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Research Article

Microbiological Examination of Infant Food and Feed Formula

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Abstract

Eighty four samples of infant food commercially available in Libya were studied for their microbiological status. The microbiological quality of these infant foods varied over the range 1.0 to 6.0 $\log_{10}$ CFU/g with the mean total viable count of $\geq$ 3.0 $\log_{10}$ CFU/g. Twenty four (19 samples) were unsatisfactory for infant consumption because they contained more than recommended limit. Bacterial isolates were identified by API system. The study was concluded with identification of Cronobacter sakazakii isolates by PCR techniques. The safety of these infant foods can be assured by a preventative approach based on the application of Hazard Analysis Critical Control Point (HACCP) in the food and food related industry.

Keywords baby food, Bacillus spp, C. sakazakii, enterobacteriaceae, infant food, Staphylococcus spp

Introduction

Global incidence of food-borne disease is increasing and difficult to estimate, as data from several countries reveals (Sakaguchi et al. 1990; Himmelright et al. 2002). A greater proportion of these can be attributed to contamination of baby food by pathogenic microorganisms at some points during production (Chantal et al. 2004; Kandhai et al. 2004). Infant foods are usually pasteurized during manufacturing; however, some of organisms do survive such heat treatment. The presence of microorganisms in the finished dried products may also have come from the factory environment. The environment such as air in drying and filling areas is often the principle contamination sources for dried products or from other sources such as the addition through ingredients not subjected to a heat treatment during the powdered infant formula manufacturing process (Caric 1993; Chantal et al. 2004). Despite the high temperature employed in the production of infant milk formula, reconstituted baby foods are considered to be a high-risk food. Notwithstanding the high temperatures employed in the manufacture of infant products during the spray drying process, there have been a number of food related illnesses where infant milk powder or infant food products have been implicated as the vehicle of infection involving C. sakazakii, Salmonella spp, B. cereus (Biering et al. 1989; Rushdy et al. 1998; Threlfall et al. 1998; Usera et al. 1998; Bar-Oz et al. 2001; Lai, 2001; Renata et al. 2004 and Duc et al. 2005).

In Libya, although cereals are normally processed in the country, powdered milk products are often imported. Combined cereal/milk products for infants which are sold in the country may have been produced locally or imported. For the locally produced infant cereal/milk products, sources of contamination may include equipment, storage conditions, raw materials, the environment and staff.

Studies regarding the microbiological quality of infant food and feed formulae are lacking in Libya, which makes such study important not only locally but also internationally; as infant food can be a risk factor for infant illness. Therefore, the main aim of the study was to evaluate the microbiological

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quality of eighty four infant food and feed formulae available in Libya, with particular attention being placed on detection of food-borne pathogens such as B. cereus, Enterobacteriaceae, Staph. aureus and toxigenic fungi. The study was concluded with identification of isolates by API® 20 E, API® staph and API® 50 CH techniques.

Materials and Methods

Microbiological examination of samples

Samples and Media

Eight four samples of baby food consumed by Libyan infants were collected from several local sources including retailers and stores. Samples which consisted of imported and locally produced products were stored dry at room temperature (21°±2°C) and examined microbiologically to determine their level of contamination. The samples contained rice flour, wheat flour, mixed grain cereal contained, wheat, rice, barley, and oat flour, skimmed milk powder or whole milk powder and in various combination. The samples were examined for bacteria species such as Bacillus spp, Staphylococcus spp, Enterobacteriaceae and fungi. Standard methods were used for isolation, enumeration and identification of bacteria and fungi (Robert et al. 2004; Samson et al. 2002).

Sample (25g) was weighed into a sterile stomacher bag and 225 ml of sterile maximum recovery diluent was added. The mixture was then mixed for 60 s in stomacher (Steward Stomacher blender 400, London, UK) and serial dilutions were made from it. Total aerobic bacterial count determined using plate count agar. Bacillus cereus was isolated and enumerated using Bacillus cereus selective agar (PEMBA) and Bacillus cereus agar base. Coliforms and Enterobacteriaceae were isolated and enumerated using Violet red bile glucose (VRBG) agar. Cronobacterr sakazakii was isolated by using Chromocult® Enterobacter sakazakii agar. Staphylococcus spp was isolated and enumerated using Baird-Parker agar. Selective enrichment broth (RV) and XLD were used for isolation of Salmonella. spp. Moulds and yeast were isolated and enumerated using malt extract agar (MEA) and potato dextrose agar (PDA). All media and diluents were purchased from Oxoid (Basingstoke, UK).

Identification of bacteria by API technique

API® 20 E, API® staph and API® 50 CH are standardized systems for identification of the Enterobacteria, Staphylococcus spp and Bacillus spp respectively, which use miniaturized biochemical tests. These were purchased from BioMérieux (La Balme-les-Grottes, France).

Statistical Analysis

Microbiological plate counts were transformed into base-10 logarithms ($\log_{10}$ CFU/g) before computing and performing statistical analyses. Minimum detection limits for bacteria count were 1.0 $\log_{10}$ CFU/g, based on the maximum sensitivity of the tests with sample diluted by 10^{-1}. Averages of triplicate samples were analyzed and the results were calculated using Microsoft Office Excel 2003 software (Microsoft Corporation, Redmont, Washington, USA).

Results and Discussion

The general microbiological quality of the infant food and feed samples is given in Table 1. The total viable counts varied over the range ≤ 1.0 to 6.4 $\log_{10}$ CFU/g with the mean total viable count ~ 3.4 $\log_{10}$ CFU/g. Of the eighty four samples examined, 76.2% were considered microbiologically satisfactory with total viable count of ≤ 4.0 $\log_{10}$ CFU/g. Nearly 23.8% of the samples were deemed unsatisfactory for infant consumption because they contained total viable counts higher than the recommended safety limit of 4.0 $\log_{10}$ CFU/g proposed by FAO and WHO and the International Dietetics Association of the European Community (IDAEC). These results appear to be similar to those reported by Scottish Food Coordinating Committee (1990), who examined 12 types of baby formula and found that bacterial count ranged from < 1.0 to 6.7 $\log_{10}$ CFU/ml. While Finoli and Rondini, (1989) examined 26 infant formulae in Italy and found that the total aerobic counts did not exceed 2.3 $\log_{10}$ CFU/g. Degree and frequency of microbial contamination of infant food products may be influenced by hygienic condition at processing stages.

Table 1: Occurrence of Bacillus spp, Enterobacteriaceae and Staphylococcus spp in infant foods in Libya

Products	Number of samples examined	Range log_{10} CFU/g of Fungi	Range log_{10} CFU/g of Total count	Number / Percentage of samples positive (%)		
				Bacillus spp	Enterobacteriaceae	Staphylococcus spp
Baby cereal with milk	7	<1.0 – 2.52	<1.0 – 3.91	3 (42.9)	2 (28.6)	3 (42.9)
A senibl with cereal grains	9	<1.0 – 3.32	<1.0 – 4.06	5 (55.6)	4 (44.4)	2 (22.2)
Maternal infant milk cereal	3	2.43 – 2.80	2.75 – 2.79	2 (66.6)	1 (33.3)	1 (33.3)
Purity	3	<1.0 - 3.67	<1.0 – 3.67	1 (33.3)	ND	ND
Inesfood (Baby lack) cereal with milk	9	<1.0 – 2.82	<1.0 – 3.39	6 (66.7)	5 (55.6)	6 (66.7)
Mothers choice	3	<1.0 2.91	<1.0 3.57	3 (100)	ND	2 (66.6)
Plasmon	5	<1.0 – 3.0	<1.0 – 3.81	3 (60)	ND	2 (40)
A senibl ground rice	5	<1.0 – 2.83	<1.0 – 3.82	3 (60)	1 (20)	1 (20)
Nestle infant cereal	8			3 (37.5)	ND	4 (50)
Farley's baby cereal with milk	3	<1.0 – 3.4	<1.0 -3.54	4 (75)	1 (33.3)	3(100)
Cehlab	14	<1.0 – 3.57	1.89 – 5.58	8 (57.1)	6 (42.9)	6 (42.9)
Ulker	7	2.36 – 3.43	<1.0 – 6.43	4 (57.1)	ND	ND
Hayati	1	2.63	3.43	1 (100)	ND	ND
RiRi flates (rice with milk)	4	<1.0 - 3.3	<1.0 – 5.28	3 (75)	2 (50)	2 (50)
A senibl with nuts	2	2.69 – 3.39	2.69 – 5.81	2 (100)	1 (50)	1 (50)
Cream rice	1	3.64	4.41	1(100)	ND	1 (100)
Total	**84**			**61.9 %**	**27.4 %**	**40.5 %**

Average counts of Bacillus spp, Staphylococcus spp and Enterobacteriaceae were 4.4, 4.5 and 3.8 $\log_{10}$ CFU/g respectively. The total mould count in most samples was equal to or less than 3.7 $\log_{10}$ CFU/g. The fungi isolated were of the genera, Aspergillus, Penicillium and several unidentified species.

Occurrence of Enterobacteriaceae, Bacillus spp. and Staphylococcus spp. in the samples was 27.4%, 61.9% and 40.5% respectively (Table 1). Figure 1 show the presence of Bacillus spp., Enterobacteriaceae and Staphylococcus spp. in locally manufactured products was 60%, 40% and 33% respectively, and 63%, 20% and 44% respectively in imported products.

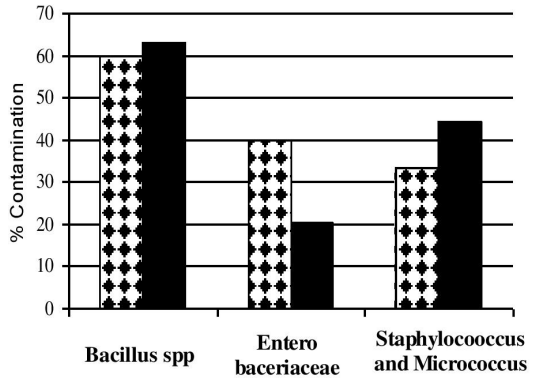

Figure 1. The percentage of infant food sample imported ■ and local. ⁘ contaminated with Bacillus spp, Enterobacteriaceae, Staphylococcus and Micrococcus spp.

In this study, it was found that more than 26.3% of the samples contained Enterobacteriaceae (≥ 2.0 $\log_{10}$ CFU/g). Salmonella was not present in any of the samples tested. Other reported studies showed occurrence of Enterobacteriaceae in different types of infant food products although Salmonella spp were not detected in any of the infant samples examined (Muytjens et al. 1988; Chantal et al. 2004; Iversen and Forsythe, 2004). Iversen and Forsythe (2004) also reported the absence of Salmonella spp in powdered infant milk formula, although Salmonella spp have been shown to be able to tolerate the spray drying, a process used in the production of a number of infant food formulae (In't Veld et al. 1994). In one study also, coliforms were detected in 3 of 124 samples of spray dried milk, 6 of 54 samples of roller dried milk and 13 of 38 samples of infant formula from 10 factories at populations of > 1.0 $\log_{10}$ CFU/g (Ghodeker et al. 1980).

The results of API 20E biochemical profiles showed all presumptive Enterobacteriaceae isolates belonged to Pantoea spp, Citro. koserilama, E. cloacae, E. amnigenus, Aeromonas. hydrophila, K. oxytoca, K. pneumoniae, E. aerogenes, E. cancerogenus and three strains were positive as C. sakazakii.

The results of API 20E biochemical profiles showed all presumptive Bacillus spp were identified as B.cereus; others were B.licheniformis, Geobacillus stearothermophilus, B. subtilis and Brevi laterosporus. The results of the present study are, in general, in agreement with Shinagawa et al., (1992); Anderton (1993) and Crielly et al., (1992) who also isolated the following species of Bacillus from milk and dried milk products including infant milk powdered: B. licheniformis, B. cereus, B. circulans, B. firmus, B. pumilus, B. subtilis, B. coagulans, B. sphaericus, B. lentus, B. polymyxa, B. caratarum, B. thuringiensis, B. pumilus and B. megaterium. Dried milk products, such as milk powder, infant milk formula and infant cereal products, contaminated with B. cereus should be considered a potential vehicle for food-borne B. cereus disease (Kramer and Gilbert 1989; Becker et al. 1994).

All isolated strains of Staphylococcus and Micrococcus spp identified as Derma nishinomiyaen, Staph. xylosus, Kocuria varians, Staph. lentus, Micrococcus spp, Kytococ sedentarius, and Kocuria rosea, Staph. aureus was not isolated in the study.

Results of this study indicated that infant food products can be contaminated by microorganisms. It is for this reason that the microbiological quality of dried infant products or its products (reconstituted foodstuffs) is of paramount importance particularly if such feeds are produced and/or used in developing countries where there seem to be inadequate quality control checks on food processing and manufacture.

Conclusions

This study has shown that some of infant foods retailed in Libya could pose a health threat to babies and infants who are fed on these. The newborn infant is susceptible to infection and infant formula requires

a high level of microbiological quality control during production, distribution and usage. It is important to ensure that infant formulae is prepared using good hygiene practice, with rapid cooling, and minimization of the time between preparation and consumption to reduce the risk of bacterial infection.

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