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Bacteriological Investigation on Milk Powder in the Egyptian Market with Emphasis on its Safety

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Abstract: One hundred and fifty random samples of milk powder, 100 whole milk powder samples [65 Brand (I) and 35 Brand (II)] and 50 Infant milk formula (IMF) were collected from different local markets and pharmacies in Dakahlia governorate, Egypt, for bacteriological examination. The obtained results revealed that the mean values of total aerobic plate count were $1.5\times10^4 \pm 3.6\times10^3$, $1.5\times10^3 \pm 2.8\times10^2$ and $1.4\times10^3 \pm 2.5\times10^2$ for whole milk powder Brand (I), (II) and IMF samples, respectively. 6 out of 65 samples of whole milk powder Brand (I) showed unaccepted count. Salmonellae could not be detected in any of the examined milk powder samples. *B. cereus* could be detected after plating on PEMBA agar using surface plate technique, confirmed *B. cereus* samples represented 44.6, 5.7 and 14.0 %, with an average count of $6.3\times10^2 \pm 1.4\times10^2$, $3.8\times10^2 \pm 2\times10^2$ and $1.4\times10^2 \pm 1.6\times10$ for whole milk powder Brand (I), (II) and IMF samples, respectively. *Staph. aureus* enterotoxins could not be detected in all of the examined milk powder samples. The results allow concluding that milk powder in spite of its low moisture content may at times be responsible for food poisoning to consumers and may contaminate other food, if it included in its preparation.

Key words: Milk powder · Infant milk formula · Total aerobic plate count · Salmonellae · *B. cereus* · *Staph. aureus* enterotoxins

INTRODUCTION

Milk powder is one of dairy products of reduced moisture content. It is produced to achieve saving of transportation and costs related to reduced volume and weight, increasing shelf life of the product and with its greater concentration of milk solids. It is used in the manufacture of some dairy products such as ice cream, cheese, evaporated milk, condensed milk and infant milk formula. Also it is used as an ingredient in bakery products, processed meats, soups, etc. The function of milk powder in these products is influenced by the components of the powder, primarily protein, fat and lactose [1].

Powdered infant milk formula belongs to a special sub-set of powdered milks, these products are formulated to be as similar to human milk as is possible then concentrated and spray dried, in some cases, specific heat-labile ingredients are added after drying. Typically, infant milk formulae contain milk, or soy proteins, or protein hydrolysates together with those forms of fat, carbohydrate, vitamins and minerals that are bioavailable to the infant [2].

The important quality parameters for milk powder are microbiological quality and sensory characteristics, beside physical and chemical properties which are mainly concerned with the content of moisture, fat, total protein and non-protein nitrogen, lactose, titratable acidity, ash and other nutrients such as calcium.

Low water content (Maximum 5%) of dried milk acts as an inhibitory factor with respect to any bacterial spores or vegetative cells that have survived the drying process. The micro flora of dried milk powders depends on many factors including the number and type of bacteria present in the raw milk or milk by-product, preheating temperatures, operating conditions of the

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evaporator and dryer and plant hygiene. High numbers of micro-organisms in the raw milk may result in high numbers in the milk powder and the decline in numbers as a result of exposure to heat, is offset by the removal of water in the powder [3].

Post-processing contamination is a major factor impacting on contamination of milk powder, as the raw material is often subjected to lethal temperatures, which eliminate vegetative cells of pathogens. Milk powder outbreaks demonstrate that failures in preventive systems such as presence of water allowing microbial multiplication, or presence of zones difficult to maintain and to clean (isolation from a drying tower) were the origin of contamination [4]. In other cases, illness has been done due to contamination and abuse of reconstituted products.

Microbial pathogens of major concern in both dried milk and infant milk formula includes salmonellae, *Bacillus cereus* and *staph. aureus*. While these organisms will not grow in the powder, they may remain viable for long periods of time and resume growth when the powder is reconstituted and stored at favorable temperature [4].

Dried milk powders have been implicated in a number of food-borne disease outbreaks involving salmonellae that causing gastroenteritis [4]. Low-level contamination of powdered infant milk formula with salmonellae has been associated with infection in infant [5, 6]. The other pathogen that joined the salmonellae as important contaminants of milk powders causing food poisoning is *Bacillus cereus*, which is able to produce spores that can survive pasteurization and survive the manufacturing process of powdered milk. It represents a problem when powdered milks are reconstituted and stored for prolonged periods at incorrect temperatures. Most B. *cereus* strains isolated from dairy products are able to grow and produce toxins below 10°C [7, 8].

Bacillus cereus is the cause of two kinds of food borne diseases, an emetic (Vomiting) intoxication due to the ingestion of a toxin (Cereulide) pre-formed in the food and a diarrheal infection due to the ingestion of bacterial cells/spores which produce enterotoxins in the small intestine. Although there is very little epidemiological evidence linking B. *cereus* to illness to infants, diarrhea is a significant cause of ill health and death among infants and children in developed countries[7].

Thermo stable *Staph. aureus* enterotoxins are considered as one of the most prevalent causes of gastroenteritis (Food-borne staphylococcal poisoning) worldwide [9], in which major symptoms are vomiting and diarrhea, occurs after ingestion of one or more preformed thermo stable staphylococcal enterotoxins (SEs) in food contaminated with *S. aureus*. SEs are normally no or only slightly, inactivated during food processing, storage, distribution or during the preparation of the food in the kitchen. Therefore, if enterotoxinogenic staphylococci are able to grow in food to high numbers (more than 10^5 to 10^6 cfu/g or /ml) before they are killed there is still a risk for intoxication with consumption. Recently, powdered milk produced in Japan, was linked to contaminated dairy products in June and July of 2000. Health authorities reported that dry skim milk powder was contaminated with staphylococcal enterotoxin A [10].

This study was planned to throw the light on the bacteriological characteristics of milk powder with emphasis on its safety.

MATERIALS AND METHODS

Collection of Samples: A total of 150 random samples of dried milk powder, representing 100 samples of whole milk powder (WMP) [65 Brand (I), 35 Brand (II)] and 50 Infant milk formula (IMF) samples. The samples were collected from local different markets and pharmacies in Dakahlia governorate, Egypt and within the accurate shelf life period, then transferred to the laboratory in their packages to be tested bacteriologically.

Preparation of Samples: Either whole milk powder (WMP) and Infant milk formula (IMF) packages were mixed well before being aseptically opened. A dry and sterile metal spatula was used for sample transferring for examination [11].

Preparation of Serial Dilutions: 11g of the mixed milk powder sample were added to 99 ml of sterile 0.1% peptone water (40-45°C) in a sterile wide mouth capped bottle and thoroughly mixed to give a dilution of 1:10 and then ten- fold serial dilutions were prepared [11].

Aerobic Plate Count: From the previously prepared dilution, 1 ml was mixed with 12ml of liquefied standard plate count medium (45°C). Inoculated and control plates were left to dry before being incubated for 48 ± 2 h at 35°C in an inverted position. Colonies of incubated plates were counted and recorded [11].

Detection of Salmonellae [12]: Pre-enrichment: Aseptically 25g of milk powder sample were weighted and poured slowly over surface of 225 ml brilliant green water (which prepared by adding 2 ml of 1% Brilliant Green dye solution to 1L distilled water) in a sterile capped container which let standing undisturbed for 60 ± 5 min. Loosely capped container was incubated, without mixing or pH adjustment, for 24 ± 2 h at 35°C.

Selective Enrichment: The incubated sample mixture was gently shacked and 1 ml of the mixture was transferred to 10 ml of Rappaport- vassiliadis broth by using sterile pipette then incubated for 24 ± 2 h at 41°C.

Selective Plating: A loopful from the incubated RV broth was streaked over the surface of pre-dried xylose lysine desoxycholate (XLD) agar plate and incubated for 24 ± 2 h at 35°C then examined for suspected salmonellae colonies which appear pink colonies with or without black centers. Atypically, a few salmonellae species produce yellow colonies with or without black centers.

At least two typical or suspected colonies of being salmonellae from XLD plate were streaked over surface of Triple Sugar Iron (TSI) agar slant and Lysine Iron Agar (LIA) using a sterile needle. TSI and LIA slants were incubated for 25±2 h and 48±2 h at 35°C, respectively.

Confirmation and Biochemical Identification of *Salmonellae* [12]

Enumeration and Isolation of *Bacillus cereus:* Enumeration and isolation of *Bacillus cereus* using polymyxine pyruvate - egg yolk- mannitol bromo thymol - blue agar (PEMBA) [13]. Surface plate technique was used.

Plating (Plate count technique) with incubation in an inverted position for 24 ± 2 h at 35°C.

Confirmation and Identification *of B. cereus* [14]: Suspected colonies were transferred to nutrient agar slants. The slants were incubated for 24 h at 30°C for further identification.

On basis of the test result, identify as *B. cereus* those isolates which are actively motile, strongly hemolytic and not produce rhizoid colonies or protein toxin crystals.

Calculation the number of *B. cereus* per gram as follows:

$$\text{Count/g} = \frac{\text{No. of colonies confirmed}}{\text{No. of colonies tested}} \text{ X } \frac{\text{Presumptive count}}{\text{Volume tested x dilution}}$$

Detection of *Staphylococcus aureus* Enterotoxins (SET-RPLA KIT, Oxoid)

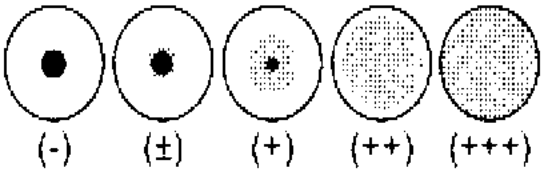
Toxin Extraction: 10 g of milk powder sample were blended with 10 ml of sodium chloride solution (0.85%) in a homogenizer. The blended sample was centrifuged at10, 000 rpm at 4°C for 30 minutes. The supernatant fluid was filtered through a 0.2µm-0.45µm low membrane binding filter. The filtrates were retained for assay of toxin content.

The SET-RPLA Assay Technique: The latex reagents were shacked thoroughly before use to ensure a homogeneous suspension. The control reagents were reconstituted by adding 0.5ml of diluent (TD910) to each vial and shacked gently until the contents were dissolved.

A microtiter plate was arranged so that each row consists of 8 wells as each sample needed the use of 5 such rows (four wells for each of the four enterotoxins (SEA, SEB, SEC and SED) and one latex control). Using a pipette, 25µl of diluents were dispensed in each well of each of the 5 rows, 25µl of test sample were dispensed to the first well of each of the 5 rows. Using a pipette and starting at the first well of each row, 25µl were picked up and perform doubling dilutions along each of the 5 rows. Stop at the 7th well to leave the last well containing diluent only.

To each well in the first row, 25µl of latex sensitized with anti-enterotoxin A were added. To each well in the second row, 25µl of latex sensitized with anti-enterotoxin B were added. To each well in the third row, 25µl of latex sensitized with anti-enterotoxin C were added. To each well in the fourth row, 25µl of latex sensitized with anti-enterotoxin D were added. To each well in the fifth row, 25µl of latex control were added. The contents of each well were mixed well, the plate was rotated by micromixer, with taking care that no spillage occurs from the wells. The plate was covered with a lid, the plate was left undisturbed on a vibration-free surface at room temperature for 20-24 hrs. The plate was placed on black paper for the duration of this incubation.

Each well in each row was examined for agglutination, against a black background. The agglutination pattern was judged by comparison with the following illustration.



Results classified as (+), (++) and (+++) were considered to be positive and

Bacteriological Specifications

Table I: Bacteriological standard of whole milk powder

Requirements	milk powder	Standard
APC	Not more than 50,000/g	USDA (U.S. Standard Grade) 2001 and Egyptian standard 2001
Salmonella	Absent	
B. cereus	10^5- 10^6	
Staph.aureus enterotoxins	Absent	

Table II: Bacteriological standard of infant milk powder

Requirements	Infant milk	Standard
APC	≤ 10.000	FDA 1996
Salmonella	Absent	
B. cereus	≤ 100/g	
Staph.aureus enterotoxins	Absent	

RESULTS

Aerobic Plate Count: The results presented in Table 1 summarized that the aerobic plate counts/g in case of whole milk powder Brand (I), (II) and infant milk formula ranged from 1×10^2 to 1.7×10^5, 1×10^2 to 6×10^3 and 1×10^2 to 1×10^4, respectively. With mean values of $1.5\times10^4 \pm 3.6\times10^3$, $1.5\times10^3 \pm 2.8\times10^2$ and $1.4\times10^3 \pm 2.5\times10^2$ respectively.

As shown in Table 2 the highest frequency distribution based on aerobic plate count of the examined whole milk powder Brand (I) samples was 53.8% and lied within the range of 10^3 - < 10^4 and for whole milk powder Brand (II) sample the highest frequency distribution was 54.3% and lied within the range of 10^2 - < 10^3, while for infant milk formula the highest frequency distribution was 52% and lied between (10^3 - < 10^4).

Salmonellae spp.

Salmonellae failed to be detected in all of the examined milk powder samples

Bacillus cereus: The results in Table 3 presented the presumptive count of *B. cereus* according to colony morphological characters on PEMBA agar plates by using surface plating technique and showed that 56.9% (37/65), 8.6% (3/35) and 20% (10/50) of the examined whole milk powder Brand (I), (II) and IMF samples were positive for *B. cereus*. *Bacillus cereus* counts ranged from 1×10^2 to 2×10^3 with an average of $4.6\times10^2 \pm 7.5\times10$ for WMP Brand (I) samples, 1×10^2 to 1×10^3, with an average of $4\times10^2 \pm 2.4\times10^2$ for WMP Brand (II) samples and 1×10^2 to 3×10^2, with an average of $1.5\times10^2 \pm 2.1\times10$ for infant milk formula samples. The highest frequency distribution for WMP (I), (II) and infant milk formula samples were 35.2, 66.7 and 90%, respectively and lied within the range of 100 - < 300 for all of the examined samples as displayed in Table 4.

The results in Table 5 pointed out that 44.6% (29/65), 5.7% (2/35) and 14% (7/50) of the examined whole milk powder Brand (I), (II) and IMF samples were confirmed as *B. cereus,* respectively after biochemical identification of the isolates with counts ranged from 1×10^2 to 2×10^3 with an average of $6.3\times10^2 \pm 1.4\times10^2$ for WMP (I) samples. Concerning to WMP (II) samples the counts ranged from 1×10^2 to 6.6×10^2, with an average of $3.8\times10^2 \pm 2\times10^2$, while for IMF, the counts ranged from 1×10^2 to 2×10^2, with an average of $1.4\times10^2 \pm 1.6\times10$ for. The highest frequency distribution for WMP (I) and infant milk formula samples were 41.4 and 100% respectively and lied within the range of 100 - < 300 as displayed in Table 6, while in case of WMP (II) one sample (50%) lied within the range of 100 - < 300 and the other sample (50%) lied within the range of 600 - < 900.

Table 1: Results of statistical analysis of aerobic plate counts in examined milk powder samples (n = 150)

Types of samples	No. of Samples	Positive samples		A.P.C (count/g)			
		No.	%	Min.	Max	Mean	S.E.M±
Whole milk powder (Brand I)	65	65	100	1×10^2	1.7×10^5	1.5×10^4	3.6×10^3
Whole milk powder (Brand II)	35	35	100	1×10^2	6×10^3	1.5×10^3	2.8×10^2
Infant milk formula	50	50	100	1×10^2	1×10^4	1.4×10^3	2.5×10^2
Total	150	150	100	1×10^2	1.7×10^5	7×10^3	1.6×10^3

Table 2: Frequency distribution of examined milk powder samples based on their aerobic plate counts

Intervals	Frequency					
	Whole milk powder (I)		Whole milk powder (II)		Infant milk formula	
	No. of samples	%	No. of samples	%	No. of samples	%
10^2 - < 10^3	12	18.5	19	54.3	23	46
10^3 - < 10^4	35	53.8	16	45.7	26	52
10^4 - < 10^6	18	27.7	0	0	1	2
Total	65	100	35	100	50	100

Table 3: Results of statistical analysis of presumptive *Bacillus cereus* counts in the examined milk powder samples on PEMBA agar media

Types of samples	No. of examined Samples	Positive samples		* presumptive count / g			
		No.	%	Min.	Max	Mean	S.E.M±
Whole milk powder (I)	65	37	56.9	1×10^2	2×10^3	4.6×10^2	7.5× 10
Whole milk powder (II)	35	3	8.6	1×10^2	1×10^3	4×10^2	2.4×10^2
Infant milk formula	50	10	20	1×10^2	3×10^2	1.5×10^2	2.1 × 10
Total	150	50	33.3	1×10^2	2×10^3	3.9×10^2	6 × 10

* According to morphological character on PEMBA agar

Table 4: Frequency distribution of the examined milk powder samples based on their presumptive Bacillus cereus counts on PEMBA agar

Intervals	Frequency					
	Whole milk powder (I)		Whole milk powder (II)		Infant milk formula	
	No. of samples	%	No. of samples	%	No. of samples	%
1×10^2 - < 3×10^2	13	35.2	2	66.7	9	90
3×10^2 - < 6×10^2	10	27	0	0	1	10
6×10^2 - < 9×10^2	4	10.8	0	0	0	0
9×10^2 - < 2×10^3	6	16.2	1	33.3	0	0
2×10^3	4	10.8	0	0	0	0
Total	37	100	3	100	10	100

Table 5: Real B. cereus counts after confirmation

Types of samples	No. of examined Samples	Positive samples		* presumptive count / g			
		No.	%	Min.	Max	Mean	S.E.M±
Whole milk powder (I)	65	29	44.6	1×10^2	2×10^3	6.3×10^2	1.4×10^2
Whole milk powder (II)	35	2	5.7	1×10^2	6.6×10^2	3.8×10^2	2×10^2
Infant milk formula	50	7	14	1×10^2	2×10^2	1.4×10^2	1.6 × 10
Total	150	38	20.6	1×10^2	2×10^3	5.2×10^2	1.1×10^2

* After biochemical confirmation

Table 6: Frequency distribution of examined milk powder samples based on confirmed Bacillus cereus counts

	Frequency					
	Whole milk powder (I)		Whole milk powder (II)		Infant milk formula	
Intervals	No. of samples	%	No. of samples	%	No. of samples	%
1×10^2 - < 3×10^2	12	41.4	1	50	7	100
3×10^2 - < 6×10^2	9	31.0	0	0	0	0
6×10^2 - < 9×10^2	1	3.4	1	50	0	0
9×10^2 - < 2×10^3	4	13.8	0	0	0	0
2×10^3	3	10.4	0	0	0	0
Total	29	100	2	100	7	100

***Staph. aureus* Enterotoxins:** Testing the samples for *Staph aureus* Enterotoxins, revealed that Enterotoxins could not be detected in all of the examined milk powder samples.

DISCUSSION

Large scale production and consumption of dry milk powder, makes it desirable to detect its hygienic quality. The results of the current aerobic plate count were in agreement with that reported by Ahmed and Anwar [15]. While, slightly higher values were obtained by El-Prince and Korashy [16]. Lower results were reported by Èanigova and Duckova [17]. According to the limits proposed by APHA [11], ES [18] and USDA [19] for dried milks that APC must not exceed 5×10^4 /g, most of the examined samples were within the acceptable limit except 6 samples out of the 65 of the examined samples of WMP Brand (I) don't comply the standards. For infant milk formula all the examined samples met the standard limit = 10^4 as recommended by FDA 20].The high aerobic count indicated neglected sanitary measures during manufacturing process, handling, packing and as well as use of low quality milk or ingredients in the production.

The Aerobic Plate Count (APC), or total viable count, is an indicator of the overall degree of microbial contamination of foods. It is defined as the total number of colony forming units (cfu) of bacteria per gram or ml. Since recent advances of the powdered milk industry have stimulated the interest of various professional groups to a degree not heretofore attained, it is not unnatural that some attention should be given to the bacterial content of this product, not only from the standpoint of numbers of living organisms present, but also from the standpoint of numbers originally contained in the milk from which it was manufactured. In fact, it has already been suggested that bacterial limits should be set for liquid milk which is to be converted into powder

The obtained result of salmonellae agreed with all the national standard Codex, European and Egyptian which reported that Salmonellae must be absent in 25g of dry milk products [2, 20, 21]. Several outbreaks of salmonellosis have been traced to dried milk products and with low-level contamination of powdered infant milk formula were epidemiologically and microbiologically detected by Jourdan *et al.* [22,23].

Proper pasteurization inactivates all known Salmonellae in fluid milk; thus its presence in dried milk indicates post-pasteurization contamination, due to lacking concerning, survival and growth characteristics in milk powders during manufacture, handling, filling process and storage. Salmonellae can grow in the reconstituted product if stored above 5 °C for a sufficient time and multiply very rapidly at room temperature. Outbreaks due to Salmonellae usually share a common factor, the accumulation of contaminated dust and powder deposits in the factory environment, which are eventually, transferred to the product by mechanical fault. The most common hazard reported is the accumulation of powder deposits in the drier insulation, which having become contaminated by environmental Salmonellae, gains access to the product via stress cracks in the inner skin of the dryer. The second most important hazard is due to contaminated air and may occur during the secondary drier stages, transport of powder to silos, or during filling and packing operations. Also failures in production, for example the presence of water in a zone that is normally dry, allowing multiplication of Salmonellae, or the presence of Salmonellae in zones that are difficult to maintain dry and clean (e.g. a drying tower), were identified as the origins of contamination [24].

Concerning to *Bacillus cereus* the obtained results of confirmed samples for whole milk powder agreed to those reported by Rangasamy *et al.* [25] and for infant milk formula, the result was similar to Cohen *et al.* [26].

Table 7: Incidence of presumptive and confirmed B. cereus in examined milk powder samples

		Presumptive *B. cereus*			Confirmed *B. cereus*			
					Samples		Isolates	
Types of	No. of Samples	No. of +ve samples	% of +ve samples	No. of isolates	No. of +ve samples	% of +ve samples Samples	No. of +ve isolates	%*
Whole milk powder (I)	65	37	56.9	68	29	44.6	48	70.6
Whole milk powder (II)	35	3	8.6	5	2	5.7	3	60
Infant milk formula	50	10	20	12	7	14	7	58.3

* form the total No. of isolates

Table 8: Isolated Bacillus spp. Other than *B. cereus* from examined milk powder samples

	Frequency					
	Whole milk powder (I)		Whole milk powder (II)		Infant milk formula	
Isolates	No. of samples	%*	No. of samples	%*	No. of samples	%*
B. mycoides	7	10.3	1	20	2	16.7
B. pumilus	1	1.4	-	-	-	-
B. subtilis	2	2.9	-	-	1	8.3
B. lichiniformis	5	7.4	-	-	-	-
Other bacillus spp.	5	7.4	1	20	2	16.7

* form the total No. of isolates

The presumptive count was recorded on basis of colonial morphology on PEMBA agar plate, but false negative result may occur or overgrown by acid producing micro-organisms make the identification of *B. cereus* colonies difficult and also false positive result may occurs so confirmation should always carried out [27]. According to HPA [28], when the total number of Bacillus colonies and the number of suspected *B. cereus* not exceed 10^4/g, they didn't need to confirm biochemically.

Generally very few organisms produce similar reactions on egg yolk-polymexin-containing media to those elicited by *B. cereus*. Specific identification of *B. creus* requires only differentiation from closely related bacillus spp. (Group IA), *B. mycoides*, *B. thuringiensis* and, *B. anthracis* [29] and other bacillus involved in food poisoning, *B. pumilus, B. subtilis, B. licheniformis* (intoxication dose more than 10^5) [27].

As explained in Tables 7 and 8, 68 isolates from examined WMP Brand (I) presumed to be *B. cereus*, but after biochemical identification only 48 isolates (70.6%) confirmed as *B. cereus* ,while 7 isolates (10.3%) confirmed as *B. mycoides*, 1 isolate (1.4%) *B. pumilus*, 2 isolates (2.9%) *B. subtilis*, 5 isolates (7.4%) *B. licheniformis and* 5 isolates (7.4%) confirmed as other bacillus species. 5 isolates were taken from WMP Brand (II) samples, 3 isolates (60%) confirmed as *B. cereus* ,while one isolate (20%) confirmed as *B. mycoides* and the other isolate (20%) confirmed as *B. subtilis*. In case of infant milk formula 12 isolates presumed to be *B. cereus*, but after biochemical identification only 7 isolates (58.3%) confirmed as *B. cereus* ,while 2 isolates (16.7%) confirmed as *B. mycoides*, 1 isolate (8.3%) as *B. subtilis and* 2 isolates (16.7%) confirmed as other bacillus species.

The infectious dose for *B. cereus* is not really decided and varied, but generally presence of *B.cereus* greater than 10^6 organisms /g in food is indicative of growth and proliferation of the organisms and considers a potential hazard to health. All the samples to be examined lied below the dangerous limit. For infant milk formula samples [30] revealed that concentrations of *B. cereus* of 10^3 to 10^5 /g can result in illness in infants or aged and infirm individuals, although [20] for microbiological standards of infant formula, recommended that *B. cereus* count should be = 100 / g. Contaminated infant milk formula samples faces this FDA limit except 3 samples in presumptive count and 2 samples in confirmed samples exceed 100 cell/g.

Vegetative cells of *B. cereus* are destroyed in the heat process used to produce dried milk powder, but spores can survive the processing. Generally low numbers of *B. cereus* and/or its spores do not cause problems unless powdered milks are reconstituted and stored for

prolonged periods at incorrect temperatures especially most *B. cereus* strains isolated from dairy products are able to grow and produce toxins below 10 °C. Growth and toxin production can be prevented by storing reconstituted products at temperatures below 4°C [31].

Presence of *B. cereus* in infant formula is associated with contamination of raw milk and other added ingredients then subsequent survival of the spores following heat treatment. The practice of preparing infant milk formula with warm tap water or boiling water is potentially unsafe. Formula prepared under these conditions with initial level of 100 cfu/g may reach the infectious dose when stored at 10°C for 24 hours or when stored at room temperature for greater than 4 hours [32].

Presence of *B. cereus* in dried milk products is considered as an indicative of carelessness during production. Dried milk products contaminated with *B. cereus*, even at low levels should be considered as potential vehicles for food-borne *B. cereus* disease. As these products contain an elevated level of carbohydrates (starch, sucrose or lactose) and minerals, they can promote proliferation and enterotoxin production when they are reconstituted and held at ambient temperature for extended periods, potentially even at refrigeration temperature [8].

S. aureus enterotoxins could not be detected in any of examined samples. Similar result was stated by Harvey and Gilmour [33]. Outbreaks due to consumption of milk powder containing preformed *S. aureus* enterotoxin were reported by Asao *et al.* [34].

Staphylococcal enterotoxin A (SEA) (= 0.38 ng/ml) was detected in low-fat milk and approx. 3.7 ng/g in powdered skim milk (34,35) detected that enterotoxin A produced by 6.5%, B by 1.6%, C by 2.4%, D by 1.6% and E by 0.8% of the isolated enterotoxigenic strains of *S. aureus* from infant milk formula.

Staphylococcal toxins in dried milk powder indicate unhygienic ingredients or unacceptable processing conditions. *S. aureus* is destroyed in the heat process. Outbreaks occur as a result of post-processing contamination or due to preformed toxins surviving the heat-processing step either in raw milk before heat treatment or in the concentrated milk before drying.

It was concluded that the final quality of the milk powder and infant formula depends on the quality of the raw milk and ingredients added to infant formula. Proper processing must be carried out to, ensure that the product is not contaminated after processing. Also, safe storage after reconstitution is recommended.

REFEERENCES

1. Robinson, R.K., 2002. Dairy Microbiology Handbook, Third Edition: Chapter 5: The Microbiology of Concentrated and Dried Milks.
2. Food standards Australia and New Zealand, 2006. A Risk Profile of Dairy Products in Australia. Attachment 2, Appendices 1-6. Draft Assessment Report, Proposal P296. Primary Production and Processing Standard for Dairy. 22 march 2006.
3. Otte, I., 1980. Mikrobiologie der trockenprodukte, Institut fur Hygiene Bundesanstalt fur Milchforscung, Keil, Germany. Sited in Dairy Microbiology Handbook, Third Edition: Chapter 5: The Microbiology of Concentrated and Dried Milks. Edited by Robinson RK (2002).
4. ICMSF, 1998. Microorganisms in Food, 6: Microbial Ecology of Food Commodities. Blackie Academic and Professional, London.
5. Bornemann, R., D.M. Zerr, J. Heath, J. Koehler, M. Grandjean, R. Pallipamu and J. Duchin, 2002. An outbreak of *Salmonella* serotype *Saintpaul* in a children's hospital. Infect Control Hosp. Epidemiol., 23: 671-676.
6. Forsyth, J.R., N.M. Bennett, S. Hogben, E.M. Hutchinson, G. Rouch, A. Tan and J. Taplin, 2003. The year of the *Salmonella* seekers, 1977. Aust. N.Z. J. Public Health, 27: 385-389.
7. Institute of Environmental Science and Research Limited, 1995. Chemical and microbiological composition of infant formula. A report for the Ministry of Health. Public Health Commission October 1995.
8. Jaquette, C.B. and L.R. Beuchat, 1998. Survival and growth of psychtrophic *B. cereus* in dry and reconstituted infant rice cereal. J. Food Prot., 61: 1629-1635.
9. Jablonski, L.M. and G.A. Bohach, 1997. Staphylococcus aureus, p. 353–375. In M P Doyle, L R Beuchat and T J Montville (ed.) Food microbiology: fundamentals and frontiers. ASM Press, Washington DC.
10. Food Safety Net, 2000. 2 September 2000, posting date. Snow Brand search uncovers suspicious milk bags. Food Safety Net archives. http://www.foodsafetynetwork.ca.
11. APHA (American public health association), 1992. Standard Methods for Examination of Dairy Products, 16th Ed, American Public Health Association, Washington, DC.

12. FDA (U.S. Food and Drug Administration), 2006. Center for Food Safety and Applied Nutrition. Bacteriological Analytical Manual Online, Chapter 5: *salmonella*, 8th ed. (Andrews and Hammack, 1998). Available at http://www.cfsan.fda.gov/ ~ebam/ bam-5.html.
13. Holbrook, R. and J.M. Anderson, 1980. An improved selective and diagnostic medium for the isolation and enumeration of *bacillus cereus* in food. Candian J. Microbiol., 26: 753-759.
14. FDA (Food and Drug Administration), 2001. Center for Food Safety and Applied Nutrition. Bacteriological Analytical Manual Online, 8th ed. Ch.14: *Bacillus cereus*, Rhodehamel and Harmon (1998) Available at http://www.cfsan.fda.gov/ ~ebam/ bam-14.html.
15. Ahmed, S. and M.N. Anwar, 2006. Microbial Counts of Dried Powder Milk Available in Local Markets of Bangladesh. Bangladesh J. Microbiol., 23(2): 162-164.
16. El-Prince, E. and E. Korashy, 2003. Microbiological quality of dried milk-based infant foods in Assiut City. Assiut. Vet. Med. J., 49: 190-203.
17. Èanigova, M. and V. Duckova, 2004. The contribution to microbiological quality of dried milk. J. Central European Agric., pp: 5.
18. Egyptian standards (ES), 2001. Dried milks, Egyptian Organization for Standardization and Quality Control.
19. USDA (United States Department of Agriculture) (US standard grade), 2001. United States Standards for Grades of Dry Whole Milk 2001.
20. FDA (Food and Drug Administration), 1996. Microbiological standards for infant formula proposed in 1996 ANPR.
21. Canadian Standards, 2003. Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Food, Interpretive Summary, Jan. 2003.
22. Jourdan, N., S. Le Hello, G. Delmas, J. Clouzeau, C. Manteau, B. Désaubliaux, V. Chagnon, F. Thierry-Bled, N. Demare, F.X. Weill and H. de Valk, 2008. Nationwide outbreak of *Salmonella* enterica serotype Give infections in infants in France, linked to infant milk formula, September 2008. Euro Surveillance 13(39). Available-online: http://www.eurosurveillance. org/ ViewArticle.aspx? ArticleId=18994.
23. Soler, P., S. Herrera, J. Rodríguez, J. Cascante, R. Cabral, A. Echeita-Sarriondia and S. Mateo, 2008. On Behalf Of The National Surveillance Network of Spain. Nationwide outbreak of *Salmonella* enterica serotype Kedougou infection in infants linked to infant formula milk, Spain, 2008. Euro Surveillance. 13(35). Available online: http://www. eurosurveillance. org/ View Article. aspx? ArticleId=18963.
24. European Food Safety Authority (EFSA), 2004. Microbiological risks in infant formulae and follow-on formulae. 113, 1-35, Opinion of the Scientific Panel on Biological Hazards on the request from the Commission related to the microbiological risks in infant formulae and follow-on formulae. Adopted on 9 September 2004.
25. Rangasamy, P.N., M. Lyer and H. Roginski, 1993. Isolation and characterisation of *Bacillus cereus* in milk and dairy products manufactured in Victoria. The Australian J. Dairy Technol., 48: 93-95.
26. Cohen, J., E. Marambio, B. Lynch and A.M. Moreno, 1984. *Bacillus cereus* infection in newborn infants. Rev. Chil. Pediatr, 55: 20-25.
27. Lund, B.M., T.C. Baird-Parker and G.W. Gould, 2001. The microbiological safety and quality of food. Springer, 2000.
28. Health protection agency (HPA), 2005. Enumeration of bacillus cereus and other bacillus species. Issue no: 1.4 Issued by Standards Unit, Evaluations and Standards Laboratory Specialist and Reference Microbiology Division. www.evaluations-standards. org.uk.
29. ICMSF, 1996. Microorganisms in Foods 5. Characteristics of Microbial Pathogens. International Commission. on Microbiological Specifications For Foods. Blackie Academic and Professional, London.
30. Becker, H., G. Schaller, W. von Wiese and G. Terplan, 1994. *Bacillus cereus* in infant foods and dried milk products. Int. J. Food Microbiol., 23: 1-15.
31. AIFST (Australian Institute of Food Science and Technology), 2003. Food borne Microorganisms of Public Health Significance. 6th Ed. AIFST (NSW Branch). Food Microbiology Group, South wood Press Pty. Ltd, Australia.
32. Food standards Australia and New Zealand, 2004. Bacillus Cereus Limits In Infant Formula. Final Assessment Report Application A454. 18 February 2004.

33. Harvey, J. and A. Gilmour, 1990. Isolation and identification of staphylococci from milk powders produced in Northern Ireland. J. Appl. Microbiol., pp: 433-438.
34. Asao, T., Y. Kumeda, T. Kawai, T. Shibata, H. Oda, K. Haruki, H. Nakazawa and S. Kozaki, 2003. An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: estimation of enterotoxin A in the incriminated milk and powdered skim milk. Epidemiol. Infection J., 130: 33-40.
35. Umoh, V.J., K.S. Obawede and J.U. Umoh, 1985. Contamination of infant powdered milk in use with enterotoxigenic Staphylococcus aureus. Food-Microbiology Volume 2, Issue 4, October 1985, pp: 255-261.