Isolation of *Cronobacter* spp. Isolates from Infant Formulas and Their Survival in the Production Process of Infant Formula

Songzhe FU[1], Jianxin GAO[1], Ying LIU[2] and Haiying CHEN[1]

[1]Nanchang Center for Disease Control and Prevention, Nanchang, P.R. China; [2]State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing, P.R. China

Abstract

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Over a 24-month surveillance, three *Cronobacter* strains, NC041, NC830, and NC1006, were isolated from 77 powder infant formulas (3.90%). No *Cronobacter* was detected in liquid milk. The prevalence of *Cronobacter* in the prefinal product and packaged final product was 3.70% and 4.35%, respectively. The isolated *Cronobacter* strains were subjected to several lethal challenges including the pH, drying, disinfectant, and simulated infant formulas manufacturing process (SIFMP). The results indicated that they exhibited unusual resistance to the dry stress and disinfectant. In SIFMP, *Cronobacter* isolates were inoculated into three possible contamination entry points involving the stages prior to heating, drying, and filling, respectively. No *Cronobacter* could survive the heating. However, a high level (10^5 CFU/ml) of *Cronobacter* was detected in the samples after the inoculation at the drying point. Furthermore, the survival of *Cronobacter* was observed during the storage at 10°C after 7 days. The results support the hypothesis that *Cronobacter* contamination occurs at the stages of drying and filling, and highlight the need to improve the disinfecting measures in the manufacturing process.

Keywords: *Cronobacter*; dry stress; powdered infant formula (PIF); contamination sources

Cronobacter, formerly *Enterobacter sakazakii*, is a recently proposed genus consisting of six genomospecies (Iversen *et al.* 2008). It has been implicated in the illness of neonatal meningitis in infants. The mortality rate of the infants who develop *Cronobacter* associated neonatal meningitis is up to 80% (Iversen & Forsythe 2003). Moreover, infections in the elderly and the patients who are immunocompromised have been reported recently (Gosney *et al.* 2006). A recent international survey has shown that *Cronobacter* spp. were isolated from 27 products. Among these, 12% infant foods and drinks were *Cronobacter*-contaminated (Chap *et al.* 2009). Both the source of *Cronobacter* and vehicle of transmission are not always clear. Surveillance studies have detected *Cronobacter* in various foods, households, and environments (Kandhai *et al.* 2004). However, powdered infant formula (PIF) has been epidemiologically linked to the cases of infants infection (Acker *et al.* 2001), thus more research has focused on the surveillance of PIF products for the presence of *Cronobacter*. The production of PIFs includes the addition of ingredients, homogenisation, pasteurisation treatment, spray drying, and packaging. The powdered milk is subsequently sold as an ingredient for food and nutritional products. It is generally assumed that *Cronobacter* appears to contaminate infant formulae and infant foods particularly at the

stages after the pasteurisation treatment, including drying and packing, or during vitamins or supplement fortification steps. Due to the organism ability to resist drying or osmotic stress (Breeuwer *et al.* 2003; Riedel & Lehner 2007; Arku *et al.* 2008), *Cronobacter* can persist in powdered infant formulae over a long period of time. To establish the possible routes of dissemination of contamination from the environmental and food sources, some authors identified and characterised *Cronobacter* spp. isolated from dried-milk and related environments (Mullane *et al.* 2007; El-Sharoud *et al.* 2009). Hein *et al.* (2009) and Craven *et al.* (2010) investigated the spatial distribution, prevalence and persistence of *Cronobacter* in the non-processing and processing environments of milk powder factories. The findings revealed that the supply air is a potential vehicle for extrinsic *Cronobacter* contamination and confirmed that *Cronobacter* is dispersed widely in milk powder factories. Mullane *et al.* (2008) also investigated microbial contamination in air filters and proposed that the effects of the air filter installation and maintenance is linked to contaminated product in powdered milk protein-processing facilities. However, the possible contamination sources of *Cronobacter* and the influence of different processing activities on the contamination levels are not very well characterised to date which enables on-going contamination to occur. Less is known regarding the entry points of *Cronobacter* in the infant formulae production process. Due to the concern about the potential introduction of pathogens into the processing environment, inoculated tests cannot be performed in the powdered milk production facility. Therefore, it is very essential to investigate the uncertain routes of *Cronobacter* contamination in a simulated manufacturing process. This work was therefore undertaken to evaluate the environmental stress resistance of *Cronobacter*, with special attention to the sources of *Cronobacter*. In this study, over a 24-month period a surveillance of liquid milk and PIF was performed and the survival of *Cronobacter* in a simulated manufacturing process was also tested.

MATERIAL AND METHODS

Sampling site and sampling plan. The samples were taken from five milk powder factories from March 2008 to April 2010. The same batches of liquid milk and powdered milk were tested for the presence of *Cronobacter* spp. A total of 144 samples were collected for analysis during the study: 5 of maltodextrin (one of the ingredients in infant formulas), 62 of liquid milk (sampled after pasteurisation treatment), and 77 of powdered infant formulas (54 sampled after spray dry and 23 sampled after packaging).

Isolation and identification of *Cronobacter* spp. The samples were tested for the presence of *Cronobacter* as described by Chap *et al.* (2009). Briefly, 25 g of liquid milk or powder infant formula were added to 225 ml of peptone water and then incubated at 37°C overnight. A ten ml aliquot was then incubated in 90 ml Enterobacteriaceae Enrichment broth (EE) at 37°C for 24 hours. From each enriched sample, 0.1 ml was streaked or spread onto Druggan-Forsythe-Iversen (DFI) agar (Oxiod, Basingstoke, UK) and incubated at 37°C for 24 hours. Blue-green colonies were picked and subjected to further biochemical and molecular confirmative techniques. Then the 16S rRNA gene of the isolates was amplified using two bacterial universal primers, 27F and 1492R, and further confirmed with species-specific PCR to detect the 16S-23S rDNA internal transcribed spacer (ITS) (Liu *et al.* 2006). The similarity test of the 16S rRNA gene sequence was performed by using BLAST program on NCBI http://www.ncbi.nlm.nih.gov.

Dehydration stress resistance of *Cronobacter* ***on stainless steel***. Sterile stainless steel coupons (type 304; 2.5 cm by 2 cm) with No. 4 finish were prepared as described by Kim *et al.* (2006). Reconstituted infant formula was made by combining powdered infant formula (Fonterra, Auckland, New Zealand) with distilled water at a ratio of 1:100 (w/v), dissolving by heating at 45–60°C, and autoclaving at 121°C for 15 minutes. Suspensions (100 ml) of *Cronobacter* in reconstituted infant formula were spot inoculated on each coupon to give ca 6 log CFU/coupon and dried for 20 h (25% ± 5% relative humidity; 100 KPa air pressure) at 20 ± 2°C in a laminar-flow biosafety cabinet. Each inoculated coupon was transferred to a sterile 50-ml tube containing 20 ml of 0.1% peptone water and 3 g of sterile glass beads every hour. The tubes containing 0.1% peptone water, coupon, and glass beads were vortexed at maximum speed for 5 min to detach the biofilm. Immediately after vortexing, the samples (0.1 ml in triplicate) were serially diluted in 0.1% peptone water and subsequently surface plated on TSA and incubated at 37°C for 24 hours. The colonies were then counted.

Resistance to acid and alkaline stresses. To investigate the resistance to acid and alkaline stresses, test strains were cultured for 12 h in TSB to the late exponential phase. TSB test media were adjusted to the target pH of 3.0, 3.5, 4.0, 4.5, 7.2, 9.0, 10.0, and 11.0 using sterile 10 mol/l of HCl or 10 mol/l of NaOH prior to autoclaving, assisted by a pH metre (Corning Incorp., Corning, USA). The cultures of *Cronobacter* isolates were inoculated into TSB with different pH levels, respectively, at a final population of 10^6 CFU/ml, and incubated at 28°C. At specific intervals during the incubation period, the viability of the test organism was determined by OD600 as specified below.

Survival of *Cronobacter* ***in simulated infant formulas manufacturing process***. To investigate the survival of *Cronobacter* in heating, drying, and filling as three possible contamination entry points on the end products, the suspension of *Cronobacter* in reconstituted infant formula was spot inoculated on stainless steel coupons to give cca 6 log CFU/coupon or inoculated into a 10 ml stainless steel tube with 5 ml suspension (final population of 10^6 CFU/ml), respectively. The inoculated coupons were dried by using a hair dryer (Philip, HP4376) (the outlet temperature is 75–90°C) in the biosafety cabinet for 20 minutes. For the stainless steel tube, the reconstituted infant formula was first pre-heated at 45°C for 20 min and then heated at 68°C for 15 minutes. Then 200 µl of the reconstituted infant formula was spot inoculated on the stainless steel coupon and dried for 20 min in the biosafety cabinet as described above. Then the viable numbers of *Cronobacter* from two groups of dried coupons were tested following the protocol by Kim *et al.* (2006). For another duplicate, two groups of dried coupons were immersed in 50 ml tube containing 10 g PIF, stored at 10°C, and tested for the presence of *Cronobacter* after 1 week. To investigate the contamination risk of filling, 0.5 g PIF were added into a stainless steel tube containing 100 µl 10^3 CFU/ml *Cronobacter*, stored at 10°C, and plated on TSA after 1 week. The colonies were then counted after incubation at 37°C for 24 hours.

Efficacy of the disinfection procedure for killing *Cronobacter* ***in bioflim.*** To investigate the efficacy of the disinfection procedure, stainless steel coupons inoculated with *Cronobacter* were immersed in the reconstituted infant formula for 6 days. The coupons were then treated using three procedures, respectively: (1) Simulated CIP cleaning: the coupons were immersed in 75°C 2.5% (w/v) NaOH, and vortexed for 3 min, then rinsed with 80°C sterile water for 10 min, immersed in 55°C 0.1 mol/l HCl for 3 min, and rinsed with 95°C sterile water for 15 min; (2) NaOH treatment: the coupons were immersed in 25°C 2.5% (w/v) NaOH or water (as control) for 3, 5, 10 min; (3) Cleaning with ethanol: coupons were wiped with 75% ethanol cotton balls for 30 s and dried for 1 min with a hair dryer, and the procedure was repeated twice. At specific intervals, the viability of the test organism was determined by plating on TSA.

Statistical analysis. The mean value and standard deviation were calculated from the data obtained from the three separate experiments. The data were analysed using unpaired two-tailed Student's *t*-test. Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The detection of *Cronobacter* in the liquid milk and powdered infant formulas of 62 liquid milk, no sample was *Cronobacter*-positive. Two *Cronobacter* strains (strain NC041 and NC830) were isolated from 54 spray dried samples, one *Cronobacter* strain (strain NC1006) was isolated from 23 packaged PIF samples. The prevalence of *Cronobacter* in the prefinal product and packaged final product was 3.70% and 4.35%, respectively. 16S rRNA sequence analysis showed that 3 strains exhibited 99.9% sequence similarity to the type strain. The GenBank accession numbers for strains NC041, NC830, and NC1006 were GU727682, GU727683, GU727684, respectively. Meanwhile, two samples of powdered maltodextrin were also found to be *Cronobacter*-polluted. Furthermore, *Enterobacter cloacae, Enterobacter agglomerans, Citrobacter sedlakii,* and *Klebsiella pneumoniae* were also detected in the PIF samples. These organisms were not further investigated.

Dehydration stress resistance

As shown in Figure 1, three strains exhibited strong resistance to dry stress. After 6 h, the reductions were only between 0.9 and 1.5 log(10) for the test strains. After 12 h, the reductions increased to 1.4 up to 2.4 log ($P < 0.05$). Then, the number of the three strains stabilised to 6 log CFU/ml in

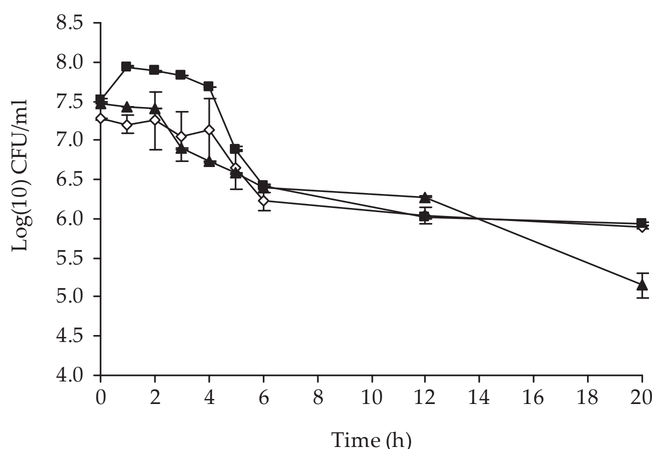

Figure 1. Survival of *Cronobacter* strains under different dry conditions. Fresh 24-h cultures of *Cronobacter* strains: NC041 (◇), NC1006 (■) and NC830 (▲) in infant formula were air-dried at 30°C for given periods of time. The dried inoculum was reconstituted in sterile peptone water and enumerated by a standard plate count technique. Error bars indicate SD calculated from triplicates

20 h, except for the strain NC830. For the strain NC041, there was even 0.5 log increase after 2 h dehydration. Then the number of NC041 gradually decreased to 6 log CFU/ml in 12 hours. These findings are in agreement with the data obtained by Breeuwer *et al.* (2003). It was reported that the viable counts of the stationary phase *Cronobacter* dried in air and incubated for 46 days at 25°C decreased only by 1–1.5 log unit. In our study, the reduction between 1.4 log CFU/ml and 2.4 log CFU/ml was higher than that mentioned above. By allowing a high velocity air stream to pass over the surfaces, the test strains were subjected to a sublethal desiccation that may ultimately lead to more cell deaths. The results demonstrated that *Cronobacter* can tolerate desiccation in a high velocity air stream and can persist for a long time during the shutdown maintenance in factories.

Resistance to acid and alkaline stresses

As shown in Figure 2, we tested the growth of *Cronobacter* isolates at high and low pH. Overall, it was found that the strains showed the resistance ability to high pH. The mean OD_{600} of *Cronobacter* isolates was the highest at pH 7.2, which was not significantly ($P > 0.05$) higher than those at pH 9.0 or pH 10.0, and especially at pH 9.0. The growth of *Cronobacter* was slightly inhibited at pH 11.0. However, the strains did not survive at pH below 4.0 (pH 3.0, 3.5, 4.0). The mean OD_{600} at pH below 4.0 was significantly lower ($P < 0.05$) than those for all other pH values tested. Likewise, the mean OD_{600} for pH 4.5 was significantly lower than the OD_{600} at pH 7.2 or pH 9.0 ($P < 0.05$). It was also found that the lag time of *Cronobacter* isolates increased as pH increased at high pH (Figure 2). The exposure of *Cronobacter* to pH below 4.0 is known to cause reductions in the population (Kim & Beuchat 2005). It is notable, however, that the exposure of *Cronobacter* to the environments at pH from 9.0 to 10.0 may not cause immediate death. Gurtler and Beuchat (2005) also reported that populations of *Cronobacter* decreased only by 0.5 log CFU/ml when the cells were exposed to an environment of pH 11.25 for 5 minutes. The results were consistent with those reports, suggesting that the acid disinfectants such as chlorine dioxide could also be considered in the design of effective control measures.

The survival of *Cronobacter* in the heating, drying, and filling

To investigate the survival of *Cronobacter* at the three stages of infant formulas manufacturing process, three *Cronobacter* isolates were inoculated in the three contamination entry points possible including the heating, drying, and filling, respectively. Entry points and final products were tested for the presence of *Cronobacter*. No *Cronobacter* was detected in the samples after heating (Table 1). However, the number of *Cronobacter* only decreased from 6 log CFU/ml to 5 log CFU/ml after drying. The effect of the filling and storage conditions of PIF on the survival of three *Cronobacter* strains was also investigated. After drying or filling, storage tests showed that there is no significant increase occurred at 10°C in 7 day ($P > 0.05$). Due to the possible lethal challenges of drying, an increase in the range of 0.12 and 0.16 log occurred in subsequent PIF storage. An

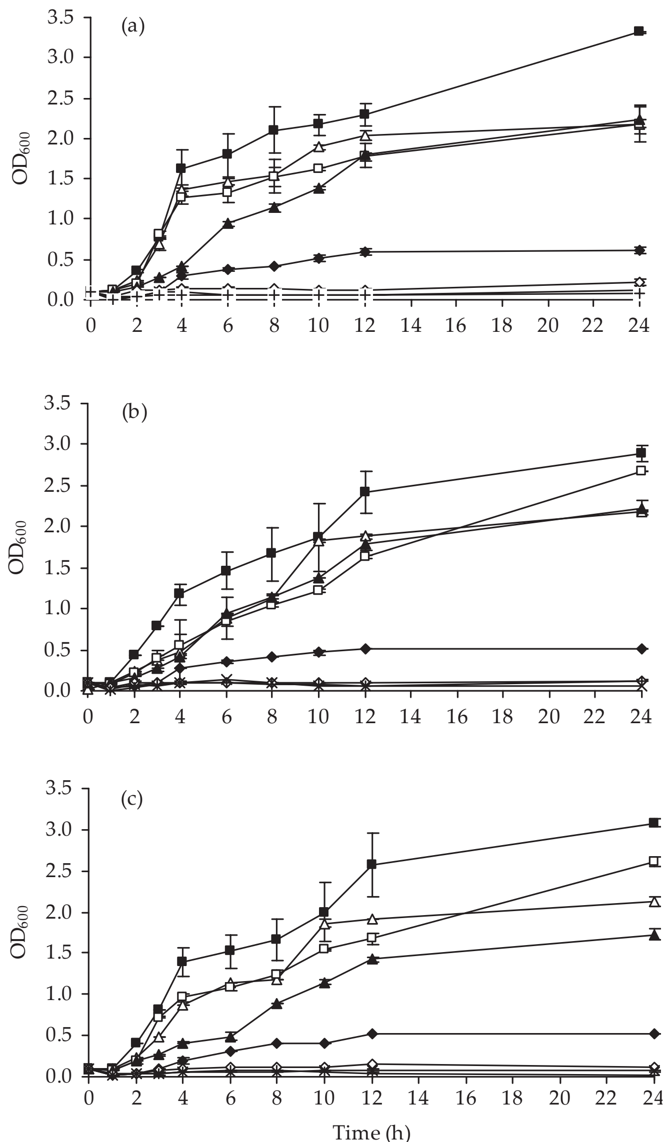

Figure 2. Growth curves of *Cronobacter* strains at different pH. Growth of *Cronobacter* strains: NC041 (a), NC1006 (b) and NC830 (c), in tryptic soy broth adjusted to pH 7.2 (■), 9 (△), 10 (□), 11 (▲), 4.5 (◆), 4.0 (◇), 3.5 (×), and 3.0 (+) was monitored by optical density measurements at 600 nm. Error bars indicate SD calculated from triplicates

increase of 0.74 log CFU/ml to 0.93 log CFU/ml in PIF also occurred after 7 days of storage. In the current study, the *Cronobacter* isolates were not speciated. However, current customer specifications for *Cronobacter* in infant formulae do not differentiate *Cronobacter* and its level on this basis. Our results showed that after the inoculation test prior to heating, drying, and filling, *Cronobacter* cannot survive the heat treatment. However, in the process of drying and filling, the organism was inhibited slightly in the high temperature drying and survived in the final products. Therefore, all isolates that survived even at low levels are considered to be a potential risk.

The environmental sources and potential transmission routes of this pathogen remain controversial. There is a possibility that the food production animals (such as cattle, pigs) and farm environment play a role in the transmission of *Cronobacter*. However, the recent research by Molloy *et al.* (2009) showed that *Cronobacter* spp. were not recovered from cattle faeces, farm soil, or trough water but were isolated from a wide range of samples indicating that *Cronobacter* spp. were not carried by certain food production animals. In another work by Molloy *et al.* (2010), no significant change was observed in the number of inoculated *Cronobacter* over a 24-h period in rumen fluid, and *Cronobacter* was undetectable after 30 min of incubation in the model abomasum. These results indicated that food production animals are unlikely to be the source of *Cronobacter*. Previous reference reported that *Cronobacter* is found in a wide range of foods and environments including infant formulas

Table 1. Survival of *Cronobacter* strains in simulated manufacturing process

Inoculated site	Test strains	Different stages of manufacturing process (CFU/ml)				
		prior to treatment	pre-heat treatment	pasteurization treatment	drying	after 7 days of storage in the PIF
Tubes (before heating)	NC041	(4.68 ± 0.32) × 10^6	(1.80 ± 0.02) × 10^6	ND	ND	ND
	NC1006	(8.14 ± 0.19) × 10^6	(1.90 ± 0.01) × 10^6	ND	ND	ND
	NC830	(5.55 ± 0.45) × 10^6	(2.30 ± 0.14) × 10^6	ND	ND	ND
Coupons (before drying)	NC041	(1.17 ± 0.03) × 10^6	–	–	(1.09 ± 0.10) × 10^5	(1.55 ± 0.45) × 10^5
	NC1006	(1.65 ± 0.05) × 10^6	–	–	(6.40 ± 0.15) × 10^5	(9.35 ± 0.65) × 10^5
	NC830	(1.28 ± 0.23) × 10^6	–	–	(1.00 ± 0.09) × 10^5	(1.33 ± 0.33) × 10^5
Tubes (after drying)	NC041	(8.9 ± 0.05) × 10^3	–	–	–	(7.5 ± 0.10) × 10^4
	NC1006	(7.2 ± 0.11) × 10^3	–	–	–	(5.7 ± 0.15) × 10^4
	NC830	(7.8 ± 0.15) × 10^3	–	–	–	(4.3 ± 0.09) × 10^4

ND = not detected; – = not done

(Chap *et al.* 2009; Jaradat *et al.* 2009), indicating that *Cronobacter* is a ubiquitous microbe. Due to its osmotolerant nature (Nazarowec-White & Farber 1997), the environments with particular association with dry environments may provide a strong selectivity and ecological advantage for the survival of *Cronobacter* in PIF under dry conditions, although the mechanism is unclear.

Although ingredients and raw materials can contain pathogens, our results support the existing data which are available to the regulatory agencies and milk producers stating that the recommended heat treatments are sufficient to reduce substantially the risk arising from *Cronobacter* which may be present in these products. The use of hot water (> or = 68°C) during reconstitution appears to be an effective means to reduce the risk of *Cronobacter* in these products. Thus, combined with the results from the surveillance, our results indicated that the presence of *Cronobacter* in packaged PIF is possibly due to the recontamination in the course of drying and subsequent processing steps (including conveying, dry mixing with additional ingredients, and final filling/packaging). The processing and preparation of powdered milk in drying and filling as possible sources of contamination, colonisation, or infection should be seriously checked.

Efficacy of disinfection procedure in killing *Cronobacter* in bioflim

The treatment with 75% (v/v) ethanol for 30 s significantly ($P < 0.05$) reduced the populations, although only by 3.13 to 4.26 log CFU/coupon, respectively (Table 2). On two treatments with 75% (v/v) ethanol, the reduction reached 3.8 log to 4.26 log ($P < 0.05$). After the third ethanol treatment, 50 CFU/coupon was still detected positive for the strains NC041 and NC1006. The treatment with 2.5% (w/v) NaOH for 10 min also significantly ($P < 0.05$) decreased the population of the strains in 6-day-old biofilm by 4.74 log to 5.40 log CFU per coupon. Simulated CIP is an effective disinfection procedure. On the treatment with 75°C 2.5% (w/v) NaOH, 5.11 to 5.72 log decrease was achieved for the test strains, while subsequent HCl cleaning caused a decrease to an undetectable level (< 1.6 log CFU/coupon) within 3 minutes. The disinfecting test showed that CIP is sufficient to reduce the risk from *Cronobacter* in the manufacturing facility. However, using ethanol or NaOH alone can not guarantee the inactivation of *Cronobacter*. Since the measures to prevent the transmission of *Cronobacter* are important to milk powder industries, our findings suggest that the widespread nature of *Cronobacter* should be taken into account in the design of effective control measures.

In the manufacturing facility, wet cleaning (by circulating cleaning solutions and water under well controlled conditions) and dry cleaning (by allowing a high velocity air stream to pass over the surfaces) were generally used to control the level of pathogens. The wet methods can involve either manual hosing of surfaces or more effectively automated cleaning-in-place systems (CIP systems). These are mainly applied for cleaning the milk collection

Table 2 Survival of *Cronobacter* strains spot inoculated and dried on the surface of stainless steel coupons at 25°C for 6 days as affected by treatment with disinfectants

Disinfectants	Treatment time (min) or order	Population recovered (log CFU/coupon) by strain		
		NC041	NC830	NC1006
Water (control)	0	7.58	7.47	7.45
	3	7.63	7.51	7.46
	5	7.61	7.52	7.48
	10	7.67	7.54	7.50
2.5% (w/v) NaOH	0	7.58	7.47	7.45
	3	3.42 (4.11[a])	3.04 (4.39[a])	2.92 (4.52[a])
	5	3.27 (4.28[a])	2.41 (5.01[a])	2.25 (5.17[a])
	10	2.75 (4.74[a])	2.00 (5.40[a])	< 1.6[b] (> 5.80[a])
CIP cleaning	initial population	7.58	7.47	7.45
	NaOH cleaning	2.41 (5.12[a])	2.31 (5.11[a])	1.7 (5.72[a])
	HCl cleaning	< 1 .6[b]	< 1.6[b]	< 1.6[b]
75% (v/v) ethanol	initial population	7.58	7.47	7.45
	1st wiping	4.45	3.34	3.19
	2nd wiping	3.78	2.17	2.75
	3rd wiping	1.7	1.7	< 1.6[b]

[a]reduction or increase in population compared to the number of *Cronobacter* recovered from stainless steel coupons treated with water (control) for the same length of time; [b]detection limit was 40 CFU/coupon (1.6 log CFU/coupon)

tank, dosing tank, and storage pipeline system, while ethanol is mainly used for powder milk storage tanks and fluidised bed cleaning. Although those control measures do indeed drastically reduce the levels present in high-hygiene areas, Hein *et al.* (2009) found such hygiene measures as inefficient to eliminate completely *Cronobacter* from all areas of the processing line.

Recontamination with *Cronobacter* possibly comes from the external and internal parts of the equipment and surroundings of the processing lines. Craven *et al.* (2010) found higher occurrence of *Cronobacter* in the non-processing (external parts of processing lines) than in the processing areas. The most prevalent and persistent clones were isolated from the external roofs over the spray driers, air treatment areas, and areas with busy foot traffic. It is currently not possible to eliminate completely this pathogen from the external parts of the equipment and surroundings. Thus, it is consistently present at very low levels in the environment, and may gain access to the processing line through the air movement. Busy foot traffic also enables it to get into the processing lines.

On the other hand, *Cronobacter* may also grow on the internal parts of the equipment. The very nature of the dairy products means that the possible formation of *Cronobacter* biofilms on the surfaces of the process equipment is always real. The surfaces always should be smooth, without pits and cracks. However, pathogens may grow in the cracks and become the blind angle that disinfectants can not reach. After the processes of drying, filling,or disinfectant treatment, the residual or outside *Cronobacter* will possibly attach to and grow on the internal surfaces of powder milk storage tanks or fluidised bed, thus becoming a source of contamination. Infant formula remaining on these surfaces also protects *Cronobacter* against the lethal effects of disinfectants.

It is also notable that the general processing activities such as spray drying and milling operations can create aerosols. Aerosols with dust particles provid the surface for the attachment of the airborne micro-organisms, and are able to travel to long distances from their source. After the final clean water wash is performed as the last stage of the cleaning process, drying out of the plant is then performed by passing warm air through the plant by activating the air heaters and fans.

Although, the air used in dry cleaning is heated up to 85°C, there is a certain blind angle that can not be heated. This process may also introduce the airborne micro-organisms in the form of

aerosols and become a source of contamination. These pathogens originating from the processing environment that is in direct contact with the product would then contaminate the next batch of the product. Further study will examine the effectiveness of the commonly used disinfectants in killing *Cronobacter* and identify good hygienic practice to prevent the contamination and spread of micro-organisms.

CONCLUSIONS

In summary, our results identified that recontamination happened in drying and filling, and emphasised the importance of proper cleaning of the surfaces soiled by rehydrated infant formulas and other foods. The highest hygienic requirements must be satisfied to avoid contamination coming from the busy foot traffic, movement of untreated air, and insufficient disinfection procedure. The Critical Control Points should be carefully identified to minimise the micro-organisms entry into the high-hygiene zones and prevent the proliferation of those that are already present. Improved disinfection procedures and extensive monitoring of the environmental samples, product contact surfaces, and finished products would contribute to reducing the dissemination of pathogens and ultimately excluding hazards from the final powdered product.

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Corresponding author:

Dr. Songzhe Fu, Nanchang Center for Disease Control and Prevention, No. 833 Lijing road, Honggutan district, Nanchang, 330038, P.R. China
tel.: + 86 791 636 32 55, e-mail: fusongzhe@hotmail.com