MICROBIOLOGICAL ASSESSMENT AND EVALUATION OF REHYDRATION INSTRUCTIONS ON POWDERED INFANT FORMULAS, FOLLOW-UP FORMULAS AND INFANT FOODS IN MALAYSIA

N. Abdullah Sani,[*1] S.H.P. Hartantyo,[*] and S.J. Forsythe[†]

[*]Food Science Programme, School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.

[†]School of Science and Technology, Nottingham Trent University, Clifton Lane, Nottingham, NG11 8NS, UK

ABSTRACT

A total of 90 samples comprised of powdered infant formulas (51), follow-up formulas (21) and infant foods (18) from 15 domestic and imported brands were purchased from various retailers in Klang Valley, Malaysia and evaluated in terms of microbiological quality and the similarity of rehydration instructions on the product label to guidelines set by the World Health Organization. Microbiological analysis included the determination of aerobic plate count (APC) and the presence of Enterobacteriaceae and *Cronobacter* spp. Isolates of interest were identified using ID 32E (bioMérieux®). In this study 87% of powdered infant formulas, follow-up formulas and infant foods analyzed had aerobic plate counts below the permitted level of $< 10^4$ cfu/g. These acceptable APCs ranged between $< 10^2$ to 7.2×10^3 cfu/g. The most frequently isolated Enterobacteriaceae was *Enterobacter cloacae* which was present in three infant formulas and one infant food tested. Other Enterobacteriaceae detected from powdered infant and follow-up formulas were *Citrobacter* spp., *Klebsiella* spp. and other *Enterobacter* spp. No *Cronobacter* species were found in any samples. Rehydration instructions from the product labels were collated and it was observed that none directed the use of water with a temperature >70°C for formula preparation as specified by the 2008 revised World Health Organization guidelines. Six brands instructed the use of water at 40-55°C, a temperature range which would support the survival and even growth of Enterobacteriaceae.

Keywords: Powdered infant formula, follow-up formula, infant foods, rehydration instructions

INTRODUCTION

In terms of food safety, infants and children are considered to be a part of the high-risk group of individuals as their immune systems may not yet be fully developed. Infants and young children are especially vulnerable to diarrheal illnesses when introduced to fluids and foods as they are weaned from breastfeeding to a mixed diet (Marino, 2007). In food, pathogens can grow at room temperature. Further, elevated temperatures that are typical in tropical countries can hasten pathogen multiplication (Tirado et al., 2010).

One of the pathogens of concern is the opportunistic *Cronobacter* (formerly *Enterobacter sakazakii*), which has gained attention in the past decade by its association with infant infections through contaminated infant formula (Joseph and Forsythe, 2011; Kucerova et al., 2011). These organisms have been observed to persist in dry environments such as powdered foods and grow rapidly in reconstitution (Iversen and Fanning, 2009). An early survey on the presence of

[1] Corresponding author: norra@ukm.my

Enterobacteriaceae in powdered infant formula (PIF) by Muytjens et al. (1988) reported that 52.2% of the samples contained the organisms. The following year, a case of infant formula milk believed to be contaminated with Enterobacteriaceae (*Cronobacter*) during the manufacturing process (Simmons et al., 1989) and three cases of neonatal meningitis caused by *Cronobacter* found in dried infant formula in Iceland (Biering et al., 1989) were reported. Isolation of *Cronobacter* in 16.6% of PIF samples was reported in 2004 (Iversen and Forsythe, 2004).

In 2008 the Food and Agriculture Organization/ World Health Organization (FAO/WHO) issued a call for data on *Cronobacter* occurrence in PIF (intended target age < 6 months) and follow-up formula (intended target age > 6 months). In response, an international survey involving eight laboratories in seven different countries (including Malaysia) was coordinated in order to determine the presence of *Cronobacter sakazakii* and other *Cronobacter* spp. in follow-up formulas and other infant foods. Initial investigations in this study were done in line with the FAO/WHO request and were subsequently published (Chap et al., 2009). However, given the lack of published information in Malaysia with regards to the presence of *Cronobacter*, the survey was extended to a wider range of PIF, follow-up formula (FOF) and infant or weaning foods (IF) available in the country.

Aside from the intrinsic presence of pathogens, improper handling of infant-related food, such as inadequate cleaning of bottles, multiple reheating or inappropriate rehydration procedures may also favor the proliferation of harmful bacteria. For this reason, the WHO in 2007 released both printed and online materials to guide the general public about safe milk handling (WHO, 2007a; WHO, 2007b). It is uncertain how widely these guidelines have been distributed and adopted for product instructions for the preparation of infant feed. It is worthwhile therefore to check whether the instructions provided on different products are in line with these WHO recommendations.

It is worth noting that the basic principles of food poisoning and food hygiene in developed and developing countries are the same. However, food safety in developing countries such as Malaysia is more challenging due to the tropical climate. Further, though the basic factors preceding foodborne illness in the tropics are the same as in other places, conditions such as high ambient temperature and humidity, general lack of refrigeration, local habits, impure water, poor sanitary facilities and profusion of intestinal pathogens and parasites can enhance the dangers (Adams, 2007). This study aims to determine the microbiological quality of PIF and related products commercially available in Malaysia in terms of their aerobic plate count and the presence of Enterobacteriaceae especially *Cronobacter*. This study will provide microbiological surveillance data that may be used to evaluate the suitability of internationally-prescribed infant formula handling and management standards to conditions in tropical developing countries.

MATERIALS AND METHODS

Milk Samples

A total of 90 samples were analysed. They were comprised of PIF (51), FOF (21) and IF (18) from 15 domestic and imported brands purchased from various retailers in Klang Valley, Malaysia. By definition, PIF is a formula intended for use by infants from 0-6 months; FOF is a formula for use by infants from 6 months onward, and infant food can be any food other than breast milk or infant formula that is made specifically for infants. Whenever available, five

samples from identical production batches were obtained. Only one sample was analyzed from some PIF brands as they were provided by local distributors. Product ingredients, reconstitution instructions and products containing special components such as probiotic cultures were recorded.

Microbiological Analysis

Microbiological analysis conducted in this study included the determination of aerobic plate count (APC), the presence of Enterobacteriaceae and *Cronobacter* spp. Following the surface spread plate method (Roberts and Greenwood, 2003) the APC in milk and infant food samples were determined. Twenty five grams of each sample was added into 225ml portions of Maximun Recovery Diluent (MRD, Oxoid Thermofisher, UK) and allowed to rehydrate at room temperature for 10 minutes. After rehydration, the rehydrated milk was serial diluted in MRD until a 10^{-5} dilution was obtained. From each MRD dilution, 0.1ml portions were spread onto Plate Count Agar (PCA, Oxoid Thermofisher). The PCA was then allowed to dry, incubated overnight at 37°C and discrete colonies thereafter counted. All samples were analyzed in duplicate.

In order to determine the presence of *Cronobacter* and other Enterobacteriaceae in the milk and infant food, samples were analysed as previously described by Chap et al. (2009). Samples were pre-enriched by suspending 25 g in 225 ml Buffered Peptone Water (BPW, Oxoid Thermofisher) and incubated at 37°C for 18-24h. After incubation, 10 ml portions were transferred into 90 ml Enterobacteriaceae Enrichment (EE, Oxoid Thermo Fisher) broth and incubated overnight at 37°C as an enrichment step.

To detect Enterobacteriaceae, 1 ml portions of EE broth were pipetted onto separate Petri dishes and mixed with 10-15ml of molten, cooled Violet Red Bile Glucose agar (VRBGA, Oxoid Thermo Fisher) and allowed to set. The solidified medium was then overlayed with an additional 10ml of molten, cooled VRBGA and allowed to set. For *Cronobacter* detection, a loopful of EE broth was streaked on Brilliance *Enterobacter sakazakii* chromogenic DFI agar (Oxoid Thermo Fisher). The inoculated plates were incubated at 37°C overnight. All samples were analyzed in duplicate. Isolates of interest were identified using phenotyping (ID 32E, BioMérieux® France).

RESULTS AND DISCUSSION

Determination of Aerobic Plate Count (APC)

The general microbial flora present in 90 samples of PIF, FOF and IF from 15 different commercial brands were determined (Table 1). From the samples analyzed, 61 had aerobic plate counts (APC) less than 10^2 cfu/g; 1 of the samples had an APC between 10^2 to $< 10^3$ cfu/g; 16 samples had APC between 10^3 to $< 10^4$ cfu/g; and 12 samples exceeded the maximum acceptable APC level of 10^4 cfu/g. It was observed that 87% (78/90) of the samples had acceptable APC limits of $< 10^4$ cfu/g, a guideline of safety against possible food poisoning (Gilbert et al., 2000; HMSO, 1995).

Very high aerobic counts ($>10^4$) were observed for seven follow-up formulas and five infant foods, in agreement with studies by Chap et. al (2009). The highest APC (4.2×10^6 cfu/g) was recorded for an infant cereal intended for babies aged 8-24months. This product was labeled to

contain the probiotic *Bifidobacterium lactis* but because of the anaerobic nature of this organism, it could not have contributed to the high APC levels. According to the Malaysian regulations (Regulation 26A, Act 281, 1983), food containing bifidobacteria should contain at least 10^6 viable cells per gram (Food Act, 2006) and it has been established that long-term consumption of infant formula milk supplemented with *B. lactis* is safe and well-tolerated by infants (Saavedra et al. 2004). Milk supplemented with probiotics results in certain immunomodulatory effects such as decreased allergic tendencies (Rautava, 2007; Viljanen et al., 2005). The level of *Enterobacteriaceae* spp. for this product was not quantified but Enterobacteriaceae, specifically *Enterobacter vulneris* was detected using the biochemical test ID 32E.

Further, an infant cereal which contained *B. lactis* cultures also yielded a relatively high APC of 5.5 x 10^4cfu/g. A follow-up formula containing *B. longum* and *Lactobacillus rhamnosus* probiotics showed a high APC level of 5.3 x 10^5. On the other hand, a probiotic-containing follow-up formula yielded an APC < 10^2 cfu/g despite having *Bifidus* cultures in its composition. In this case, the exact *Bifidus* species was not mentioned.

For PIF, APCs ranged between < 10^2 to 7.3 x 10^3 cfu/g. Approximately 78% of these samples had APCs < 10^2 cfu/g, which is reported as 'undetected' according to CODEX regulations. For follow-up formulas, 43% of the samples had an APC < 10^2 while 14% (3/21) of the samples had APCs >10^4, and therefore not meeting standard regulations. Of the 18 IF tested, the APC range was between < 10^2 to 4.6 x 10^6 cfu/g, the highest aerobic plate count for all samples tested. Around 72% of the IF samples were compliant to CODEX regulations for aerobic plate count.

***Detection of* Enterobacteriaceae spp.**

The Enterobacteriaceae level is one of the microbiological criteria (ISO 21528-1, 2004; EC Regulations, 2005) prescribed for dried infant formula and dried dietary foods for special medical purposes intended for infants below six months of age; and it should not be present in 10g of the mentioned food category. These regulations further state that if Enterobacteriaceae are present, the presence of *Cronobacter* should be tested. In this study, Enterobacteriaceae was detected in 13/90 samples but none were confirmed to contain *Cronobacter*, following the prescribed method of detection using *Cronobacter* chromogenic medium (Iversen and Forsythe, 2004).

Further biochemical profiling was conducted using the bioMerieux ID 32E system, in place of the prescribed API 20E system, as preliminary studies using well characterized *Cronobacter* strains cultures found ID 32E to more accurately identify the organism. The most frequent organism detected was *Enterobacter* spp. (5/90 samples); followed by *Citrobacter* spp. (5/90) and *Klebsiella* spp. (3/90 samples) (Table 2). These results are similar to those obtained by Iversen and Forsythe (2004) who isolated Enterobacteriaceae including *Enterobacter* spp., *Pantoea* spp., *Escherichia coli* and *Klebsiella* spp. from various infant milk and infant food samples.

The dose-response relationship of Enterobacteriaceae in milk powders has not been established but its absence in the product provides extra protection to newborns, especially to premature, immuno-compromised, low (< 2500g) and very low (< 1500g) birth weight babies in case multiplication of the organism occurs during preparation, storage or administration of the infant feed (FAO/WHO, 2004; Muytjens et al., 1988). The impact of infection largely depends on the disease contracted by the neonates (Reij et al., 2009) but Bowen and Braden (2008) have reported that of infants suffering from meningitis, a considerable percentage do not survive while

those who do survive suffer severe sequellae. More recently, Joseph and Forsythe (2011) reported the association of *C. sakazakii* ST4 with the majority of neonatal meningitis cases over the past 30 years. Despite the presence of Enterobacteriaceae in the samples, no outbreak or documented reports have been made in Malaysia pertaining to neonatal infection following consumption of any of the Enterobacteriaceae-positive products mentioned herein, or of any powdered infant formula for that matter.

Cronobacter was not detected in any of the samples analyzed in this study. Other studies have reported the presence of *Cronobacter* from various food products, including infant food and milk but usually, the organism was found in a very low percentage of the total samples tested. Reports by the FAO/WHO (2006) indicate a 2-22% incidence of *Cronobacter* spp. in PIF from various studies. Tudela et al. (2008) reported the absence of pathogenic bacteria in 156 rehydrated milk formulas examined in a hospital.

It is standard procedure that 10g of sample be used for analysis. However, given the low frequency of *Cronobacter* incidences, Hoque et al. (2010) states that the organism may be better traced if larger volumes of sample are used. In addition to using larger volumes of sample, Iversen and Forsythe (2004) suggested the use of *Cronobacter* chromogenic medium to better detect the organism. This suggestion was made after chromogenic medium was observed to more effectively isolate *Cronobacter* (67/485 positive samples), as compared to the conventional VRBGA method (Muytjens et al., 1988) then adopted by the FDA which yielded only 19/485 positive samples.

Evaluation of Rehydration Instructions on Product Packaging

The ease of application, clarity and consistency of rehydration instructions provided on infant milk products are an important consideration when addressing guidance needs for the general public during preparation and management of infant food. By definition of the Codex Alimentarius Commission (CAC 1981, 2007), infant formula is 'a breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding'. The CAC describes FOF as 'food intended for use as a liquid part of the weaning diet for the infant from the 6^{th} month on and for young children'. On the other hand, IF is described as 'food processed and manufactured for the nutritional health of children in their first year of life' (Anon, 2010).

The WHO has issued a set of guidelines on the safe preparation, storage and handling of powdered infant formula; which includes two sets of guidelines: preparation of PIF in care settings (WHO 2007a) and preparation in the home (WHO 2007b). The latter guideline has been tabulated and how each powdered infant formula (PIF) and FOF product conforms to it was evaluated (Table 3). While the WHO publication is a guideline and not a standard by which manufacturers must comply with, it is important to note that some rehydration instructions on product labels may either be insufficient, ambiguous or difficult to follow and may cause the improper handling of infant formula milk.

The 90 samples evaluated in this study consisted of 24 different PIF and FOF products. From these, 21/24 rehydration instructions specified that bottles and utensils should be sterilized by boiling (Step A.4). All sample labels directed that the proper amount of boiled water be transferred to a clean sterilized bottle (Step B.4); all but one specified that water should be brought to boil for use in formula preparation (Step B.3) and that the exact amount of formula should be added to it (Step B.5).

The FAO/WHO (2006) and WHO (2007a; 2007b) recommended the use of water >70°C for reconstitution of powdered infant formula but none of the collated rehydration instructions indicated the use of water at this temperature. When milk is prepared at the recommended 70°C, milk handlers should be aware of the importance of rapid cooling in order to avoid the multiplication of bacteria. From the different PIF and FOF product types, nine mentioned specific temperatures ranging from 40-55°C. These temperatures, at which feed may be given to infants, may have been recommended so that no further cooling would be required, as per Step B.6 of the WHO guidelines. However, it is important to note that at these temperatures, *Cronobacter* and other Enterobacteriaceae can grow (Chap et al., 2009). All other brands only mentioned for previously boiled water to be 'cool' or 'lukewarm' prior to addition of formula. These subjective temperature descriptions may contribute to the mishandling of infant formula and if subsequent growth and multiplication of microorganisms occur due to these temperature errors, it would be difficult to trace and take corrective action.

The potential growth of *Cronobacter* in bottled reconstituted infant formula milk depends on several factors such as initial water temperature, temperatures of the rooms wherein the milk was prepared and stored, reheating temperature and time (Rosset et al., 2007). Because of the small volumes of IFM distributed to infants (roughly 30ml), Rosset et al. (2007) further stressed the importance of temperature control as smaller volumes are more sensitive to temperature changes.

Six of the product types tested contained probiotic cultures, three were follow-up formula and the others were infant cereals. For a probiotic culture to maintain its beneficial characteristics in a food product, its viability should be maintained. Generally speaking, lower temperatures account for better stability and the higher the temperature, the shorter time required for the number or probiotic bacteria to decrease, ranging from several hours to minutes at 40-55°C and seconds at higher temperatures (Lee and Salminen, 2009). In cases where the infant product contains probiotic bacteria, special consideration must thus be given in terms of rehydration procedure as well as the handling of the rehydrated product. All the infant cereals with probiotics specified that water were to be heated and cooled to 40°C, while two of the FOF (Samples 5 and 14) product labels instructed the use of boiled water cooled to 45°C. The other probiotic-containing FOF (Sample J) label did not specify any temperature nor was any special instructions provided.

Furthermore, 16/24 of the product labels did not provide specific keeping and disposal instructions for unused formula. The WHO recommends that formula that has not been consumed within 2 hours should be discarded. Only five products gave specific instructions for handling unconsumed formula while an additional two (Samples 8 and 9) specified that a fresh batch of formula should be prepared for each feeding.

In Malaysia in the late nineties, it was reported that 9.6% of infants were born with a low weight (< 2,500g) and represents the group that is at risk of consuming contaminated feed (Estuningsih and Abdullah Sani, 2008). Given this situation, possible *Cronobacter* contamination in developing countries such as this should all the more be given attention because hygienic conditions and facilities (such as clean running water) may not be at par with those in exporting countries, or may not always be available; thus increasing the risk of contamination implicating high-risk groups.

Contaminated water and contaminants on bottles and nipples are significant health concerns for formula-fed infants (Morais et al., 1998; Morais et al., 2005). A study of over 2,000 infants less than 6 months of age in the Philippines showed that consumption of even small amounts of contaminated liquids nearly doubles their risk of diarrhea as compared to fully breastfed infants

(VanDerslice et al., 1994). Thus, when breastfeeding is not possible, it is suggested to minimize possible contamination of formula by constantly monitoring both raw materials and the production environment. Rehydration instructions for all infant-related products should be simple and easy to apply. For multiracial and multiethnic nations such as Malaysia, it is also ideal that rehydration illustrations be included on product packaging to assist those who do not understand the language on the product label and those who are not literate.

CONCLUSIONS

Results of this study showed that around 13% of PIF, follow-up formula and infant food samples (n=90) commercially available in Malaysia had viable counts greater than the permitted 10^4 cfu/g level. Enterobacteriaceae was detected in 14% (13/90) of the infant products analyzed. Rehydration instructions provided on product labels are generally comprehensive but could be further improved to foster consistency with guidelines prescribed by the WHO and to cater to special consumer groups such as the less-educated.

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Table 1. Aerobic plate counts of different infant milk and feed samples

Product type	No. of samples	Aerobic plate count (cfu/g)[1]			
		$< 10^2$	10^2-$< 10^3$	10^3-$< 10^4$	$>10^4$
Infant Formula	51	41	0	10	0
Follow-up Formula	21	9	0	5	7
Infant Food	18	11	1	1	5
Total	90	61	1	16	12

[1]Colony-forming units per gram of sample

Table 2. Types of Enterobacteriaceae detected in various milk samples

Product type/sample code	Enterobacteriaceae detected
Infant Formula E	*Citrobacter freundii*
	Citrobacter amalonaticus
Infant Formula F	*Enterobacter cloacae*
	Klebsiella terrigena
	Citrobacter freundii[1]
Infant Formula G	*Enterobacter cloacae*[1]
Infant Formula H	*Klebsiella pneumoniae*
Follow-up Formula J	*Citrobacter freundii*
	Klebsiella pneumoniae
Infant Food 2	*Enterobacter cloacae*
Infant Food 11	*Enterobacter vulneris*

[1]Organism detected in two samples of the same product type

Table 3. Similarity of PIF and FOF rehydration instructions to WHO guidelines (2007) for preparation of infant formula

Recommended steps[a]	Sample compliance to WHO guidelines[b]																							
	4[c]	5*	6	7	8	9	10	14*	20	21	22	30	A	B	C	D	E	F	G	H	I	J*	K	L
A. Cleaning and sterilizing feeding and preparation equipment																								
1. Hands should always be washed thoroughly with soap and water before cleaning and sterilizing feeding and preparation equipment	✓	✓	✓	✓	X	✓	✓	✓	X	✓	X	✓	X	X	✓	✓	✓	✓	X	X	✓	✓	X	X
2. Wash feeding and preparation equipment (e.g. cups, bottles, teats and spoons) thoroughly in hot soapy water.	✓	✓	✓	✓	X	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓	✓	✓	✓
3. After washing the feeding and preparation equipment, rinse thoroughly in safe water.	p	p	X	✓	X	X	X	P	X	X	X	X	✓	✓	X	X	p	X	X	X	X	p	✓	X
4. Sterilizing: if using a commercial home sterilizer (e.g. electric or microwave steam sterilizer, or chemical sterilizer), follow manufacturer's instructions. Feeding and preparation equipment can also be sterilized by boiling.	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓	✓	✓	✓
5. Hands should be washed thoroughly with soap and water before removing feeding and preparation equipment from a sterilizer or pan. The use of sterilized kitchen tongs for handling sterilized feeding and preparation equipment is recommended.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
6. Remove feeding and preparation equipment just before it is to be used. If equipment is removed from the sterilizer and not used immediately, it should be covered and stored in a clean place. Feeding bottles can be fully assembled.	✓	✓	X	X	X	X	X	✓	X	X	X	X	X	X	X	X	✓	X	X	X	✓	✓	X	X

[a] According to WHO Guidelines for safe preparation, storage and handling of powdered infant formula (2007b)
[b] Key: ✓= Guideline specified on product label; X= Guideline not specified on product label; p= Guideline partially mentioned on product label
[c] Numbers and letters in this row represent sample codes
* Contains probiotic bacteria

Table 3. (Continued) Similarity of PIF and FOF rehydration instructions to WHO guidelines (2007) for preparation of infant formula

Recommended steps[a] (continued)	Sample compliance to WHO guidelines[b]																							
	4[c]	5*	6	7	8	9	10	14*	20	21	22	30	A	B	C	D	E	F	G	H	I	J*	K	L
B. Preparing a feed using powdered infant formula																								
1. Clean and disinfect a surface on which to prepare the feed.	X	X	X	X	X	X	X	X	X	X	X	X	✓	✓	X	X	X	X	X	X	X	X	✓	X
2. Wash hands w/ soap, water; dry using a clean cloth or single-use napkin.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3. Boil a sufficient volume of safe water. If using an automatic kettle, wait until kettle switches off; make sure that the water comes to a rolling boil.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓
4. Taking care to avoid scalds, pour the appropriate amount of boiled water that has been allowed to cool to no less than 70 °C, into a cleaned and sterilized feeding cup or bottle.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5. To the water, add the exact amount of formula as instructed on the label.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓
6. Immediately after preparation, quickly cool feeds to feeding temperature by holding the bottle or feeding cup under running tap water, or by placing in a container of cold or iced water	X	X	X	✓	X	X	✓	X	X	P	X	X	X	X	✓	X	X	X	X	X	✓	X	X	X
7. Dry the outside of the feeding cup or bottle with a clean or disposable cloth.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
8. Because very hot water has been used to prepare the feed, it is essential that the feeding temperature is checked before feeding in order to avoid scalding the infant's mouth. If needed, continue cooling as outlined in step 6.	X	X	X	X	X	X	✓	X	X	X	X	✓	✓	✓	✓	X	X	X	X	X	✓	X	✓	X
9. Discard any feed that has not been consumed within two hours.	X	X	X	X	p	p	X	X	✓	X	X	X	✓	✓	X	X	X	X	X	X	X	X	✓	✓

[a] According to WHO Guidelines for safe preparation, storage and handling of powdered infant formula (2007b)
[b] Key: ✓= Guideline specified on product label; X= Guideline not specified on product label; p= Guideline partially mentioned on product label
[c] Numbers and letters in this row represent sample codes
[d] Contains probiotic bacteria