Original Article

The Survey of *Cronobacter* spp. (formerly *Enterbacter sakazakii*) in Infant and Follow-up Powdered Formula in China in 2012*

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Abstract

Objective To determine *Cronobacter* spp. contamination in infant and follow-up powdered formula in China.

Methods All of 2282 samples were collected from the retail markets in China from January 2012 to December 2012, and analyzed for *Cronobacter spp*. by the Chinese *National Food Safety Standard*. Characterization of the isolates was analyzed by pulsed-field gel electrophoresis (PFGE) with *Xba*I and *Spe*I restriction enzymes.

Results *Cronobacter* spp. strains were isolated from 25 samples, and the positive rates in infant powdered formulas and follow-up powdered formulas were 0.90% (10/1011) and 1.18% (15/1271), respectively. Analysis of variable data regarding different purchasing store formats, seasonality, and production locations as well as comparison of infant versus follow-up formulas did not reveal statistically significant factors. During the sampling period, one of six surveillance zones did exhibit a statistically significant trend towards higher positive rate. PFGE characterization of *Cronobacter* spp. to elucidate genetic diversity revealed only three pairs of *Cronobacter* spp. out of 25 having the same PFGE patterns.

Conclusion The current investigation indicated a lower positive rate of *Cronobacter* spp. in the powdered formula in China. This evidence suggested contamination originating from multiple different sources during the manufacturing process.

Key words: *Cronobacter* spp.; Contamination; PFGE; Powdered formula

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INTRODUCTION

Cronobacter spp. is a ubiquitous gram-negative, non-spore-forming opportunistic pathogen frequently isolated from food and environmental samples[1-3]. On occasion, it has been associated with sporadic cases or small outbreaks of sepsis, meningitis, cerebritis, and necrotizing enterocolitis. Although *Cronobacter* spp. is associated with illness in all age groups, analysis of age distribution in reported cases indicates that infants (children <1 year) are at particular risk.

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Among infants, those at greatest risk for *Cronobacter* spp. infection are neonates (<28 days), particularly low-birth weight infants or immunocompromised infants. *Cronobacter* spp. has been identified as one of the microorganisms of greatest concern with infant powdered formula[2,4].

The first 2 known cases of *Cronobacter* spp. neonatal meningitis were reported by Urmenyi and Franklin in 1961[5]. Since 1961, an increasing number of *Cronobacter* spp. infections have been reported amongst neonates, infants and children with exposure and outbreaks of infection associated with infant formula[1,6]. Prior to 2004, incidence of food contamination and related infection from *Cronobacter* spp. had not been reported in China[7]. At the inception of field surveillance for this pathogen contamination levels were observed at 12.64% (11/87) in counterfeit and low-quality powdered infant formula in Fuyang, Anhui province in 2004 and 5.19% (11/212) in powdered formula in the Chinese retail market in 2005[8-9]. The Chinese government strengthened the inspection and regulation of powdered infant and follow-up formula factories after the above two surveys[8-9], especially Sanlu milk scandal[10]. This survey was conducted to collect information about the contamination of *Cronobacter* spp. in powdered infant and follow-up formula sold in national market in all the provinces in order to obtain the contamination level and the effect of government regulation. All these isolates were further characterized using the PFGE to learn the relevance of this contamination.

MATERIALS AND METHODS

Sampling Plan

About 20 cities were investigated in each surveillance area and these cities were selected hierarchically based on economic development as measured by per capita gross domestic product (GDP).

Local retail sales data were used to develop a sampling plan that is representative of daily consumption patterns in both urban and rural markets. The sampling points should include all the retail locations for local residents where these products may be bought. Only one sample can be picked randomly from each batch powdered formula sold in the market. All brands of powdered formula produced within the surveillance area were considered for evaluation. Powdered formulas suitable from birth to 36 months were collected from markets. In total, 2282 powdered formula samples were collected during quarterly collections from January 2012 to December 2012 (Table 1).

Isolation and Identification

For this investigation, laboratories were qualified for participation if they had completed blinded control proficiency testing within the last two years and were capable of having positive isolates re-identified by BioMérieux VITEK 2 GN ID card. All the data were submitted by the National Wed-Based Reporting System of Food Microbiology Surveillance[11].

The examination of the powdered formula was performed according to Chinese *National Food Safety Standard-Food Microbiological Examination: Cronobacter* spp. GB 4789.40-2010[12]. Using a three-tube most probable number (MPN) procedure, triplicate 100 g, 10 g, and 1 g samples were aseptically weighed out for analysis and transferred into 90 mL culture media and 9 mL sterile buffered peptone water (BPW) (Huankai Micorbiology Technologies Co., Ltd., China), respectively, and incubated at 36 °C for 18±2 h. Then, 1 mL pre-enrichment medium transferred into 10 mL of mLST/vancomycin medium (Luqiao Technologies Co., Ltd., China) and incubated at 44±0.5 °C for 24±2 h. The incubated broth samples were streaked onto the

Table 1. Samples in Powdered Formula in Different Surveillance Areas

Surveillance Areas	Provinces	No. of Samples Tested
Northeast China	Liaoning, Jilin, Heilongjiang	258
North China	Beijing, Hebei, Shanxi, Neimenggu	344
East China	Jiangsu, Zhejiang, Anhui, Fujian, Jiangxi, Shandong	403
Northwest China	Shaanxi, Gansu, Qinghai, Ningxia, Xinjiang	508
Southwest China	Chongqing, Sichuan, Guizhou, Yunnan	339
South Central China	Henan, Hubei, Hunan, Guangdong,Guangxi , Hunan	430
Total		2282