Sains Malaysiana 40(4)(2011): 345–351

Enterobacteriaceae, *Cronobacter* (*Enterobacter*) *sakazakii* and Microbial Population in Infant Formula Products in the Malaysian Market

(*Enterobacteriaceae*, *Cronobacter (Enterobacter) sakazakii* dan Populasi Mikroorganisma dalam Produk Rumusan Bayi di Pasaran Malaysia)

NORRAKIAH ABDULLAH SANI* & LIM YEN YI

ABSTRACT

This study was carried out to detect and identify the presence of Enterobacteriaceae*, and* Cronobacter sakazakii*, and determine the microbial population of infant formula products obtained from hypermarkets and a private hospital in Malaysia. Sixteen infant formulas and 14 special infant formulas from eight manufacturers were tested.* Enterobacter cloacae*,* E. asburiae*,* Klebsiella pneumoniae *spp*. pneumoniae*,* K. planticola *and* Pantoea *sp., 3 were confirmed present in five samples using ID 32E biochemical test (Biomerieux).* C. sakazakii *was not detected in any of the infant formulas tested. Five samples failed to comply with the microbiological criterion for aerobic plate count. The infant formula and special infant formula samples with different ingredients and nutrient composition did not show any significant difference in terms of aerobic plate count. Although one of the samples contained probiotic, the high microbial count for the other samples could have been contributed by the above identified* Enterobacteriaceae *since the infant formula samples non-sterile and contamination could have occurred during milk production and/ or milk preparation. It is imperative to prepare the infant formula milk samples according to the manufacturer's instruction and in an aseptic condition.*

Keywords: Cronobacter (Enterobacter) sakazakii*;* Enterobacteriaceae*; infant formula product; microbial population*

ABSTRAK

Kajian ini dilakukan untuk mengesan dan mengenal pasti kehadiran Enterobacteriaceae*, dan* Cronobacter sakazakii*, serta menentukan populasi mikroorganisma dalam produk rumusan bayi yang terdapat di pasaran serta hospital swasta di Malaysia. Enam belas sampel produk rumusan bayi dan 14 produk rumusan bayi istimewa daripada lapan pengeluar susu telah dikaji. Bagi pengenalpastian* Enterobacteriaceae*,* Enterobacter cloacae*,* E. asburiae Klebsiella pneumoniae *spp*. pneumoniae*,* K. planticola *dan* Pantoea *sp. 3 telah dikesan dalam 5 sampel rumusan bayi selepas menggunakan ID 32E (Biomerieux).* C. sakazakii *tidak dikesan di dalam sampel susu rumusan bayi. Lima sampel telah gagal memenuhi kriteria mikrobiologi bagi hitungan piring aerobik. Sampel produk rumusan bayi dan rumusan bayi istimewa yang berbeza komposisi kandungan dan nutrien tidak menunjukkan perbezaan bererti daripada segi hitungan piring aerobik. Walaupun satu daripada sampel tersebut mengandungi probiotik, populasi mikroorganisma yang tinggi bagi sampel yang lain mugkin disumbang oleh* Enterobacteriaceae *yang dikenalpasti oleh sebab susu rumusan bayi adalah tidak steril dan kontaminasinya mungkin berlaku semasa penghasilan dan/ atau penyediaan susu. Adalah penting menyediakan sampel susu mengikut panduan pengeluar dan dalam keadaan aseptik.*

Kata kunci: Cronobacter (Enterobacter) sakazakii*;* Enterobacteriaceae*; populasi mikroorganisma; produk rumusan bayi*

INTRODUCTION

Enterobacteriaceae is a group of organisms which includes several that cause primary infections of the human gastrointestinal tract. Bacteria that affect the gastrointestinal tract include certain strains of *Escherichia coli* and *Salmonella*, all 4 species of *Shigella*, and *Yersinia entercolitica*. Members of this family are major causes of opportunistic infection (including septicemia, pneumonia, meningitis and urinary tract infections). Examples of genera that cause opportunistic infections are: *Citrobacter*, *Enterobacter*, *Escherichia, Hafnia*, *Morganella*, *Providencia* and *Serratia* (Fox 2010).

Currently, the neonatal pathogen *Enterobacter sakazakii* is taxonomically reclassified into a new genus, "*Cronobacter"* together with four other species including *C. turisensis*, *C. muytjensii*, *C. dublinensis* and *C. genomospecies* 1 (Iversen et al. 2007). *C. sakazakii* is a rod-shaped Gram-negative bacterium which is motile and does not produce spore. It was successfully isolated from 2 in 82 (2.4%) powdered infant formula samples (Iversen & Forsythe 2004). According to Norrakiah et al. (2007), *C. sakazakii* were isolated from powdered infant formula with an incidence percentage of 12.5%. Total plate count for 21% (n=38) of powdered infant and children formulas

in Malaysia were > 10^4 cfu/g and thus, not complying with the microbiological criteria as stated in the Malaysian Food Act 1983 (2007) (Fauziah et al. 2008) while some bacteria from the family of *Enterobacteriaceae* including *E. cloacae*, *E. gergoviae* and *C. freundii* were detected in powdered infant formulas and children formulas in Malaysia (Norrakiah & Chan 2005).

According to Gutler et al. (2005), research should be more focus in detection, enumeration and identification for the presence of *C. sakazakii*, that is more effective and also identify the factors that influence the growth and death of *C. sakazakii* in infant formulas or reconstituted infant formulas. This is because the contamination usually happened after processing procedure that would cause infection. Food and Drug Administration (FDA/CFSAN, 2002) and ISO (ISO/ TS 22964, 2006) methods are not effective in detecting *C. sakazakii* as some ingredients used to prepare the particular selective and differential medium had prevented the recovery of injured cells (Al-Holy et al. 2008). Hence, it is important to identify which enrichment and differential medium combination are more selective and specific for detection of *C. sakazakii* in powdered infant formulas in order to lower the exposure risk of neonates and infants towards this organism that may lead to fatal infections such as meningitis, sepsis, necrotising enterocolitis and bacteremia. Therefore, the objectives of this study were to detect and identify the presence of *Enterobacteriaceae* and *C. sakazakii* and determine the microbial population in infant formula products.

Materials and Methods

Infant formula samples

A total of 30 powdered infant formula samples (16 normal infant formulas and 14 special infant formulas) from 8 manufacturers were obtained from hypermarkets and a private hospital in Malaysia. RB 1 - RB 16 represented normal infant formulas while RB 17 - RB 30 represented special infant formulas. The samples obtained were kept at room temperature (25°C) and in dry condition during the whole study.

Detection of *Enterobacteriaceae*

A modified version of ISO 21528-3 (ISO 2001) and BS 5763 part 10 (BSI 1986) methods were used. A total of 25 g for each sample was homogenized with 225 mL of maximum recovery diluent (MRD) using a stomacher for 50 s. Serial dilution (10^{-2} - 10^{-4}) was then carried out by using 9 ml MRD. For primary isolation of *Enterobacteriaceae*, overlay method was carried out where 1.0 mL of each dilution was pipetted into petri dish and then mixed with 10 - 15 mL molten violet red bile glucose agar (VRBGA, Oxoid). After that, about 10 mL VRBGA was poured onto the solidified medium. The plate was then incubated at 37°C for 24 h. After incubation, five suspected colonies were sub cultured from VRBGA to tryptone soya agar (TSA, Oxoid). TSA was incubated at 37°C for 24 ± 4 h. Finally, species of *Enterobacteriaceae* present in infant formulas were identified by using ID 32E biochemical test kit (Biomerieux).

Detection of *Cronobacter sakazakii*

The identification of *C. sakazakii* was carried out using a modified version of ISO/ TS 22964 (ISO 2006) method. A total of 25 g for each sample was pre-enriched with 225 mL buffered peptone water (BPW) and incubated at 37°C for 18 ± 2 h. After that, 10 mL and 0.1 mL of pre-enriched cultures were transferred to 90 mL *Enterobacteriaceae* Enrichment (EE) broth (Oxoid) and 10 mL *Cronobacter* screening broth (CSB, Oxoid), respectively following Iversen et al. (2008). Both were incubated at 37°C and 42°C for 24 h, respectively. Suspected *C. sakazakii* were indicated by colour changes of CSB from purple to yellow. It was because the strain could ferment sucrose that further lower the pH and causing colour changed from purple to yellow. The enriched cultures were further streaked onto Oxoid's *Brilliance™ Enterobacter sakazakii agar (formerly chromogenic Enterobacter sakazakii agar,* DFI formulation) and Merck's Chromocult® *Enterobacter sakazakii* (CES) agar and incubated at 37°C and 44°C, 24 ± 2 h, respectively. Suspected positive colonies of *C. sakazakii* were indicated by blue-green or green colonies that grew on DFI and CES agars, respectively. Typical colonies were sub cultured to TSA and incubated at 37°C for 24 ± 4 h. The identification of the typical colonies of *C. sakazakii* was then carried out by using ID 32E biochemical test kit (Biomerieux).

Microbial population - aerobic plate count

A total of 25 g for each sample was homogenized with 225 mL MRD using a stomacher for 50 s. Then, 1.0 mL homogenate was serially diluted from 10^{-1} to 10^{-6} by using 9.0 mL MRD. 0.02 mL from each dilution was pipetted as a drop on each sector of the plate count agar (PCA). The plates were incubated at 30°C for 48 h and the microbial colony counts were determined in cfu/gm.

Results and Discussion

Detection of *Enterobacteriaceae*

As shown in Table 1, *E. cloacae* were detected in two normal and one special infant formula milk. *Klebsiella pneumoniae* spp. *pneumonia* was detected in one normal and one special infant formula milk. *E. asburiae, K. planticola* and *Pantoea* sp. 3 were each detected in special infant formula milk. The results obtained were similar with the study of Anderton et al. (2004) which detected *Enterobacter* spp. and *Klebsiella* spp. in infant formula samples obtained from both hospital and house. A range of other bacteria were isolated from follow up formulas, including *Acinetobacter baumannii*, *E. cloacae*, *K. pneumoniae, Citrobacter freundii*, and *Serratia*

TABLE 1. Identification of *Enterobacteriaceae* species present in powdered infant formula samples

Sample (dilution)	Suspected colony	% ID32E test	Identification code	Organism
RB 3 (10^{-1})	1*	98.40	75074757330	*Klebsiella pneumoniae* sp. *pneumoniae*
RB 11 (10^{-1})	1	86.20	34064743011	*E. cloacae*
RB 12 (10^{-1})	1	99.30	34074745231	*E. cloacae*
RB 19 (10^{-1})	1	90.50	34064741211	*E. cloacae*
	2	84.80	04074745230	*Pantoea* sp. 3
RB 29 (10^{-1})	1	99.90	45074755331	*K. pneumoniae* sp. *pneumoniae*
	2	98.50	45174757333	*K. planticola*
	4	84.20	34074701010	*E. asburiae*

* Category of typical colony with different shape and size isolated from VRBGA
10^{-x}: Dilution at which purple colony surrounded by purple-red halo grew in VRBGA

*ficari*a based on a coordinated survey for related organisms in powdered infant formula, follow up formula and infant foods undertaken by 8 laboratories in 7 countries (Chap et al. 2009). Oonaka et al. (2010) identified 52 strains of *Enterobacteriaceae* isolated from powdered infant formula using VITEK2 compact system (Biomerieux). In 36 (24.2%) of the 149 samples, 11 bacterial types were isolated: 13 *Pantoea* spp., nine *E. sakazakii*, seven *K. pneumonia* subsp. pneumonia, six *E. cloacae*, four *Leclercia adecarboxylata*, three *Escherichia coli*, two *E. vulneris*, two *K. pneumonia* subsp. *ozaenae*, one *Buttiauxella agrestis*, one *E. hermannii* and one *Ewingella americana*. The other three strains could not be identified.

Fauziah et al. (2008) conducted a study to assess the presence of *Enterobacteriaceae* in powdered infant formula and children's milk which were taken from three nurseries, supermarkets and neonatal intensive care units (NICU) in two hospitals (A and B). A total of 38 samples were tested for this study. Isolates were presumptively detected by culture on differential selective DFI agar and identified using biochemical profiles based on API 20E (Biomerieux), RapID One (Remel) and Microgen GN-ID (Microgen Bioproducts). The *Enterobacteriaceae* spp. detected in the samples of powdered infant formula and children's milk were *E. cloacae*, *E. coli, K. pneumonia*, *K. ozaenae*, *E. agglomerans*, *C. freundii*, *E. aerogenes*, *C. koseri*, *E. asburiae*, *Shigella* spp. and *E. amnigenus*. Non-*Enterobacteriaceae* spp. detected in the samples includes *Pseudomonas aeruginosa*, *A. iwoffii* and *A. haemolyticus*. Non blue-green colonies produced on the *Brilliance E. sakazakii* chromogenic agar were biochemical tested by using Microgen GN-ID (Microgen Bioproducts) to identify the *Enterobacteriaceae* and other bacteria that were present in the raw and pasteurized milk (Norrakiah et al. 2008). *E. asburiae*, *E. dissolvens, E. cloacae, K. ozaenae*, *C. younge*, *H. alvei* and *Proteus vulgaris* were identified in raw milk samples. While for pasteurized milk samples, the *Enterobacteriaceae* identified were *K. ozaenae*, *C. younge*, *H. alvei*, and *P. vulgaris*. *Xanthomonas maltophilia*, a strain that was not from the family *Enterobacteriaceae* was also detected in pasteurized milk samples obtained from restaurant and farms.

DETECTION OF *CRONOBACTER SAKAZAKII*

Two types of chromogenic agars (DFI and CES) were used in this study instead of VRBGA, even FDA/ CFSAN (2002) has used VRBGA as primary isolation medium and TSA as detection medium for suspected *C. sakazakii*. This is because VRBGA is only selective to *Enterobacteriaceae* and colifoms but not specific for the detection of pathogens such as *C. sakazakii* and *Salmonella*. Besides that, TSA is not specific for the detection of *C. sakazakii* as many other *Enterobacteriaceae* could also grow as yellow-pigmented colonies on this agar after incubation.

No *C. sakazakii*-positive samples were detected and so it was not possible to calculate the detection-sensitivity of the different media combinations. As shown in Table 2, the combination of CSB and CES recorded the highest specificity (90.9%), followed by CSB and DFI (88.2%), EE + CES (81.1%) and EE + DFI (78.9%). These results showed that CSB was more effective in determining the presence of *C. sakazakii* in the infant formula products within 48 hours. The use of CSB increased the specificity of both DFI and CES. This is because CSB has the ability to inhibit the growth of other *Enterobacteriaceae* spp. as it contains sucrose that could act as humectant that lowers the water activity of the broth. Moreover, most *α-glucosidase* positive *Enterobacteriaceae* are not able to ferment sucrose (Al-Holy et al. 2008). Although some *Enterobacteriaceae* can ferment sucrose, most are *α-glucosidase* negative that could be differentiated by using chromogenic agar (Iversen & Forsythe 2007).

CES acted as a better selective medium for *C. sakazakii* as compared to DFI because less false positive results were obtained by using CES. In contrast, the combination of EE broth and DFI recorded more detection of false positive colonies. According to Iversen and Forsythe (2007), some isolated *C. sakazakii* were not able to grow in EE broth. EE broth and DFI combination was less selective and may lead to error during the enumeration of true *C. sakazakii* present because some isolated *C. sakazakii* especially cells that had been exposed to stress were sensitive to sodium desoxycholate in DFI or sensitive to ox bile purified and brilliant green in EE broth (Al-Holy et al. 2008).

TABLE 2. Efficiency of different combination of enrichment and selective medium in detecting *Cronobacter sakazakii* in the powdered infant formula samples

Method	True positive	False positive	True negative	False negative	Sensitivity[a]	Specificity[b]
EE + DFI	0	8	30	0	-	78.9%
EE + CES	0	7	30	0	-	81.1%
CSB + DFI	0	4	30	0	-	88.2%
CSB + CES	0	3	30	0	-	90.9%

[a] Sensitivity is the number of true positive samples divided by the total of true positive and false negative samples
[b] Specificity is the number of true negative samples divided by the total of false positive and true negative samples (Manafi & Lang 2005)

As shown in Table 3, no *C. sakazakii* positive samples (n=30) were identified by using four different combinations of enrichment and selective medium following confirmation of suspected blue green/ green colonies with ID 32E biochemical test (Biomerieux), showing satisfactory microbiological condition of the infant formula. The ID 32E biochemical test (Table 3) confirmed the suspected colonies to be *E. cloacae*, *K. pneumoniae* spp. *pneumoniae* and *K. planticola*. Two strains (*E. cloacae* and *K. pneumoniae* spp. *pneumoniae*) were found to give false positive results on CES agar while three other strains (*E. cloacae*, *K. planticola* and *K. pneumoniae* spp. *pneumoniae*) gave false positive results on DFI agar.

The Codex Alimentarius Commission (CAC 2008) criteria require that no *C. sakazakii* should be present in 10 g of powdered infant formula after primary packaging until the opening of the can for consumption. Although CSB for nine infant formula samples (RB 2, RB 3, RB 6, RB 7, RB 8, RB 10, RB 13, RB 14 and RB 16) and seven special infant formula samples (RB 21, RB 29, RB 12, RB 20, RB 25, RB 27 and RB 30) changed from purple to yellow colour after being incubated at 42°C for 24 h, no *C. sakazakii* was detected in all 16 samples. This was because other

TABLE 3. Identification of suspected *C. sakazakii* colonies that was isolated from chromogenic agars by using ID 32E biochemical profile

Method	Sample	ID32E %	Identification Code	Organisms
EE + DFI	RB 10	94.00	75074755331	*Klebsiella pneumoniae* sp. *pneumoniae*
	RB 13	99.90	45074757331	*K. pneumoniae* sp. *pneumoniae*
	RB 14	94.00	75074755331	*K. pneumoniae* sp. *pneumoniae*
	RB 16	84.10	75074757331	*K. pneumoniae* sp. *pneumoniae*
	RB 21	98.50	45174757333	*K. planticola*
	RB 26	99.90	45074755331	*K. pneumoniae* sp. *pneumoniae*
	RB 29	99.90	45074757331	*K. pneumoniae* sp. *pneumoniae*
EE + CES	RB 6	95.20	75074757130	*K. pneumoniae* sp. *pneumoniae*
	RB 7	94.00	75074755331	*K. pneumoniae* sp. *pneumoniae*
	RB 13	99.90	45074757371	*K. pneumoniae* sp. *pneumoniae*
	RB 14	94.00	75074755331	*K. pneumoniae* sp. *pneumoniae*
	RB 21	94.00	75074755331	*K. pneumoniae* sp. *pneumoniae*
	RB 26	94.00	75074755331	*K. pneumoniae* sp. *pneumoniae*
	RB 29	99.90	45074757331	*K. pneumoniae* sp. *pneumoniae*
CSB + DFI	RB 3	99.30	34074745231	*E. cloacae*
	RB 6	84.10	75074757331	*K. pneumoniae* sp. *pneumoniae*
	RB 12	99.30	34074745231	*E. cloacae*
	RB 25	82.90	34074741011	*E. cloacae*
CSB + CES	RB 3	71.20*	34074741211	*E. cloacae*
	RB 6	67.30	45075757373	*K. planticola*
	RB 25	71.20	34074741211	*E. cloacae*

* Identification results of ≤ 80% using ID 32E were not reliable

Cronobacter strains such as *C. malonaticus*, *C. turicensis*, *C. muytjensii*, *C. dublinensis* or *C. genomospecies* 1 might have grown and fermented the sucrose in CSB but the identification database of ID 32E was limited to the identification of *C. sakazakii* in *Cronobacter* genus. Usage of CSB only could detect 12.9% (n=104) *C. sakazakii* positive samples but the application of the combination of CSB and other chromogenic agars (mDFI dan ESIA) could detect 1.9% (n=104) *C. sakazakii* positive samples (Joosten & Iversen 2009). This explains why CSB for 16 samples had changed to yellow colour but only four and nine samples producing suspected *C. sakazakii* positive colonies on chromogenic agars after enriched by using CSB and EE broth, respectively.

A coordinated survey for *Cronobacter* and related organisms in powdered infant formula, follow up formula and infant foods was undertaken by 8 laboratories in 7 countries in recognition of and in response to the data needs identified in an FAO/WHO call for data in order to develop global risk management guidance for these products (Chap et al. 2009). *C. sakazakii* was isolated from 27 products; 3//91 (3%) follow up formulas (as defined by CAC), and 24/199 (12%) infant foods and drinks. Hence *C. sakazakii* was less prevalent in follow up formula than other foods given to infants over the same age range. Zink (2009) reported that there was no relationship between samples that were *C. sakazakii* positive with processing methods (for e.g. spray-dried wet mixing vs. dry mixing) and types of product (for example soy vs. milk).

Fauziah et al. (2008) conducted a study to assess the presence of *C. sakazakii* and *Enterobacteriaceae* in powdered infant formula and children's milk which were taken from three nurseries, supermarkets and neonatal intensive care units (NICU) in a two hospitals. A total of 38 samples were tested for this study. Isolates were presumptively detected by culture on differential selective DFI agar and identified using biochemical profiles based on API 20E (Biomerieux), RapID One (Remel) and Microgen GN-ID (Microgen Bioproducts). Identification was subsequently confirmed by real - time polymerase chain reaction (PCR). *C. sakazakii* was isolated from 7 samples (18%) which were 5 samples from the nurseries and 2 samples from NICU Hospital B. Four of the samples were infant formula and others were children's milk. Norrakiah et al. (2008) determined the presence of *C. sakazakii* of 32 milk samples (16 raw and 16 pasteurized milk), obtained from four places in the area of Kajang and Bangi, Selangor, Malaysia. The places included a restaurant, a market and two farms. *C. sakazakii* were detected in 63% of the raw milk samples and in 13% of the pasteurized milk samples.

MICROBIAL POPULATION - AEROBIC PLATE COUNT

According to Table 15 (regulation 39) as stated in the Malaysian Food Act 1983 (2007), total plate count of powdered infant formula should be less than 10,000 cfu/g or 4 log cfu/g. In year 2008, Codex Alimentarius Commission (CAC 2008) has decided that aerobic plate count (APC) for powdered infant formula should be ≤ 500 - 5,000 cfu/g. As shown in Figure 1, only two samples (RB 11 and RB 13) did not comply with the microbiological standard stated in Food Act 1983 whereas three samples (RB 3, RB 11 and RB 13) did not comply with the microbiological criteria stated in CAC (2008). Three special infant formula samples (RB 18, RB 19 and RB 29) did not comply with the microbiological standards set by both the Malaysia Food Act 1983 (2007) and CAC (2008). APC in this case did not take into consideration of the probiotics that were intentionally added into infant formulas (CAC 2008). RB11 is a casein predominant infant formula that is suitable form birth. It has undergone a recent reformulation and now contains *Lactobacillus reuteri*, an active probiotic which can help support infant' digestive system.

In a coordinated survey for related organisms in powdered infant formula, follow up formula and infant

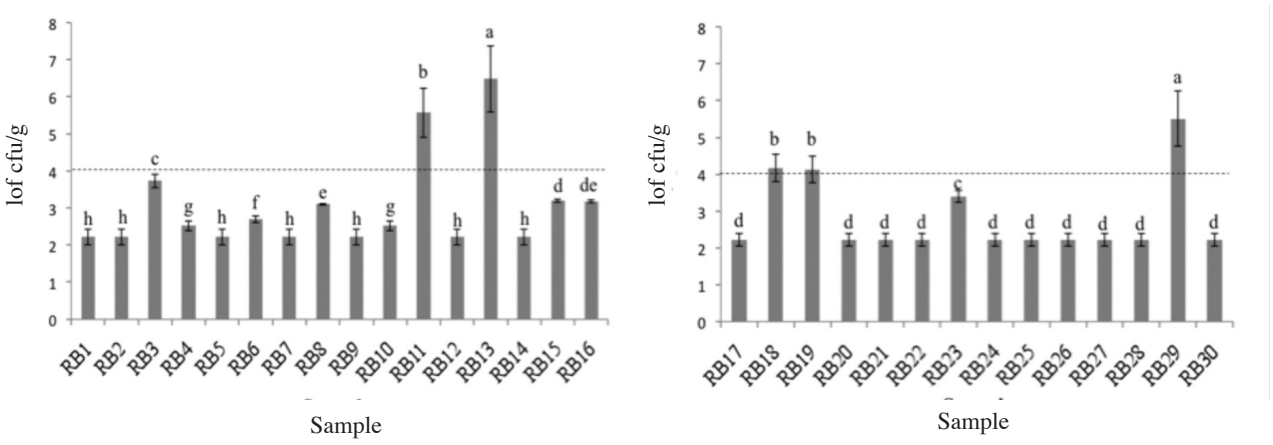

FIGURE 1. Aerobic plate count for (a) normal powdered infant formulas (n=16) and (b) special powdered infant formulas (n=14)
a - h: Same alphabet for different milk samples shows that there was no significant difference at $p > 0.05$

foods was undertaken by 8 laboratories in 7 countries. Fourteen samples had APC>10^5 cfu/g, 3 of which contained probiotic cultures. Malaysia found high APC values for 7/12 follow up formulas; four were measured at 10^4–10^5 cfu/g and three were measured at >10^5 cfu/g (Chap et al. 2009). Two samples with APC>10^5 cfu/g contained a probiotic culture.

Norrakiah et al. (2008) showed that both raw and pasteurized milk had high total plate count. FDA had set bacteriological standards through Pasteurized Milk Ordinance whereby total plate count for raw milk and its products and pasteurized milk as ≤ 100,000 cfu/mL and ≤ 20,000 cfu/mL, respectively (US Public Health Service 1995). Pasteurized milk should have total plate count of lower than 10^5 cfu/mL as stated in the Malaysian Food Act 1983 (2007) Table 15 Regulation 39. The pasteurized milk samples tested did not comply with the microbiological standards set by both FDA (US Public Health Service 1995) and Malaysia Food Act 1983 (2007).

Normal powdered infant formulas and special infant formulas did not show significant difference in terms of APC despite differences in nutrient composition, ingredients and function of the formulas (Zink 2009). Contamination of powdered infant formula only could be reduced or prevented by monitoring the critical control points (CCPs) and taking appropriate action during the processing (Nazarowec-White & Farber 1997). The high microbial count could have been contributed by the identified *Enterobacteriaceae* since the infant formula samples are unsterile and contamination could have occurred during milk processing and/ or preparation. Although, there were different packaging instructions for follow up formulas which advised reconstituting with water at a temperature of 50–55°C, 40–45°C and lukewarm (Chap et al. 2009), it is imperative to prepare the infant formula milk powder according to the manufacturer's instruction and in an aseptic condition.

Conclusions

For the identification of *Enterobacteriaceae*, *E. cloacae*, *E. asburiae*, *K. pneumoniae* spp. *pneumonia*, *K. planticola* and *Pantoea* spp. 3 were detected in five samples of infant formula products (three normal and two special infant formulas). *C. sakazakii* was not detected in any of the infant formula milk products. A total of five samples failed to comply with the microbiological criteria for aerobic plate count as stated in the Malaysian Food Act 1983 and CAC (2008). Although one of the samples contined probiotic, the high microbial count for the other samples could have been contributed by the above identified *Enterobacteriaceae* since the infant formula samples are unsterile and contamination could have occurred during milk production and/ or preparation. It is imperative to prepare the infant formula milk samples according to the manufacturer's instruction and in an aseptic condition.

Acknowledgements

The authors acknowledge the funding from MOSTI for Science fund 02-01-02-SF0476 and UKM-ST-07-FRGS0034-2009 for this project.

References

Al-Holy, M.A., Lin, M., Al-Qadiric, H.M. & Rasco, B.A. 2008. A comparative study between overlay method and selective-differential media for recovery of stressed *Enterobacter sakazakii* cells from infant formula. *Food Microbiol*. 25: 22-28.

Anderton, A., Nwoguh, C.E., McKune, I., Morrison, L., Greig, M. & Clark, B. 2004. A comparative study of the numbers of bacteria present in enteral feeds prepared and administered in hospital and the home. *J. Hosp. Infect*. 23: 43-49.

British Standards Institution (BSI). BS 5763. 1986. *Microbiological Examination of Food and Animal Feeding Stuffs. Part 10 enumeration of Enterobacteriaceae*. London: BSI.

CAC (Codex Alimentarius Commission). 2008. *Code of Hygienic Practices for Powdered Formulae for Infants and Young Children***.** CAC/RCP 66 - 2008. (on line) http://www.codexalimentarius.net/download/standards/11026/cxp_066e.pdf accessed on 4 March 2009

Chap, J., Jackson, P., Siqueira, R., Gaspar, N., Quintas, C., Park, J., Osaili, T., Shaker, R., Jaradat, Z., Hartantyo, S.H.P., Abdullah Sani, N., Estuningsih, S. & Forsythe, S.J. 2009: International Survey of *Cronobacter sakazakii* and Other *Cronobacter* spp. in Follow Up Formulas and Other Infant Foods. *Int. J. Food Microbiol*. 136: 185-188.

Fauziah, T., Norrakiah, A.S., Uma Priya, K. & Norizan, J. 2008. Detection of C*ronobacter* (*Enterobacter*) s*akazakii* and *Enterobacteriaceae* in Powdered Infant Formula and Children's Milk. *Proceeding of the Seminar on Food Biotechnology: Perspectives, Challenges and Opportunities*, pp. 352-360.

FDA/CFSAN. 2002. Isolation and enumeration of Enterobacter sakazakii from dehydrated powdered infant formula. (on line) http://www.cfsan.fda.gov/~lrd/tpinf.html accessed on 18 July 2008

Fox, A. 2010. *Enterobacteriaceae*, Vibrio, Campylobacter and Helicobacter. In Bacteriology – Chapter Eleven. Microbiology and Immunology on-line. University of South Caroline School of Medicine. http://pathmicro.med.sc.edu/fox/enterobact.htm accessed on 1 August 2010

Gutler, J.B., Kornacki, J.L. & Beuchat, L.R. 2005. *Enterobacter sakazakii*: A coliform of increased concern to infant health. Int. J. Food Microbiol. 104: 1-34.

International Organization for Standardization. ISO 21528-3. 2001. *Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of Enterobacteriaceae - Colony count technique*. Geneva: ISO.

International Organization for Standardization (ISO). ISO/TS 22964. 2006. *Milk and milk products - Detection of Enterobacter sakazakii*. Geneva: ISO.

Iversen, C., Druggan, P., Schumacher, S., Lehner, A., Feer, C., Gschwend, K., Joosten, H. & Stephan, R. 2008. Development of a novel screening method for the isolation of "*Cronobacter*" spp. (*Enterobacter sakazakii*). *Appl. Environ. Microbiol*. 74: 2550-2553.

Iversen, C. & Forsythe, S.J. 2004. Isolation of Enterobacter sakazakii and other *Enterobacteriaceae* from powdered formula milk and related products. *Food Microbiol*. 21: 771-777.

Iversen, C. & Forsythe, S.J. 2007. Comparison of media for the isolation of *Enterobacter sakazakii*. *Appl. Environ. Microbiol*. 73: 48-52.

Iversen, C., Lehner, A., Mullane, N., Marugg, J., Fanning, S., Stephan, R. & Joosten, H. 2007. Identification of "*Cronobacter*" spp. (*Enterobacter sakazakii*). *J. Clin. Microbiol*. 45: 3814-3816.

Joosten, H. & Iversen, C. 2009. *Development of a CEN-ISO horizontal standard method for detection of Cronobacter* (on line) http://civersen.tripod.com/joosten.pdf accessed on 27 February 2009

Malaysian Food Act 1983. 2007. *Akta Makanan* 1983. Bahagian VIII. 82. Petaling Jaya: Golden Books Centre Sdn. Bhd.

Manafi, M. & Lang, K. 2005. *Comparison of three chromogenic media for detection of Enterobacter sakazakii; a preliminary study* (on line) http://www.univie.ac.at/hygiene-aktuell/asm2005.pdf [19 August 2008]

Nazarowec-White, M. & Farber, J.M. 1997. Thermal resistance of *Enterobacter sakazakii* in reconstituted dried-infant formula. *Lett.Appl. Microbiol*. 24 (1): 9-13.

Norrakiah, A.S. & Chan, C.K. 2005. *Enterobacter sakazakii* and *Enterobacteriaceae* in powdered infant formula milk and powdered children's milk. *Proceedings of the 8th Symposium of Applied Biology*.

Norrakiah, A.S., Ghassem, M. & Babji, A.S. 2007. *Enterobacter sakazakii* and growth characterization in infant formula milk. *Proceedings of the 10th Asean Food Conference 07*.

Norrakiah A.S., Noorzatul Shuhada M.Z. & Lim Y.Y. 2008. *Cronobacter* (*Enterobacter*) *sakazakii*, *Enterobacteriaceae* and Total Plate Count in Raw and Pasteurized Milk. *Proceedings of the 10th MSAB Symposium, Kuching*.

Oonaka, K., Furuhata, K., Hara, Motonobu & Fukuyama, M. 2010. Powder infant formula milk contaminated with *Enterobacter sakazakii*. *Jpn. J. Infect. Dis*. 63: 103-107.

United States Public Health Service. 1995. *Grade A Pasteurized Milk Ordinance*. Publication No. 229, Washington DC. Food and Drug Administration.

Zink, D.L. 2009. FDA field survey of powdered formula manufacturing. (on line) http://www.fda.gov/ohrms/dockets/AC/03/slides/3939s1_Zink.ppt accessed on 14 August 2008.

School of Chemical Sciences and Food Technology
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
43600, UKM Bangi, Selangor D.E.
Malaysia

*Corresponding author; email: norra@ukm.my

Received: 9 December 2009
Accepted: 7 August 2010