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Potential reservoirs and routes of *Cronobacter* transmission during cereal growing, processing and consumption

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ABSTRACT

Cronobacter are opportunistic bacterial pathogens of both infants and adults. We investigated the incidence and distribution of *Cronobacter* in 1245 samples of cereal and related environments. 39.1% (101/258) rice-related and 46.9% (98/209) wheat-related samples tested positive for *Cronobacter*, and the positive rate differed notably according to processing method. *Cronobacter* was found in rice and wheat plants at the tillering, filling and mature stages. Soil, water and swab samples from nearby milling plants were assayed, and results revealed that 6.3% (7/122) of water from paddy fields, 49.1% (28/57) and 62.1% (41/67) of swab samples from rice and wheat flour milling plants were *Cronobacter* positive. Pulsed-field gel electrophoresis (PFGE) subtyping indicated that some strains had a common profile, which suggested their persistence in the environment, potential transmission routes and cross-contamination in processing. Finally, we surveyed 18 families to evaluate potential risks. None of the families who primarily ate rice cooked with water tested positive for *Cronobacter*, though of 66.7% families (6/9) whose food staples were produced from wheat flour tested positive. Taken together, our results are important for understanding *Cronobacter* transmission and will aid in the development of additional control measures to reduce the risk of infection by these opportunistic pathogenic bacteria.

1. Introduction

Cronobacter (formerly *Enterobacter sakazakii*) are an important group of emerging opportunistic foodborne pathogens. Serious *Cronobacter* infections in infants can lead to necrotizing enterocolitis, bacteremia and meningitis, with long-term complications for those who survive, including delayed neurological development, hydrocephalus and permanent neurological damage (Holy and Forsythe, 2014). Low-birth-weight or immunocompromised infants are particularly at risk of infections caused by this pathogen (Hunter and Bean, 2013). In addition, the mortality rate of *Cronobacter*-associated meningitis may be as high as 40–80% (Friedemann, 2009). However, rates of *Cronobacter* infection are not well understood, and typically only 4–6 cases of such infections in infants are reported annually to the Centers for Disease Control and Prevention in the United States (https://www.cdc.gov/Cronobacter/statistics.html). The majority of *Cronobacter* infections occur in the adult population, especially in immunocompromised individuals, the elderly, and in those with medical implants, extended hospital visits or acute, chronic, or serious illnesses (Alsonosi et al., 2015; Patrick et al., 2014).

Cronobacter infections among infants have been epidemiologically linked to contaminated powdered infant formula (PIF) for many decades, and large surveys of PIF samples to assess the presence of *Cronobacter* have confirmed the occasional existence of this contaminant (Norberg et al., 2012; Pei et al., 2016). Thus, surveillance of PIF products for the presence of *Cronobacter* has become routine for manufacturers and government administrators. As infant formulae are pasteurized during manufacturing and *Cronobacter* cannot survive this heat treatment (Iversen et al., 2004), the observed contamination of final PIF products suggests that the bacteria likely originate from the factory environment or heat-sensitive micronutrients added after pasteurization. Furthermore, the occurrence of infections in individuals who did not consume or handle formula (Alsonosi et al., 2015; Patrick et al., 2014) suggests that the pathogen must originate from the environment or from food other than PIF. Indeed, investigations of the presence of *Cronobacter* species in non-infant formula powdered foods, such as plant material and other dry sources, have been performed (Sani and Odeyemi, 2015). In addition, PIF can also become

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contaminated in the household environment or elsewhere after the container is opened. In several investigations of outbreaks or cases of *Cronobacter* infection in premature babies and neonates, the microorganism was isolated from blenders, bottle-cleaning brushes, and opened containers of infant formula (Kandhai et al., 2004; Friedemann, 2009).

Overall, an understanding of the distribution of *Cronobacter* in food and in the environment and their further characterization is of great importance to better understand their epidemiology and transmission. *Cronobacter* can be isolated from a wide range of foods and environments. Investigations of *Cronobacter* isolated from food have included studies of powdered infant formula (Gicova et al., 2014; Norberg et al., 2012), flour (Cetinkaya et al., 2013), dry powdered foods, cereals, cereal products (Brandao et al., 2017), spices (Li et al., 2017), herbs (Garbowska et al., 2015; Jaradat et al., 2009), vegetables (Berthold-Pluta et al., 2017; Chen et al., 2016), ready-to-eat foods (Vaconcellos et al., 2018), and various foods of animal origin (Sani and Odeyemi, 2015). With respect to environmental surveys of *Cronobacter*, research has primarily focused on the PIF production environment (Sani and Odeyemi, 2015), the household environment (Jaradat et al., 2009), and farms (Vojkovska et al., 2016). The ubiquitous distribution of *Cronobacter* has been confirmed, and the majority of *Cronobacter* isolates worldwide originate from plant sources (Chen et al., 2016). However, the ecological characteristics of *Cronobacter* are poorly understood, and an in-depth systematic investigation of *Cronobacter* habitats needs to be performed to identify the occurrence and characteristics of these pathogens present in the various food categories and diverse environments in which they can be identified.

Several studies have shown that the majority of *Cronobacter* isolates worldwide originate from plant sources (Chen et al., 2016; Sani and Odeyemi, 2015). It has also been reported that a relatively high rate of cereals test positive for *Cronobacter* (Brandao et al., 2017; Friedemann, 2007). Wheat and rice are the most favored staple foods, and rice alone feeding almost half of the world's population. In 2016, global wheat production was 749.5 million tonnes, with China producing 131.7 million tonnes, and global rice (paddy) production was 741.0 million tonnes, with China producing 211.1 million tonnes (FAOSTAT). In our study, the incidence and distribution of *Cronobacter* in rice, wheat, and their related products, as well as in their growing-, processing- and consumption-related environments, were investigated. Our aim was to identify contamination pathways and gain an understanding of how to minimize or eradicate such contamination.

2. Material and methods

2.1. Sample collection

2.1.1. Food sample collection

We collected 258 rice-related samples (37 rice spike, 22 rice kernel, 86 brown rice, 32 white rice, 11 rice flour, 21 rice noodles, 14 infant nutrition rice flour, 18 flash-frozen rice flour products, and 17 ready-to-eat product samples) and 209 wheat-related samples (83 wheat spike, 15 wheat kernel, 21 wheat flour, 30 dry noodles, 31 frozen product, and 29 ready-to-eat product samples) for *Cronobacter* isolation and identification. Samples other than spikes were purchased from local markets or online shops from October to December in 2014; rice and wheat spikes were collected directly in the field from April to June in 2016.

2.1.2. Cereal plants and environmental sample collection

Over a 2-year period (2015–2016), cereal plants and related environmental samples were collected from fields and milling plants. The relevant wheat fields and wheat flour milling plants are located in Huaibei City, Anhui Province, China (116°49′21.59″E, 33°59′34.32″N), and the relevant rice fields and rice milling plants are located in Zhuji City, Zhejiang Province, China (120°07′31.44″E, 29°42′43.26″N). Wheat at the tillering (N = 54) and jointing (N = 78) stages and rice at the tillering (N = 50) stage were sampled, with each sample consisting of approximately 500 g of leaves. Wheat at the filling (N = 59) and mature (N = 33) stages and rice at the filling (N = 55) and mature (N = 61) stages were collected, with each sample consisting of approximately 500 g of spikes. Soil from wheat fields (N = 28) and rice paddy fields (N = 34), and rice paddy water (N = 112) was collected randomly from sample sites. Environmental samples from flour milling plants (N = 67) and rice milling plants (N = 57) were collected by swabbing areas of interest (100 cm^2) (Vojkovska et al., 2016), including floors, walls, and machine surfaces. Each of the sample described above was placed into a steriled, autoclaved polypropylene bags.

2.1.3. Domestic environmental sample collection

To evaluate the potential risk of the production of staple foods from wheat and rice, we selected 18 families living in Hangzhou and divided them into two groups. Families in group 1 were originally from the north of China and primarily consumed foods prepared from wheat flour. Families in group 2 were originally from the south of China, and rice cooked with water was their primary staple. Swabs of surfaces (100 cm^2) were collected from four locations (floors, worktops, sinks, and kitchenware) (N = 72) and placed in individual, sterile screw-top containers. Vacuum cleaner dust samples (N = 18) (more than 10 g) were also collected. All samples were transferred to the laboratory in a cooler with ice (2 ± 2 °C). Sample analysis was performed within 72 h.

2.2. Isolation and identification of bacterial strains

2.2.1. Isolation and identification of Cronobacter in rice- and wheat-related samples

Cronobacter was isolated from rice, wheat and their related products according to the national food safety standard method for food microbiological examination in China (GB4789.40–2010; Ministry of Health of the People's Republic of China, 2010). Briefly, 100 g of each sample was added to 900 mL of prewarmed buffered peptone water (BPW, Beijing Land Bridge Biotechnology Co. Ltd., China) and then incubated at 37 ± 1 °C for 18 ± 2 h. Next, 1 mL of overnight culture was inoculated into 10 mL of modified lauryl sulfate tryptose (mLST, Beijing Land Bridge Biotechnology Co. Ltd., China) broth with a final concentration of 10 μg/mL vancomycin, followed by further selective cultivation at 44 ± 0.5 °C for 24 ± 2 h. From each enriched sample, a loopful of the culture broth was streaked onto agar-solidified Druggan Forsythe Iversen medium (DFI, Oxoid, UK) and incubated for 24 ± 2 h at 36 ± 1 °C. Typical blue-green colonies were selected and presumptively identified using the API ID 32E system (BioMerieux, France, Ref. 32400). The identity of the strains was further confirmed by polymerase chain reaction (PCR).

2.2.2. Isolation and identification of Cronobacter from environmental samples

A dilution series of each soil sample was made ($10^{-6} \sim 10^{-8}$) and 100 mL of each dilution was added to 900 mL BPW in a stomacher bag. The swabs from the milling plants and domiciles were placed in 100 mL BPW, and the vacuum cleaner dust samples were placed in a 1:10 (w:v) dilution of BPW. The subsequent procedures were identical to those used for *Cronobacter* isolation and identification described above, except that the incubation temperature for mLST-Vm cultures was 42 ± 1 °C (Vojkovska et al., 2016).

2.3. DNA extraction

All isolates (further investigation was conducted on one isolate of each *Cronobacter*-positive sample) were incubated in brain-heart infusion (BHI, Beijing Land Bridge Biotechnology Co. Ltd., China) broth at 37 °C for 18 h. Subsequently, cultures were streaked onto Tryptic Soy Agar (TSA, Beijing Land Bridge Biotechnology Co. Ltd., China) plates and incubated at 37 °C for 24 h to obtain isolated colonies. A single colony of each strain was inoculated into BHI and grown at 37 °C for 18 h, after which 2 mL of culture was used to extract genomic DNA with a QIAamp DNA Mini Kit in accordance with the manufacturer's instructions (Qiagen, Germany).

2.4. PCR confirmation of *Cronobacter*

Triplex PCR targeting the 16s rRNA gene, the ITS sequence and the OmpA gene was performed with an optimized primer concentration ratio of 2:7:3. Three pairs of primers targeting the ITS sequence (Liu et al., 2006), the OmpA gene (Mohan Nair and Venkitanarayanan, 2006) and the 16S rRNA gene (Hassan et al., 2007) were synthesized by Sangon Biotech, Shanghai. A TaKaRa Ex Taq® PCR kit (Catalog Number: RR001A) was used for PCR, which was performed in 50-μL reaction mixtures containing 5 μL 10 × PCR buffer with $MgCl_2$, 4 μL dNTP (2.5 mmol/L), 0.3 μL of Ex Taq polymerase (5 U/μL), 2 μL of primer (20 μmol/L), and 2 μL of DNA template. Amplification was carried out as follows: an initial denaturation for 5 min at 94 °C, followed by 30 amplification cycles of 30 s at 94 °C, 30 s at 55 °C and 1 min at 72 °C, with a final extension of 8 min at 72 °C. Subsequently, 5 μL of each PCR product was separated on a 1.5% agarose gel in 1 × TBE buffer. After staining with GelRed™ Nucleic Acid Gel Stain (Biotium, USA, Catalog Number: 41003) and visualization by ultraviolet (UV) illumination, the gels were photographed and interpreted visually. When all three gene markers tested positive, the strain was judged positive; if there only one or two gene markers tested positive, 16S rDNA sequencing was performed for confirmation. DNA extracted from *Cronobacter* ATCC 25944 and sterilized water were used as positive and negative controls, respectively.

2.5. Determination of molecular subtypes by PFGE and band analysis

Cronobacter isolate subtypes were determined by PFGE using previously described methods (Mullane et al., 2007); *Salmonella* strain H9812 was used as a molecular weight marker. DNA fingerprints were stored as TIFF (tagged image file format) files and imported into BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium). A dendrogram was created using the Dice coefficient and the unweighted-pair group method with arithmetic means. The genetic diversity of the *Cronobacter* isolates was compared with a position of 1.5% tolerance and optimization settings.

2.6. Data analysis

All data were analyzed using a Chi-square test with Excel 2010 and SPSS Version 19.0 (SPSS. Inc., Standard Version).

3. Results and discussion

Our previous investigation showed that wheat flour is a likely reservoir and/or transmission route of *Cronobacter* because of the extremely high rate (100%) at which this pathogen was detected (Lou et al., 2014). In the present study, we sought to investigate the prevalence of *Cronobacter* in rice, wheat and their related products, including their distribution and characteristics in different processing-related samples, to understand the ecological niche of *Cronobacter* in different cereals.

Our evaluation of the presence of *Cronobacter* in rice and related products (N = 258) revealed that 39.1% of the samples (101 positive tests) contained these bacteria. For wheat and related products (N = 209), *Cronobacter* was detected in 46.9% samples (98 of 209) (Table 1). A careful comparison revealed an interesting phenomenon (Table 1). Rice and wheat spike samples collected directly from the field showed relatively low positive rates of *Cronobacter* (18.9 and 3.6%) compared to the average levels observed in their own complete categories. The presence of *Cronobacter* changed notably depending on the procedure for handling raw materials. The positivity rate for rice and wheat kernel samples was 59.1 and 53.3%, respectively, which may have been due to cross-contamination when the spikes were threshed and dried. After wheat kernels had been processed into flour, 100% of the samples tested positive for *Cronobacter*, and 100% of the dry noodle and frozen product samples produced from wheat flour were also contaminated. The manners in which rice kernels are processed are more variable than those for processing wheat; rice kernels can be processed to produce brown rice, white rice and rice flour. Our results showed a decrease in the *Cronobacter* detection rate from brown rice (48.8%) to white rice (21.9%), with higher rates observed in the food categories rice flour (90.1%) and flash-frozen rice flour products (94.4%). Unlike the wheat-related products assayed, the rates at which *Cronobacter* were detected in rice noodles and infant nutrition rice flour were relatively low. Brown rice is whole-grain rice with the inedible outer hull removed; white rice is the same grain with the hull, bran layer and cereal germ removed, and rice flour is produced from finely milled white rice. Because the production of white rice removes more of the outer layers from the rice kernels compared to brown rice, it is unsurprising that *Cronobacter* was detected at a much higher rate in brown rice than in white rice. The increased presence of the pathogen in rice flour and flash-frozen rice flour products compared to white rice led to the conclusion that cross-contamination must have occurred during the rice flour processing. It is interesting and notable that the rates at which *Cronobacter* were detected in rice noodles and infant nutrition rice flour were relatively low. As a matter of fact, most of the processing for these foods in China involves a steaming or sterilization step (Li et al., 2015).

Table 1
Occurrence of *Cronobacter* in rice, wheat and their related products tested.

Origin of sample (food categories)	Number of samples analyzed	Number of positive samples (%)
Rice and rice-related products		
Rice spikes	37	7/37 (18.9)
Rice kernels	22	13/22 (59.1)
Brown rice	86	42/86 (48.8)
White rice	32	7/32 (21.9)
Rice flour	11	10/11 (90.1)
Rice noodles	21	3/21 (14.3)
Infant nutrition rice flour	14	0/14 (0)
Flash-frozen rice flour products	18	17/18 (94.4)
Ready-to-eat products	17	2/17 (11.8)
Total	258	101/258 (39.1)
Wheat and wheat-related products		
Wheat spikes	83	3/83 (3.6)
Wheat kernels	15	8/15 (53.3)
Wheat flour	21	21/21 (100)
Dry noodles	30	30/30 (100)
Frozen products	31	31/31 (100)
Ready-to-eat products	29	5/29 (17.2)
Total	209	98/209 (46.9)

(Note: All the samples collected in the rice flour, rice noodles, infant nutrition rice flour and flash-frozen rice flour product categories were produced from white rice according to the Chinese tradition.).

These findings suggest that cross-contamination may easily occur during cereal processing procedures, especially during the production of cereal flour, prompting several questions. How do they get contaminated? Where are the origin of *Cronobacter* in them? Will they pose high risk to the indoor environment contamination? Our further investigation focused on answering these questions, which highlight key gaps in the general knowledge of *Cronobacter* biology, and we attempted to identify routes of *Cronobacter* transmission.

We first attempted to identify the origin of *Cronobacter* in wheat, rice and their related products. Wheat and rice samples at different growth stages were selected to test for the presence of *Cronobacter*. In total, 22 of 224 samples of wheat at different growth stages were

Table 2
Occurrence of *Cronobacter* in different growth stages of wheat and rice related samples tested.

Origin of sample	Number of samples analyzed	Number of positive samples (%)
Wheat at the tillering stage	54	6/54 (11.1)
Wheat at the jointing stage	78	13/78 (16.7)
Wheat at the filling stage	59	2/59 (3.4)
Wheat at the mature stage	33	1/33 (3.0)
Total	224	22/224 (9.8)
Rice at the tillering stage	50	13/50 (26.0)
Rice at the filling stage	55	4/55 (7.3)
Rice at the mature stage	61	6/61 (9.8)
Total	166	23/166 (13.9)
Soil from wheat fields	28	0/28 (0)
Soil from paddy fields	34	0/34 (0)
Water from paddy fields	112	7/112 (6.3)
Total	174	7/174 (4.0)
Wheat flour milling plant	67	41/67 (61.2)
Rice milling plant	57	28/57 (49.1)
Total	124	69/124 (55.6)

positive (9.8%). *Cronobacter* was detected in 6 of 54 wheat samples at the tillering stage (11.1%) and in 13 of 78 wheat samples at the jointing stage (16.7%). In addition, 2 of the 56 wheat samples at the filling stage (3.4%) and 1 of the 33 wheat samples at the mature stage (3.0%) contained *Cronobacter*. Twenty-three of 166 samples of rice at different stages were positive (13.9%). *Cronobacter* was detected in 13 of 50 samples (26.0%) at the tillering stage, in 4 of 55 samples (7.3%) at the filling stage, and in 6 of 61 samples (9.8%) at the mature stage (Table 2). These results show that samples testing positive for *Cronobacter* were identified for all the growth stages of wheat and rice, and cereal leaves (at the tillering and jointing stages) were much more contaminated than spikes (at the filling and mature stages). Thus, we concluded that the pathogen may originate from the soil. To test this hypothesis, we collected soil samples from the area where our wheat and rice samples were collected. Unexpectedly, *Cronobacter* was not detected in any of the 28 wheat field soil samples or in the 34 rice field soil samples (Table 2). Our results are consistent with several publications (Kandhai et al., 2004; Molloy et al., 2009) reporting that *Cronobacter* could not be isolated from soil samples. Nevertheless, Shigeko Ueda (2017) was able to isolate *Cronobacter* from rice soil (1 of 25 samples), vegetable fields (4 of 26 samples) and sandboxes (8 of 24 samples). The number and species of microbes in a single gram of soil was large, particularly bacteria (Gans et al., 2005). Because of the large diversity and number of bacteria in soil samples, combined with the very low abundance of *Cronobacter*, the approach we adopted of enriching and isolating members of this genus may be not suitable for soil and requires further optimization. Then we collected 122 water samples from paddy fields, 7 of which (6.3%) contained *Cronobacter* (Table 2). This finding suggests that these pathogens may originate from soil, which acts as a microorganism bank, even though we were unable to isolate *Cronobacter* from soil samples.

We were eager to understand why this contamination was occurring in processing plants. We swabbed rice milling plants and wheat flour plants located near the rice and wheat fields: 67 samples from wheat flour milling plants, of which 41 (62.1%) tested positive for *Cronobacter*; 28 of 57 (49.1%) samples from rice milling plants tested positive. In our previous study, we indicated that wheat flour may be a possible reservoir and route of transmission for *Cronobacter*, as it was found to be highly contaminated (100%) with this microorganism and can easily diffuse through the air and settle as dust. This is a typical scenario of wheat flour plants, and our results supported this idea. The same risk is also present for rice during processing in rice milling plants.

We further subtyped these *Cronobacter* isolates from water, cereal leaves (at the tillering and jointing stages), spike (at the filling and mature stages) and milling plant samples by PFGE, generating indistinguishable PFGE profiles based on a criterion of greater than 90% pattern similarity. For isolates obtained from wheat and related environments, 31 isolates produced 26 distinguishable PFGE profiles. Of the 26 different PFGE profiles, four were common to multiple isolates, whereas the remaining 22 were distinct (Fig. 1). All four PFGE profile clusters were common to multiple isolates recovered from either the wheat leaves or swab samples from the wheat flour milling plant (Fig. 1). For isolates from rice and related environments, 34 showed 25 distinguishable PFGE profiles. Of the 25 different PFGE profiles, five were common to multiple isolates; the remaining 20 were distinct (Fig. 2). Two of five PFGE profile clusters were common to three

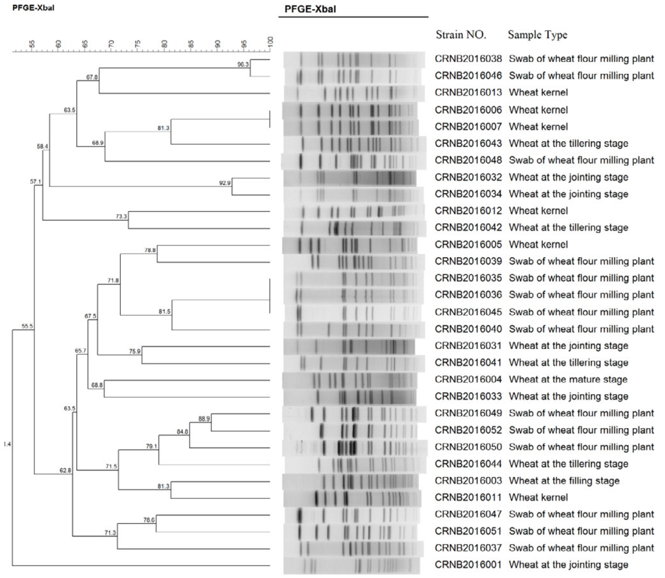

Fig. 1. Dendrogram showing *Xba*I-mediated pulsed-field gel electrophoresis (PFGE) profiles of Cronobacter strains in wheat and related environments. The dendrogram was obtained using the unweighted pair group method with arithmetic mean (UPGMA) and the DICE coefficient with 1.5% tolerance.

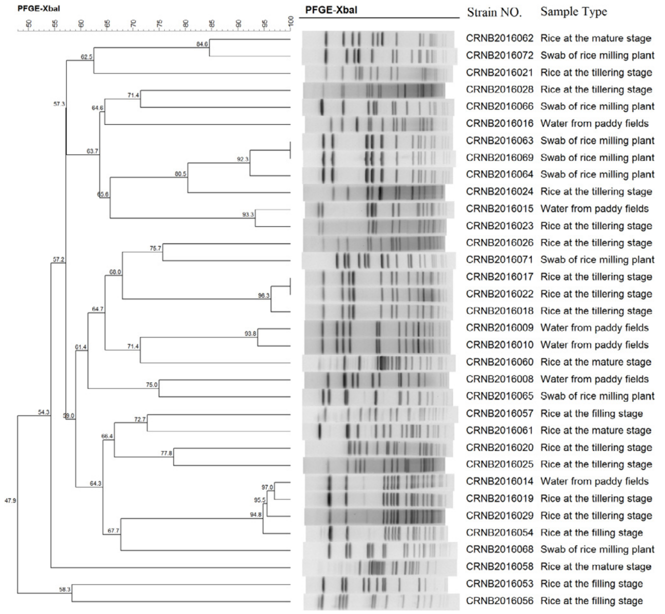

Fig. 2. Dendrogram showing *Xba*I-mediated pulsed-field gel electrophoresis (PFGE) profiles of Cronobacter strains in rice and related environments. The dendrogram was obtained using the unweighted pair group method with arithmetic mean (UPGMA) and the DICE coefficient with 1.5% tolerance.

Table 3
Occurrence of *Cronobacter* in samples collected in different primary staple foods families.

	Floor	Worktop	Sink	Kitchenware	Vacuum cleaner
Group1					
NF1	–	–	–	+	+
NF2	–	–	–	–	–
NF3	–	–	–	–	–
NF4	–	–	–	–	+
NF5	–	–	+	+	–
NF6	+	–	–	+	+
NF7	–	–	–	–	+
NF8	–	–	–	–	–
NF9	+	+	–	+	+
Group2					
SF1	–	–	–	–	–
SF2	–	–	–	–	–
SF3	–	–	–	–	–
SF4	–	–	–	–	–
SF5	–	–	–	–	–
SF6	–	–	–	–	–
SF7	–	–	–	–	–
SF8	–	–	–	–	–
SF9	–	–	–	–	–

(Note: Families in group 1 were originally from the north of China and primarily consumed foods prepared from wheat flour. Families in group 2 were originally from the south of China, and rice cooked with water was their primary staple.).

isolates from swab samples from rice milling plant and rice at the tillering stage, respectively. Another cluster was common to three isolates from rice paddy water samples. Interestingly, there was a profile cluster common to two isolates from water sample and rice at the tillering stage. What's more, one of five profile clusters (totally consist of four isolates) contained one isolate from rice paddy field water samples, two isolates from rice at the tillering stage, and one isolate from rice at the filling stage. This may indicate that these strains had a common contamination source and persist in the environment for a long time, indicating that this pathogen might be transmitted during plant growth. The profile cluster contained three isolates recovered from swab samples of rice milling plant, indicating that processing might cause cross-contamination (Fig. 2).

Staple foods of a specific society may vary by location according to local weather patterns, local farming constraints, acquired tastes and ecosystems. Individuals in northern China commonly live on a diet based on wheat, whereas individuals in southern China typically subsist on a rice-based diet. In general, wheat is often milled into flour and then used to prepare foods for household use. Whole-grain rice is typically milled into brown rice or white rice, and rice is most commonly cooked with water and then eaten. In contrast, rice flour prepared from finely milled white rice is not always available for household use. Our results show that dry flour produced from cereals has a high occurrence of *Cronobacter* and poses a risk for *Cronobacter* infections because the product can easily diffuse through the air and settle as dust in indoor environments. In fact, it is difficult to restrict the distribution and transmission of this pathogen during food preparation. Furthermore, kitchen surfaces or equipment that is in contact with *Cronobacter*-contaminated flour may become contaminated during the dough-mixing process, and further cross-contamination may easily occur during the process of washing and storing equipment.

We collected swab samples of floors, worktops, sinks, kitchenware and dust (in vacuum cleaners) from the residence of each family to test for *Cronobacter*. Interestingly, none of the samples from the residences of group 2 families tested positive, whereas samples from the residences of six of nine families (66.7%) did. Specifically, the pathogen was recovered from group 1 swab samples of floors (2 of 9), a worktop (1 of 9), a sink (1 of 9), kitchenware (4 of 9) and vacuum cleaner dust samples (5 of 9) (Table 3). This notable discrepancy suggests that *Cronobacter* contamination in domestic environments is closely related to wheat flour usage. The very high prevalence of *Cronobacter* in the domestic environment of families originally from the North of China (66.7%) is of concern, as individuals in these families are at risk of *Cronobacter* infection due to contaminated items in the kitchen area where infant formula is prepared and stored. Similar to the findings of other researchers (Jaradat et al., 2009; Kandhai et al., 2004; Reich et al., 2010), *Cronobacter* was recovered from vacuum cleaner dust. However, few studies have reported isolation of *Cronobacter* from swab samples of domestic environments (Kilonzo-Nthenge et al., 2012). The isolation of *Cronobacter* from swab samples in our study confirms that this pathogen is present in the general domestic environment of households that frequently use wheat flour.

4. Conclusions

The primary conclusion is that *Cronobacter* can be transmitted via cereals (wheat and rice) resulting from contamination that occurs during growth, processing and consumption. Frequent use of wheat flour appears to be a crucial factor for the presence of *Cronobacter* in domestic environments, and special care should be taken while preparing infant foods or formulas to avoid cross-contamination from this source. Reducing the use of dry cereal flour in residential homes with low-birth-weight or immunocompromised infants may further decrease the potential risk of *Cronobacter* infection.

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