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Bacteriological Quality of Infant Milk Formulae Examined under a Variety of Preparation and Storage Conditions

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ABSTRACT

One hundred infant milk formulae (IMFs), representative of the 10 leading brands available in the UK, were subjected to a variety of preparation and storage conditions. Each IMF was the subject of triplicate trials in which duplicate samples were analyzed. All IMFs analyzed immediately after reconstitution were of satisfactory bacteriological quality, exhibiting a total aerobic count of $<10^4$ CFU g^{-1} (mean 2.3×10^2 CFU g^{-1}) and a *Bacillus cereus* count of $<10^3$ CFU g^{-1} of powder (mean 1.3×10^2 CFU g^{-1} for formulae containing this bacterium). Seventeen percent of all dried IMF examined contained *B. cereus;* subsequent reconstitution and storage over a 24-h period at ≥30°C resulted in this organism being detected in a further 46% (63 of 100), so that the majority of these foods exceeded the International Dietetics Association of the European Community (IDAEC) proposed reconstitution safety limit of 10^3 CFU g^{-1}. Variations in preparation conditions did not significantly influence the numbers of *Bacillus* CFU present ($P < 0.05$). The bacteriological quality of an IMF depended on the type and number of organisms initially present and on product temperature and duration of product storage. Microbial numbers in IMFs were influenced by storage temperatures of ≥20°C for 14 h, while incubation at ≤10°C for 24 h had no effect ($P < 0.05$). Although the microflora of dried IMFs predominantly consisted of *B. licheniformis* (46%) and *B. subtilis* (30%), subsequent reconstitution and incubation resulted in the shift to *B. cereus* I (31%) and II (38%) as dominant organisms. The latter often grew to the exclusion of the former two *Bacillus* spp. Diarrheagenic enterotoxin was detected in 4% of IMFs analyzed after 14 h of storage at ≥25°C.

Key words: Infant milk formulae, bacteriological quality, *Bacillus* spp., diarrheagenic enterotoxin, preparation and storage effects

Despite the strong supporting evidence for breast milk as the choice food for the nutritional and immunological development of infants, not all mothers are eager or medically capable of breast-feeding *(19).* For babies who do not receive breast milk, a powdered or ready-to-feed infant milk formula (IMF) substitute is required *(18).*

Reconstituted baby foods are however considered to be a food class of high risk due to the susceptibility of infants to enteric bacterial pathogens, their severe response to toxins, and increased mortality *(12).* Despite the elevated temperatures employed in the manufacture of IMF, there have been a number of food-related illnesses where infant milk powder has been implicated as the vehicle of infection *(7, 16).* The numbers of *Salmonella* cells in IMFs implicated in previous food-borne infections were very low; e.g., in the 1985 UK outbreak only three *S. ealing* cells kg^{-1} were present *(20).* The vulnerability of infants to low numbers of pathogenic organisms may be due to the host's underdeveloped immune system *(2).*

Generally, dried milk-based infant foods are known to be contaminated with aerobic sporeformers of the genus *Bacillus* via raw milk that frequently contains these bacteria in low numbers. Of particular concern is the occurrence of enterotoxigenic *B. cereus* in these products *(3).* These authors reported that 54% of 261 samples of infant food distributed in 17 countries were contaminated with *B. cereus,* reaching levels of 0.3 to 600 viable cells per g. When samples contaminated with approximately 100 cells per ml were reconstituted and incubated at 27°C, levels of 10^5 organisms per ml were reached in 7 to 9 h. While the infectious dose of *B. cereus* is in the range of 10^5 to 10^7 cells, diarrheal enterotoxin is produced before cells reach the level of 10^7 cells per ml *(11).* Outbreaks associated with infants, aged, and/or infirm persons have been attributed previously to the consumption of foods containing low numbers of *B. cereus* in the range of 10^3 to 10^5 cells per g *(8).* Granum et al. *(10)* suggested that the food industry should be concerned about levels as low as 10^3 to 10^4 cells per ml or g of food, as it is likely that food intoxication is caused by ingestion of *B. cereus* cells or spores rather than of preformed enterotoxin. Aas et al. *(1)* revealed that ingestion of cells and/or spores ($>10^4$ ml^{-1}) was the main source of *B. cereus* food poisoning in Norway.

In the past, it has been the practice of many clinical laboratories to simply discard isolates of *Bacillus* spp. (often described as "inconsequential aerobic spore-forming bacteria") other than *B. anthracis* or *B. cereus,* as contaminants of the skin, hair, etc., which were in fact of unappreciated relevance to the infections from which they were isolated *(15).* However, several reports have recently implicated other members of the genus *Bacillus* (i.e., *B. subtilis, B. licheniformis, B. pumilus, B. brevis, B. thuringiensis,* and *B.*

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sphaericus) as etiological agents in proven food-borne illness outbreaks *(9, 13)*.

The principle objectives of this research were to determine the type and number of *Bacillus* spp. present in infant milk formulae available in the UK, to examine the effects of various methods of preparation, cooling, and storage on the microflora of these products, and to examine these infant formulations for the presence of diarrheal enterotoxin.

MATERIALS AND METHODS

Preparation of samples

Each month for 12 months, 8 to 10 infant milk formulae (IMF) products representative of the main brands available in the UK were purchased and analyzed. Care was taken to ensure that contamination of the infant powder did not occur by wearing vinyl gloves and swabbing the outer package with 70% alcohol. The package integrity of each sample container was checked prior to analysis. Infant powder (25 g) was aseptically added into duplicate 500-ml Duran bottles containing 225 ml of sterile distilled water and 6 to 8 glass beads to aid mixing. The IMF was reconstituted at a water temperature of either 56°C and/or 90°C (±0.2°C) by shaking 25 times through an excursion of 30 cm. These temperatures were achieved by equilibrating the Duran bottles containing the sterile water in preheated waterbaths (Techne Tempette Junior TE-8J) prior to reconstitution.

Cooling and storage of the reconstituted infant milk formulae

The cooling procedures used were table-top cooling (where the foods were left to cool at room temperature), water cooling (where the bottles of foods were held under cold running water), and immediate refrigeration (where the foods were placed directly into a refrigerator). Following a 30-min cooling period, the reconstituted formulae were incubated at either 4, 10, 20, 25, 30, and/or 35°C for periods up to and including 24 h, in order to simulate the "temperature abuse" which may be encountered in hospital wards *(2; 4)* and in the home.

Bacteriological analysis

The bacteria present were enumerated and identified at 0, 8, 14, and 24 h sample time intervals by spread and spiral plating (Spiral plater model B, Spiral Systems Inc.) duplicate samples onto tryptone soya agar supplemented with 0.6% yeast extract (TSYEA), nutrient agar no 2 supplemented with 0.5 mg liter^{-1} $MnSO_4 \cdot H_2O$ (NAMS), blood agar no. 2 supplemented with 7% defribrinated horse blood (BA) and *Bacillus cereus* selective agar (BCSA) (Oxoid products). Undiluted samples were also analyzed in TSEYA using the pour plate technique. The plates were incubated aerobically at 25 and 30°C for 48 or 72 h. This procedure was repeated in duplicate for 3 separate samples analyzed from each infant formulation.

Cultures obtained on the above media were examined for the following morphological and/or biochemical properties: Gram and catalase reactions, cell width and length determined via an image analyzer (Solitaire 512, Seescan Plc.) (TSYEA), lecithovitellin and lecthinase production (BCSA), hemolytic reaction and gross colony morphology (BA), and spore stain to determine shape, position and swelling of sporangium (NAMS). Other morphological and physiological tests performed included examination for motility, hydrolysis of starch, casein and/or gelatin, growth in the presence of 7.5% NaCl or 0.001% lysozyme, formation of acetoin from glucose, and growth under anaerobic conditions. The identity of each *Bacillus* isolate was confirmed using the API 50 CHB and API 20 E galleries (bioMérieux Ltd.).

Detection of diarrheagenic enterotoxin

The bacterial isolate was inoculated into brain heart infusion broth supplemented with 0.25% filter-sterilized glucose and incubated at 30°C for 18 h on a rotary shaker (250 rpm). After growth, duplicate 1-ml samples were centrifuged (Microcentaur MSE) at 11,500 × *g* for 10 min at 4°C. The filtrate was retained for subsequent assay of enterotoxin via the *Bacillus cereus* enterotoxin reverse passive latex agglutination test system (BCET-RPLA, Oxoid). The infant foods were assessed for the presence of enterotoxin by using the BCET-RPLA system after initially obtaining a fat-free fraction via the Filtron® Stirred Cell Ultrafiltration System fitted with a membrane having a 300-kDa molecular weight cutoff point (Filtron® Technology Corporation).

Statistical analysis

The Fisher's exact test was used to compare the bacteriological quality of the 10 leading brands of infant powder. The effects of IMF preparation temperature, cooling method, and storage temperature on microbial numbers (where total aerobic counts for 100 IMFs were pooled and compared as a unit under these conditions) were examined using three-way ANOVA analysis (Minitab version 11, Minitab Ltd). All significant differences were reported at the 95% level of confidence ($P < 0.05$).

RESULTS

Bacteriological quality of reconstituted infant milk formulations before incubation

All 100 IMFs examined immediately after reconstitution were of satisfactory bacteriological quality, having total aerobic counts less than the International Dietetics Association of the European Community (IDAEC) proposed safety limit of 10^4 CFU g^{-1} (Table 1) and a *B. cereus* count less than 10^3 CFU g^{-1} of power (Table 2) *(3)*. In the subsequent text these recommended values are referred to as the "reconstitution safety limit." The IMFs examined, which were representative of the 10 leading brands currently available in the UK, were of similar bacteriological quality ($P < 0.05$).

The temperature of the water used for formula reconstitution (Tables 1, 2 and 3) and/or the cooling method (Table 3) did not affect the type or number of organisms in IMFs examined collectively under brand type (Tables 1 and 2) or when examined as a unit of 100 infant milk products (Table 3) ($P < 0.05$). Large variations in microbial numbers shown in tables 1 and 3 were due to the wide range of total aerobic counts obtained for IMFs examined under brand type or as a unit of IMF (data not shown); e.g., microbial numbers in brand A products ranged from the detection limit of $\geq 1.0 \times 10^1$ to 6.1×10^3 CFU g^{-1} (Table 1). The temperature of the water used for IMF preparation and/or the cooling method did not result in products of different bacteriological quality from that of the dry samples ($P < 0.05$) when analyzed individually (data not shown).

The largest concentration of organisms present in any IMF product was 6.1×10^3 CFU g^{-1} (consisting solely of *B. licheniformis*), while the mean total aerobic count for all

TABLE 1. *Variation in total aerobic counts exhibited by 100 IMFs examined immediately after reconstitution at a water temperature of either 56 or 90°C*

IMF brand	No. samples (*n*)	No. IMF with total aerobic count (log CFU g^{-1}) after reconstitution in the range: <1.0[a] 56°C	<1.0[a] 90°C	≤2.0 56°C	≤2.0 90°C	≤3.0 56°C	≤3.0 90°C	≤4.0 56°C	≤4.0 90°C	Total aerobic count (log CFU g^{-1})[b] Mean 56°C	Mean 90°C	SD 56°C	SD 90°C
A	13	3	3	5	3	4	6	1	1	2.8	2.9	2.4	2.4
B	12	2	2	4	1	5	8	1	1	2.9	2.9	2.7	2.8
C	12	0	0	3	6	8	5	1	1	2.5	2.4	2.7	2.7
D	11	1	1	4	4	3	5	3	1	2.8	2.6	2.5	2.3
E	14	1	1	6	8	7	5	0	0	2.3	2.2	1.9	1.9
F	11	0	0	6	7	5	4	0	0	2.0	2.0	1.9	1.9
G	11	2	2	6	7	3	2	0	0	2.1	2.2	1.6	1.7
H	11	0	0	2	3	6	7	3	1	2.7	2.3	2.8	2.4
I	3	0	0	2	1	1	2	0	0	1.9	1.9	1.8	1.8
J	2	0	0	1	0	1	2	0	0	1.7	1.8	1.9	1.9
All	100	9	9	39	40	43	46	9	5	2.4	2.3	2.2	2.2

[a] IMF with total aerobic counts below the detection limit of 1 log CFU g^{-1}.

[b] Mean and standard deviation refer to the variation in total aerobic counts (log CFU g^{-1}) among IMF in each brand prepared at either 56 or 90°C. IMF with counts lower than the detection limit were not included in the calculation of the mean and standard deviation. No significant difference in microbial numbers was observed between brands ($P < 0.05$).

infant foods analyzed was 2.3×10^2 CFU g^{-1} (Table 1). Although *B. cereus* was present in 17% of the IMFs examined, of which 6 foods (35.3%) contained the enterotoxigenic form of this organism, diarrheal enterotoxin was not detected in these infant powders. The largest number of *B. cereus* recovered from any formulation was 4.8×10^2 CFU g^{-1}, while the mean *B. cereus* count for IMFs shown to contain this organism was 1.3×10^2 CFU g^{-1} (Table 2).

The microbial flora of the IMF consisted mainly of aerobic sporeformers of the genus *Bacillus,* with the most prominent species isolated belonged to members of the subgroup *B. subtilis,* such as *B. subtilis* (30%), *B. licheniformis* (46%), and *B. pumilus* (9%) (Table 4).

Bacteriological quality of reconstituted infant formulae after periods of storage abuse

As no differences were observed in the bacteriological qualities of individually examined IMFs ($P < 0.05$) or among these products when they were compared under brand type (Tables 1 and 2), microbial numbers in each reconstituted IMF were pooled and examined collectively as a unit of 100 foods under various preparation and storage

TABLE 2. *Variation in* Bacillus cereus *counts among IMFs examined immediately after reconstitution at a water temperature of 56 or 90°C*

IMF brand	No. samples (*n*)	No. IMF with *B. cereus* recovered in the range: (log CFU g^{-1}) <1.0[a] 56°C	<1.0[a] 90°C	≤2.0 56°C	≤2.0 90°C	≥2.01 to <3.0 56°C	≥2.01 to <3.0 90°C	*Bacillus cereus* (log CFU g^{-1})[b] Mean 56°C	Mean 90°C	SD 56°C	SD 90°C
A	13	9	9	4	4	0	0	1.9	1.9	0.07	0.08
B	12	12	12	0	0	0	0	LDL[c]	LDL	LDL	LDL
C	12	7	7	4	4	1	1	1.9	2.0	0.39	0.27
D	11	10	10	1	1	0	0	1.9	1.9	0	0
E	14	13	13	0	0	1	1	2.4	2.4	0	0
F	11	10	10	1	1	0	0	2.2	2.1	0	0
G	11	11	11	0	0	0	0	LDL	LDL	LDL	LDL
H	11	8	8	3	3	0	0	1.9	1.9	0.08	0.03
I	3	2	2	0	0	1	1	2.6	2.6	0	0
J	2	1	1	1	1	0	0	2.0	2.0	0	0
All	100	83	83	14	14	3	3	2.1	2.1	0.07	0.05

[a] IMF with *B. cereus* counts below detection limit of 1 log CFU g^{-1}.

[b] Mean and standard deviation refer to the variation in *B. cereus* counts (log CFU g^{-1}) among IMF in each brand prepared at either 56 or 90°C. IMF with counts less than detection limit were not included in calculations of mean and standard deviation.

[c] LDL: IMF with *B. cereus* counts less than the detection limit. No significant difference in microbial numbers was observed between brands ($P < 0.05$).

TABLE 3. *Total aerobic counts for 100 IMFs examined under a variety of preparation, cooling, and storage conditions*

Storage temperature (°C)	Preparation temperature (°C)	Mean (SD) total aerobic counts: log CFU g^{-1} for 100 reconstituted IMF stored 24 h											
		Tap cooled				Table top cooled				Refrigerated			
		0 h	8 h	14 h	24 h	0 h	8 h	14 h	24 h	0 h	8 h	14 h	24 h
35	56	2.4 (2.1)	3.4AB[a] (2.4)	4.2AB (2.5)	8.0AB (3.1)	2.4 (2.3)	3.5AB (2.5)	4.4AB (2.6)	8.3AB (2.9)	2.4 (2.1)	3.4AB (2.4)	4.5AB (2.5)	8.2AB (3.1)
	90	2.4 (2.2)	3.4AB (2.5)	4.3AB (2.5)	8.1AB (3.1)	2.4 (2.2)	3.4AB (2.4)	4.3AB (2.4)	8.0AB (3.0)	2.3 (2.2)	3.4AB (2.5)	4.4AB (2.6)	8.0AB (2.8)
30	56	2.5 (2.2)	3.1AB (2.3)	3.7AB (2.5)	6.9AB (2.8)	2.4 (2.1)	3.2AB (2.4)	3.7AB (2.5)	6.9AB (3.0)	2.4 (2.3)	2.9AB (2.4)	3.7AB (2.7)	7.2AB (2.9)
	90	2.4 (2.2)	3.2AB (2.4)	3.8AB (2.4)	7.0AB (2.9)	2.3 (2.2)	2.9AB (2.5)	3.7AB (2.5)	7.0AB (2.8)	2.4 (2.1)	2.9AB (2.5)	3.9AB (2.7)	7.2AB (3.0)
25	56	2.4 (2.3)	2.8AB (2.3)	3.1AB (2.4)	6.3AB (2.7)	2.4 (2.0)	2.7AB (2.4)	3.1AB (2.4)	5.8AB (2.7)	2.4 (2.2)	2.8AB (2.5)	2.9AB (2.5)	5.9AB (2.8)
	90	2.3 (2.2)	2.8AB (2.4)	3.2AB (2.5)	6.1 (2.7)	2.3 (2.2)	2.9AB (2.5)	3.3AB (2.5)	5.8AB (2.4)	2.4 (2.0)	2.8AB (2.3)	3.0AB (2.6)	5.7AB (2.8)
20	56	2.4 (2.1)	2.6 (2.2)	2.8AB (2.4)	3.2AB (2.5)	2.5 (2.2)	2.6 (2.4)	2.8AB (2.5)	3.1AB (2.6)	2.3 (2.2)	2.5 (2.3)	2.8AB (2.5)	3.2AB (2.7)
	90	2.4 (2.2)	2.6 (2.4)	2.9AB (2.4)	3.3AB (2.6)	2.3 (2.0)	2.5 (2.2)	2.9AB (2.3)	3.0AB (2.5)	2.4 (2.1)	2.5 (2.3)	2.8AB (2.5)	3.3AB (2.6)
≤10	56	2.3 (2.3)	2.4 (2.0)	2.4 (2.2)	2.4 (2.1)	2.3 (2.2)	2.4 (2.2)	2.3 (2.2)	2.4 (2.1)	2.4 (2.2)	2.4 (2.3)	2.4 (2.2)	2.4 (2.2)
	90	2.4 (2.1)	2.4 (2.1)	2.3 (2.2)	2.4 (2.3)	2.4 (2.2)	2.5 (2.2)	2.4 (2.1)	2.3 (2.3)	2.3 (2.3)	2.4 (2.2)	2.3 (2.1)	2.4 (2.2)

[a] A: IMF differing at $P < 0.05$ level compared to these 100 infant foods treated under the same preparation, cooling conditions for shorter storage times and B: for shorter time periods at lower storage temperatures.

TABLE 4. *Number of IMF from which different* Bacillus *spp. were isolated over a 24 hour incubation period at temperatures ≥20°C*

Bacillus spp. isolated	No. IMF in which species was isolated after incubation period of:			
	0 h	8 h	14 h	24 h
B. cereus I	10	30 (3)[b]	31 (3)	31 (3)
B. cereus II	7	34 (5)	37 (6)	38 (6)
B. licheniformis	46	46	47	31
B. subtilis	30	36	36	32
B. pumilus	9	13	13	11
B. megaterium	3	12 (2)	13 (3)	13 (3)
B. sphaericus	8	20	21 (5)	21 (6)
B. amyloliquefaciens	1	2	2	2
B. lentus	ND[a]	3	3	3
B. coagulans	6	8	8	8
B. circulans II	4	6	6	6
B. brevis	1	3	3	3
B. laterosporus	ND	ND	1	1
B. polymyxa	3	4	4	4
B. firmus	4	4	4	4
B. mycoides	5	12 (2)	13 (2)	13 (2)
Non-*Bacillus* spp. (cocci)	3	6	6	6
Bacillus spp. not detected	9	2	1	0

[a] ND: the named *Bacillus* spp. was not detected at this sample period.

[b] Numbers in parentheses: number of IMF prepared at 90°C supporting growth of *Bacillus* spp. not isolated from the same feeds prepared at 56°C.

conditions. The type and/or number of organisms in IMF examined under conditions of storage abuse were not altered by either the temperature of water used for formulae preparation and/or subsequent cooling conditions (Table 3). Some individually examined IMFs differed however, in microbial numbers when reconstituted at 90°C (±0.2°C) and incubated at ≥20°C for ≥14 h ($P < 0.05$): 11 and 7 formulations exhibited either lower or higher total aerobic counts compared to the same products prepared at 56°C respectively (data not shown). Incubation of IMFs at temperatures ≥20°C for ≥14 h resulted in an increase in the number of organisms present in each formulation (Table 3).

Improper storage of reconstituted IMF at ≥20°C for 24 h (or ≥25°C for ≥14 h) resulted in the microbiological quality of a number of formulations exceeding potentially hazardous levels (Fig. 1 and 2), with all 63 foods containing *B. cereus* (6 foods contained both *B. cereus* I and II) being above the reconstitution safety limit of 10^3 cells per g after 24 h at 30°C (Fig. 2). Products held at these higher storage temperatures were found to contain greater microbial numbers sooner; e.g., 20% of foods exceeded the satisfactory reconstitution limit of 10^3 *B. cereus* cells per g after only 8 h at 35°C (Fig. 2). While incubation of IMFs at ≤10°C for 24 h did not alter the bacteriological quality of these formulations ($P < 0.05$), an increase in the number and/or type of organisms present correlated with longer exposures at higher temperatures (Table 3). The bacteriological quality of each IMF depended on the number of organisms initially

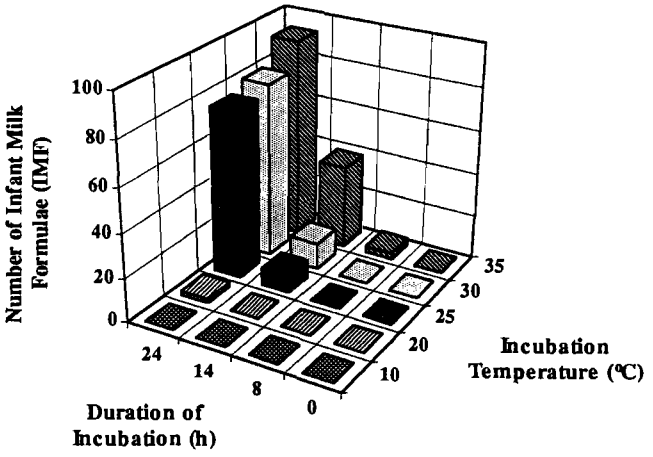

FIGURE 1. *The effects of temperature and duration of incubation on the number of infant milk formulae having a total aerobic count which exceeds the reconstitution safety limit of 10^4 CFU g^{-1}.*

present and on the product temperature and duration of product storage (Table 3).

While incubation of formulations at 20°C for 8 h did not affect microbial numbers ($P < 0.05$) (Table 3), storage at ≥20°C for ≥8 h resulted in an increase in the number of foods containing different types of *Bacillus* spp.; e.g., of *B. lentus* and *B. laterosporus,* which had not been recovered from earlier samples, emerged (Table 4). Some IMFs prepared at 90°C contained organisms that were not isolated from the same IMF products prepared at 56°C, such as *B. cereus, B. mycoides, B. sphaericus,* and *B. megaterium* (Table 4). *Bacillus licheniformis* and *B. subtilis* were initially predominant in IMF examined immediately after reconstitution. Additional storage of IMF resulted in the emergence of *B. cereus* I and II as dominant organisms, often growing to the exclusion of the former *Bacillus* spp. (Table 4).

Of the 38 IMFs supporting the growth of *B. cereus* II, diarrheal enterotoxin was detected in 4 IMFs after 14 h at ≥25°C. Additional storage at higher temperatures for longer

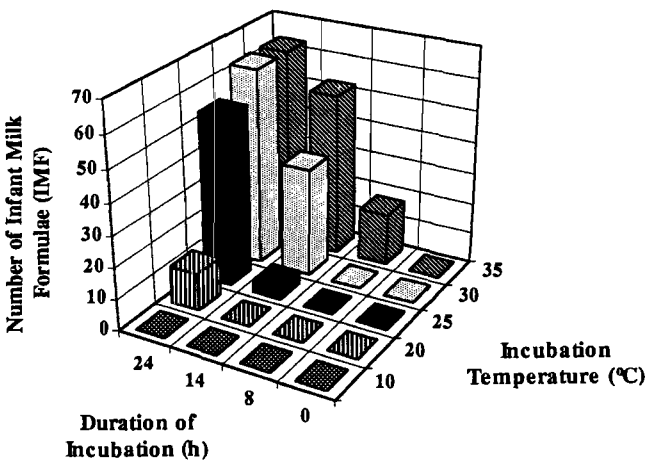

FIGURE 2. *The effects of temperature and duration of incubation on the number of infant milk formulae containing* B. cereus *at a level exceeding the reconstitution safety limit of 10^3 CFU g^{-1}.*

periods did not alter the number of products which contained toxin. However, subsequent enterotoxin studies involving the cultivation of all *B. cereus* II isolates in BHI broth supplemented with 0.25% glucose at 25°C for 14 h or more resulted in a further six isolates exhibiting diarrheal toxin production. Infant formulations produced by the 10 leading brands did not significantly differ in bacteriological quality when examined under varying conditions of preparation and storage ($P < 0.05$).

DISCUSSION

The bacteriological quality of infant milk formulae currently available in the UK is superior to that of infant formulations sold in Scotland in 1987, where a preliminary survey by the Scottish Food Co-ordinating Committee reported that the numbers of organisms present in these dried products ranged from 0 to 4.5×10^6 CFU ml^{-1} *(21).* In a later study carried out by Anderton *(2),* the author reported that milk-based powders used in the preparation of nasogastric feeds in Scottish hospitals contained 50 to 300 *Bacillus* spp. cells per g. These findings are consistent with the quality of dried infant formulae examined in other countries, where the mean total aerobic counts obtained from 26 Italian and 78 Japanese infant formulae did not exceed the recommended 10^4 CFU g^{-1} *(5, 23).*

Becker et al. *(3)* reported that of 261 samples of infant food distributed in 17 countries, 54% were contaminated with levels of *B. cereus* in the range of 0.3 to 600 CFU g^{-1}. While only 17% of UK dried IMF products contained *B. cereus,* a similar concentration of the organism was recovered, i.e., ≥10 to 480 CFU g^{-1}.

The microbial flora of the infant formulations are consistent with the type of organisms isolated by previous researchers; Lovell *(17)* and Kwee et al. *(14)* established that the bacterial flora of powdered milks consisted primarily of aerobic sporeformers, thermoduric cocci, and/or members of the genus *Corynebacterium.* Veda et al. *(23)* also showed that the most frequently isolated organisms from dried baby formulae in Japan were *B. licheniformis* and *B. subtilis,* while other *Bacillus* spp. recovered included *B. cereus, B. pumilus, B. megaterium, B. circulans,* and *B. coagulans.*

By far the greatest factors influencing the bacteriological quality of each infant feed were the number of organisms initially present and the temperature and duration of incubation. Formulae containing approximately 10^2 *B. cereus* spores per g may become unfit for consumption when subjected to storage at or above 25°C for 14 h, reaching levels of 1.3×10^3 CFU g^{-1}. Becker et al. *(3)* revealed that reconstituted infant formulae containing the same initial concentration of viable cells may reach levels as high as 10^5 *B. cereus* cells per g in 7 to 9 h when incubated at 27°C.

While the variation in IMF preparation and cooling method did not influence the number of *Bacillus* spp. present, incubation of IMF which had been initially reconstituted at 90°C often resulted in the emergence of aerobic sporeformers that were not recovered in the same samples prepared at the lower water temperature of 56°C. It is

possible that these *Bacillus* spp. emerged from slow-germinating endospores that require higher preparation temperatures in order to bring about germination. Stadhouders et al. *(22)* showed that heating milk at temperatures from 65 to 95°C for various holding times heat activated slow-germinating endospores of *B. cereus*, which for the main part did not germinate within 24 h in HTST (high-temperature short-time treated) milk stored under similar conditions.

Incubation at ≥25°C of reconstituted IMF which initially contained members of the *B. subtilis* subgroup often resulted in emergence of *B. cereus* as the dominant organism, which frequently grew to the exclusion of the former *Bacillus* spp. Wong et al. *(24)* reported that when *B. cereus* organisms started to multiply in milk products, the growth of other bacteria was inhibited. They attributed this inhibitory effect to the bacteriostatic activity of the organic acids produced by *B. cereus*.

Diarrheal enterotoxin was detected in 4 infant formulations after a 14 h incubation period at or above 25°C. All these foods had been supplemented with maltodextrin by the food companies. While maltodextrin is a harmless by-product of starch hydrolysis, Garcia-Arribas and Kramer *(6)* detected that besides glucose, starch is a good carbon source for both *B. cereus* growth and subsequent diarrheal toxin production. By supplementing IMF with maltodextrin in order to enhance the nutritional value of this product, the food industry may have inadvertently provided a suitable environment where improperly stored IMF containing enterotoxigenic *B. cereus* may produce toxin.

Of the aerobic sporeformers isolated from the dried IMF samples during this study, *B. subtilis, B. licheniformis, B. pumilus, B. cereus* I and II, *B. mycoides, B. brevis, B. megaterium, B. circulans* II, and *B. coagulans* have been occasionally implicated in either food-borne-related illness and/or opportunist infections *(13, 15)*.

In conclusion, dried infant milk formulae commercially available to Scottish retailers is of satisfactory microbiological quality and should not present any health problems to consumers if properly reconstituted (at water temperatures ≥56°C) under hygienic conditions. As the bacterial flora of inadequately stored IMF may proliferate to unacceptable levels, with possible production of diarrheal enterotoxin, these foods should be consumed within 4 h of preparation and not retained as leftovers for future use, storage of feeds during this period should occur in a properly maintained refrigerator (i.e., at ≤8°C), leftover feeds should never be re-used or topped up with fresh formulae, and feeding bottles (and teat) should be thoroughly cleaned and sterilized before re-use.

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