Cronobacter spp.

Enterobacter sakazakii
Family of *Enterobacteriaceae*

Bacterium

anses
French agency for food, environmental and occupational health & safety

Characteristics and sources of *Cronobacter* spp.

Main microbial characteristics

Cronobacter spp., responsible for human infections, are motile rod-shaped bacteria from 1 to 3 µm in length, which are facultatively-anaerobic, Gram-negative, non-spore forming, thermoresistant, oxidase negative and catalase positive. A phenotypic feature useful for their detection is alpha-glucosidase activity. No serotyping scheme is known. Their ability to form biofilms, and their relative resistance to osmotic stress and desiccation compared to other enterobacteria, facilitate their persistence especially in dry environments, such as in powdered milk and its production environment.

These microorganisms, initially classified in the single species Enterobacter sakazakii, were reclassified in 2007 in a new genus: *Cronobacter*, which comprises six species: *C. sakazakii* (type species), *C. malonaticus*, *C. turicensis*, *C. muytjensii*, *C. dublinensis* (divided into 3 subspecies) and *C. geno-mospecies* 1. Apart from the latter, all these species, isolated in humans, are classified as opportunistic pathogens. Isolates associated with neonatal meningitis cases belong to the species *C. sakazakii*, *C. malonaticus* and *C. turicensis*.

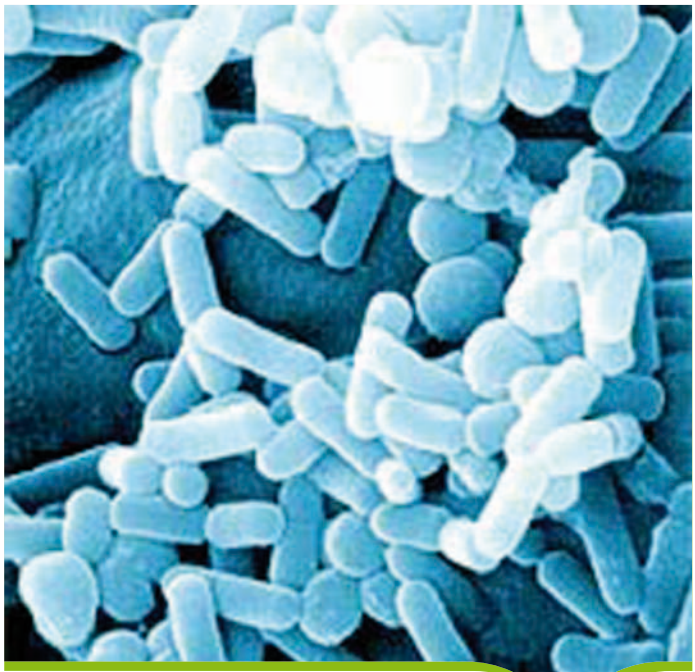
Since the first human case in 1958, knowledge of the mechanisms of virulence and pathogenicity of this organism has remained very partial and there have been few studies on the toxins produced by *Cronobacter*. Generally, the synthesis of enterotoxins may cover 22-27% of tested strains and food strains may be less virulent than human strains.

The pathogen can survive for up to two years at an a_w of 0.14-0.27 (dry product). Generation time is 300 min at 10°C, 40 min at 23°C, and 20 min at 37°C (Table 1).

Table 1. Growth characteristics

Parameters	Growth	
	Optimum	Extremes
Temperature (°C)	around 39	5.5 - 47
pH	5-9 depending on the strains	3.89 - 10
NaCl	/	1.2 mol L^{-1}

(1) Susceptible population group: people with a higher than average probability of developing symptoms of the disease, or severe forms of the disease, after exposure to a foodborne hazard [definition used for the ANSES data sheets].



Enterobacter sakazakii

Sources of the hazard

Cronobacter is a telluric microorganism, ubiquitous and found in water, soil, plants, dust and many living organisms. Rodents and insects such as flies can be vectors of contamination. *Cronobacter* has been isolated from many foods of plant or animal origin, whether dried, smoked, frozen, fermented, raw or cooked.

Transmission routes

Transmission to humans occurs solely through food. Direct transmission between humans has not been demonstrated. Asymptomatic carriage and the relative proportion of nosocomial infections are still poorly understood.

Data sheet on foodborne biological hazards
April 2011

Human foodborne illness

Nature of the disease (Table 2)

Susceptible population groups: mainly infants under two months old, with retarded growth and/or born prematurely, and very young children. To a lesser extent, the elderly and immunocompromised subjects or those with a predisposition or severe underlying disease.

Dose-effect[2] and dose-response[3] relationships

These relationships are little understood, although the dose-response relationship is assumed to be linear at low doses and the infectivity per cell (number of cases/number of exposures to a single organism) has been estimated at 8.9 10^{-6}. However this relationship is probably overestimated and is currently being reassessed.

Epidemiology

About six new cases of *Cronobacter* infections are reported each year across the world, but the incidence is probably underestimated. Approximately 130 cases of *Cronobacter* infections, including 20 cases in adults, have been documented worldwide in the last 50 years including 27 deaths. According to FoodNet, the American active surveillance network for foodborne diseases, in 2002, the rate of invasive infections caused by *Cronobacter* was estimated at 1/100,000 for infants under 12 months, rising to 8.7/100,000 for low-weight newborns.

There is no specific surveillance system for this microorganism in France and Europe. Globally, surveillance is provided by the WHO Collaborating Centre for Research, Reference and Training on *Cronobacter* (University College Dublin, Ireland). These infections are however indirectly recorded in France by the reporting system for nosocomial infections (Decree No. 2001-671 of 26 July 2001). Three major clusters or outbreaks have been reported worldwide: in Belgium in 1998 (12 children, 2 deaths), in the USA in 2001 (Knoxville, 49 children) and in France in 2004. The French episode of clustered cases, which occurred in five different hospitals, was related to consumption of powdered infant milk formula intended for special medical purposes. Nine cases were identified, among which there were four infections (including two fatal cases of meningitis) and five cases of digestive colonisation. The total number of French cases recorded to date is 22.

(2) The relationship between the dose (the amount of microbial cells ingested during a meal) and the effect on an individual.

(3) For a given effect, the relationship between the dose and the response, i.e., the probability of this effect appearing in the population.

Role of food

Main foods to consider

All powdered formulae intended for infants, young children or the elderly, including formulae for special medical purposes, and including the additives incorporated in these preparations, represent a risk. Only sterile liquid products are safe.

As *Cronobacter* does not survive pasteurisation, contamination of powdered formula occurs *via* the production environment. Consequently, studies of production facilities have identified frequent environmental contamination, particularly airborne. Raw materials that, because of their heat sensitivity, have not undergone any heat treatment prior to being incorporated in dehydrated food at the end of processing, have also been responsible for the contamination of the final product and/or its production environment.

Despite the fact that there may be a high level of contamination of the production environment, contamination of powdered formula is low: from <0.001 to a few CFUs per 10 kg in powders marketed in France in 2007. Reconstitution and storage of the product under conditions that favour *Cronobacter* growth lead to the occurrence of human infections.

Table 3. **Inactivation treatments in industrial environments**

Disinfectants	Heat
Susceptible to many disinfectants • 1% sodium hypochlorite, • 70% ethanol, • 2% glutaraldehyde.	$D_{60°C}$** = 0.9-4.4 min. $D_{70°C}$ = 0.07 min. Some strains have higher thermotolerance ($D_{58°C}$: 9.9 min) than most enterobacteria.
High pressure	**Irradiation**
600 MPa, 1 min: 3 to 6.8 $\log_{10}$ reduction*. 350-400 MPa, 10-15 min at 25°C: 7 $\log_{10}$ reduction.	D_{10}*** = 0.24 to 0.37 kGy in liquid medium. However, ionisation is not used on dehydrated formulae, due to the organoleptic and nutritional changes it causes.

* Varies depending on the strains studied.

** D is the time needed to divide by 10 the initial population of the microbiological hazard.

*** D_{10} – value: dose of radiation required to reduce the viability of a population by 90% (1 $\log_{10}$ cycle) under the stated conditions.

Table 2. **Caractéristiques de la maladie**

Mean incubation period	Target populations	Duration of shedding	Duration of symptoms
6-1[illegible]ries dep[illegible]g on the dose ingested and the host status	All consumers of foods implicated, see points "Epidemiology" and "Main foods to consider" irrespective of age and gender Especially infants fed with non-sterile infant formula milk Elderly, rather immunocompromised people	Unknown (one study shows shedding possible for up to 18 weeks)	Varies depending on the clinical forms
Main symptoms		**Complications**	**Asymptomatic forms**
Newborns	Meningitis: 41% Septicaemia, bacteraemia: 18% Necrotising enterocolitis: 16% Other (abscesses, conjunctivitis, etc.): 25%	Severe neurological sequelae (hydro-cephalus, etc.) Retarded development Estimated lethality: 40 - 80%	Yes
Adults	Abscesses: 10% Colonisations: 35% Bacteraemia: 20% Osteomyelitis: 5% Pneumonia: 20% Other (ulcers, etc.): 10%	Lethality: not estimated	Yes

Monitoring in food

Regarding powdered infant formula and powdered dietetic foods for special medical purposes in infants under six months, Commission Regulation (EC) No 2073/2005 as amended establishes a process hygiene criterion (absence of Enterobacteriaceae in 10 g, 10 samples analysed per batch) and a safety criterion (absence of *Cronobacter* in 10 g, 30 samples analysed per batch). The latter criterion is equivalent to that set by the *Codex Alimentarius*. The regulation also states that tests should be performed in parallel for Enterobacteriaceae and *Cronobacter*, unless a correlation can be established by the manufacturer, at its plant. Commission Regulation (EC) No 2073/2005 requires the use of the technical specification ISO/TS 22964.

Although water is used for the reconstitution of powdered formula, there are no analytical methods for *Cronobacter* in the field of water microbiology.

New chromogenic culture media have recently been developed and commercial methods for screening samples by molecular biology (real-time or end-point PCR) are available to detect this bacterium, but they have not all been validated against a reference method. From the perspective of molecular monitoring of *Cronobacter*, pulsed-field gel electrophoresis (PFGE) is being standardised at the level of the international PulseNet network to become the reference method.

Recommendations to operators

- **Ensure control of environmental contamination in manufacturing units for powdered formula. Bacterial growth in the production environment must be controlled by a strict hygiene plan, and moisture should be eliminated as thoroughly as possible. Effective measures therefore include detection of the presence of Enterobacteriaceae in products and ingredients, zoning (clear separation of wet and dry areas) and use of dry cleaning methods instead of those using water.**
- **Establish very strict hygiene criteria for powdered formula production lines to control Enterobacteriaceae. Although the correlation is not understood, this leads to a significant reduction in the prevalence of *Cronobacter*.**
- **Supplement the sampling recommended by the regulations so as to increase its efficacy, while bearing in mind that this alone cannot ensure product safety. The quality of production from a given production line can usefully be estimated by combining monitoring data from successive batches and analysing trends, as proposed by Commission Regulation (EC) No. 2073/2005.**
- **Avoid "pooling" (grouping test samples at a constant dilution rate for microbiological testing) because it has a negative impact on the performance of the *Cronobacter* detection method.**
- **Establish acceptance criteria for raw materials and dry ingredients added to powdered formula.**

Domestic hygiene

External contamination at home or in hospitals during the reconstitution of powdered milk is possible (by transfer of contamination using, for example, contaminated utensils). In clustered cases observed in Israel in 2001, contamination of a food mixer was responsible. Poor reconstitution and storage conditions can also allow the growth of the pathogen and the occurrence of infections.

AFSSA in 2005 and the WHO in 2007 published recommendations on good practices for the reconstitution, storage and handling of powdered infant formula; these documents are intended for facilities caring for pre-school children, hospitals and individuals.

The WHO concluded that reconstituting bottles of formula at temperatures greater than or equal to 70°C could significantly reduce the risk of contamination of powdered formula by destroying *Cronobacter*. However this method of control is controversial, not only because of the risk of burns from ingestion in the event of inadequate cooling of bottles, but also because of the potential destruction of certain nutrients and the possible germination of *Bacillus cereus* spores.

Recommendations to consumers

- **Wash hands before preparing food and before eating.**
- **Use clean utensils and baby bottles.**
- **Reduce to a maximum of one hour the time between preparation and consumption if the product is at room temperature, and 30 minutes if it has been heated.**
- **Keep reconstituted meals/bottles of formula at temperatures not exceeding 4°C and for a maximum of 48 hours.**
- **After use: empty the bottle, rinse under the cold water tap and then plunge the bottle and its accessories in warm water and washing-up liquid, clean the bottle with a brush and rinse. Allow to dry without wiping. Thorough cleaning and draining are enough. It is not necessary to sterilise the bottle.**
- **Use water intended for human consumption.**
- **Preferably use sterile formula in liquid form for the infants who are most susceptible to infection.**

References and links

General references

- AFSSA (2005). Hygiene recommendations for the preparation, handling and storage of feeding bottles. http://www.anses.fr/cgi-bin/countdocs.cgi?Documents/MIC-Ra-BIB.pdf
- AFSSA (2007). Microbial contamination of powdered formulae for infants and the elderly. http://www.anses.fr/cgi-bin/countdocs.cgi?Documents/MIC-Ra-PoudresLait.pdf
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- FAO-WHO (2007). Safe preparation, storage and handling of powdered infant formula: Guidelines. http://www.fao.org/ag/agn/agns/files/pif_guidelines.pdf
- FAO-WHO (2008). *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered follow-up Formulae. Meeting Report. Microbiological Risk Assessment Series 15. http://whqlibdoc.who.int/publications/2008/9789241563796_eng.pdf

Useful links

- WHO Collaborating Centre for Research, Reference and Training on *Cronobacter* (University College Dublin, Ireland). http://www.ucd.ie/cfs/whocollaboratingcentre/