Enetric Agar (Oxoid, CM 419), Xylose Lysine Deoxycholate Agar (Oxoid, CM 469) selective agars and incubated for 18-24 hours at 37 °C. Ten typical colonies were picked from selective medium and stock in Tryptone Soya Agar (Oxoid, CM 131). Following biochemical tests were performed for identification; urea test, formation of acid and gas from glucose, utilization of lactose and sucrose and the formation of H_2S in Triple Sugar Iron Agar, lysine decarboxylase test, Voges/Proskauer and Indole tests. The Salmonella latex test (Oxoid, FT 203) was applied to the isolates for serological analysis (Andrews and Hammack 1995; ISO 2002).

Isolation of *Listeria monocytogenes*

Twenty-five grams were weighed from the sample, homogenized in 225 ml of Listeria Enrichment Broth (Oxoid, CM 862) and incubated for 24 hours at 30 °C. At the end of the incubation period, the enriched culture was inoculated on Listeria Selective Agar (Oxoid, CM 856) using the spread plate technique and incubated for three days at 37 °C. Ten typical colonies were picked from selective medium and stocked in Tryptone Soya Agar. Following biochemical tests were performed for identification; Gram staining, catalase, oxidase, Indole, Methyl Red, Voges/Proskauer, nitrate reduction, motility, CAMP and carbohydrate fermentation test (Seeliger and Jones, 1986; Hitchins, 2011).

Isolation of *Escherichia coli* O157:H7

Twenty-five grams were weighed from the sample, homogenized in 225 ml of modified EC broth (mEC+novobiocin/14582, Merck) and incubated for 18 hours at 37 °C. After the enrichment stage, it was spread on Sorbitol MacConkey (SMAC) agar having cefixime and tellurite supplement (CT-SMAC Agar/109207, Merck) and incubated for 18-24 hours at 41-42 °C. Sorbitol negative colorless colonies were picked and inoculated on MacConkey Agar (Oxoid, CM007) containing 4-methylumbelliferyl-D-glucuronide (Oxoid, BR0071). After incubation for 18 hours at 41-42 °C, MUG negative colonies were selected and stocked in Tryptone Soya Agar. The following biochemical tests were conducted on the isolates: Gram reaction, Indole, Methyl Red, Voges/Proskauer, citrate, hydrogen sulfide production, gas production from glucose, lactose, motility and lysine decarboxylase (Harrigan 1998; Halkman 2005; Feng and Weagant 2011).

Isolation of *Cronobacter sakazakii*

Samples of 100 g, 10 g and 1 g were weighed and then completely dissolved in sterile distilled water at a ratio of 1:10. This solution was kept overnight at 36 °C. At the end of this time, 10 ml of overnight cultures were homogenized in 90 ml of Enterobacteriaceae Enrichment Broth (Oxoid, CM 1115) and incubated for 24 hours at 36 °C. After incubation, 0.1 ml of samples were spread on Violet Red Bile Glucose Agar (Oxoid, CM 485) and incubated for 24 hours at 36 °C. Typical purple colonies were picked and inoculated on Tryptone Soya Agar. The plates were incubated at 25 °C for 24-48 hours and typical yellow colonies were identified by applying lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, and carbohydrate fermentation tests (Farmer and Kelly 1992; Anon 2002a,b).

RESULTS and DISCUSSION

The microbiological analyses showed that the average microorganism counts of the samples were as follows; $1.0x10^3$ CFU/g for total aerobic mesophilic bacteria, $1.6x10^2$ CFU/g for coliforms, $1.8x10^2$ CFU/g for yeast and molds, $6.6x10^1$ CFU/g for *B. cereus* and $1.5x10^2$ CFU/g for Lactic acid bacteria. In twenty one of the samples, the count of tested microorganisms were under detection limit while *L. monocytogenes* was identified in three (6%), *Salmonella* spp in two (4%), *B. cereus* in five (10%) and *E. coli* in seven samples (14%). However, *C. perfiringens, E. coli* O157:H7 and *C. sakazakii* were not detected in any of them. The results of the microbiological analysis were presented in Table 2.

Table 2. Results of microbiological analysis

	Total aerobic mesophilic bacteria count	Coliform	Total yeast and mold	*B.cereus*	Lactic acid bacteria count
Number of sample below the detectable limits	21	39	42	45	42
Lowest value (CFU / g)	$1.0x10^2$	$1.6x10^2$	$1.2x10^2$	$4.0x10^2$	$1.3x10^2$
Highest value (CFU / g)	$1.4x10^4$	$4.0x10^3$	$3.2x10^3$	$8.3x10^2$	$3.4x10^3$
*** Number of samples not in compliance with regulation**	1 (powder)	11 (2 jars-9 powders)	8 (4 jar–4 powder)	5 (powder)	-

* Code of hygienic practice for powdered formulae for infants and young children (Codex Alimentarius, 2008) * Turkish Food Codex baby formulas directives and baby-infant supplements regulation (Anon 2009a, 2009b).

According to the microbiological criteria specified in the Codex Alimentarius Commission and Turkish Food Codex Regulation for Baby Formulas and Baby - Infant Supplements, one sample (2%) was found to be unacceptable for its total aerobic mesophilic organism count, 11 samples (22%) for the number of coliform bacteria, eight samples (16%) for total yeast and mold counts and five samples (10%) for the *B. cereus* count. Furthermore, *L. monocytogenes* was identified in three samples (6%), *Salmonella* spp in two samples (4%), *B. cereus* in five samples (10%) and *E. coli* in seven samples (14%). In conclusion, thirteen (26%) of the total fifty samples had poor hygienic quality according to Turkish Food Regulations and had risks for baby health.

Feeding infants with ready to use infant foods from birth through the first few months is getting more common for a number of reasons, such as the increasingly fast pace of daily life, lack of time, the more active participation of women in the work force. This kind of nutrition could bring some health risks for babies with sensitive immune systems in terms of microbiological quality of the infant milk and follow - on formula (Hanson et al. 2003). Therefore, from raw material to final product, regular and detailed quality control analyses must be conducted at each stage of production. Rising of the consumer's awareness could be helpful for decreasing of the health troubles originated from infant foods. Regular controls of baby formulas on the shelves of the markets are also important for keeping the both producers and the consumers updated about current situation.

In this study, the total average count of aerobic mesophilic organisms in the samples was found to be $1.0x10^3$ CFU/g, and one of the samples failed to comply with the Turkish Regulation. In a similar study, Iversen and Forsythe (2004) reported that 56% of the powdered infant food samples had the total aerobic bacteria count at $<10^2$ CFU/g level, while the others had $>10^3$-10^4. Ergün and Ergün (1994) also declared that 12% of the domestic samples they tested had over 10^4 CFU/g bacterial load for the same microorganism group.

The most important changes in total organism count of infant foods and follow-on formulas occur during the stage between preparation and consumption. Therefore, it is essential to give the mothers and hospital employees a special training to ensure that prepared infant food is consumed as quickly as possible under suitable conditions.

In this study, pathogens were also investigated in the samples and *Salmonella* spp was detected in two samples (4%). A number of studies have emphasized that powdered infant formulas are a significant source of Salmonella in newborn infections. The Salmonellosis cases in France between 2004 and 2005 affected 141 babies less than one year old, and the organism have been isolated in powdered infant foods (Brouard et al. 2007). Similarly, in Spain, there are some reports declaring that 48 babies most of whom were younger than 7 months have been effected by the infection and powdered infant foods those they consumed were contaminated with Salmonella (Usera et al. 1996).

In this study, *B. cereus* was another pathogen detected in five of the samples (10%) with the average contamination level at $6.6x10^1$ CFU/g which was not acceptable according to Turkish Regulations. In a similar study (Ergün et al. 2002), researchers reported that 3.3% of the samples they tested were contaminated with *B. cereus*. Shadlia et al. (2008) also found that over 64.3% of the tested samples contained high counts of *Bacillus* spp (2 log10 CFU/g).

Even though *L. monocytogenes* causes disease in infants (systemic infections; specifically meningitis, dangerous diarrhea, etc.), it has not been isolated in infant food, or even if it has been identified in these products, there is not

enough information to link it directly with the infections. In 2007, it was categorized in Group C in the microorganisms that poses a risk in ready-to use and powdered infant foods by WHO (Anon 2007). In this study, *L. monocytogenes* was identified in three of the analyzed samples (6%) which was quite remarkable.

E. coli was also identified in seven samples (14%) while *C. perfringens, E. coli* O157:H7 and *C. sakazakii* were not isolated in any samples.

C. sakazakii is becoming increasingly important, especially in infant foods. There are quite amount of studies investigating the presence of Cronobacter in infant foods, and most of them are focusing on isolation techniques. In one of these studies, Iversen and Forsythe (2004) tested 404 samples of different kinds infant food with various conventional techniques and detected the organism in 3-5% of the samples while In another study, researchers reported that none of the tested infant formulas was contaminated with *C. sakazakii* (Baumgartner et al. 2009).

In conclusion, thirteen of the fifty samples (26%) analyzed in this study failed to comply with the Turkish Regulation having the pathogens that could cause serious health problems in babies. Our findings demonstrated that baby foods and follow-on formulas can be a potential source of food poisoning for infants.

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