

INFORME BREVE

First clinical isolates of *Cronobacter* spp. (*Enterobacter sakazakii*) in Argentina: Characterization and subtyping by pulsed-field gel electrophoresis

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Subtyping

Abstract

Cronobacter species are opportunistic pathogens associated with severe infections in neonates and immunocompromised infants. From January 2009 through September 2010, two cases of neonatal infections associated with *Cronobacter malonaticus* and one case associated with *Cronobacter sakazakii*, two of them fatal, were reported in the same hospital. These are the first clinical isolates of *Cronobacter* spp. in Argentina. The objective of this work was to characterize and subtype clinical isolates of *Cronobacter* spp. in neonate patients, as well as to establish the genetic relationship between these isolates and the foodborne isolates previously identified in the country. Pulsed-field gel electrophoresis analysis showed a genetic relationship between the *C. malonaticus* isolates from two patients. Different results were found when the pulsed-field gel electrophoresis patterns of clinical isolates were compared with those deposited in the National Database of *Cronobacter* spp.

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PALABRAS CLAVE

Cronobacter;
Cronobacter malonaticus;
Cronobacter sakazakii;
Enterobacter sakazakii;
 Electroforesis en campo pulsado;
 Subtipificación

Primeros aislamientos clínicos de *Cronobacter* spp. (*Enterobacter sakazakii*) en Argentina: caracterización y subtipificación por electroforesis en campo pulsado
Resumen

Las especies del género *Cronobacter* son patógenos oportunistas asociados a infecciones graves en neonatos y niños inmunocomprometidos. Entre enero de 2009 y septiembre de 2010, en un mismo hospital se hallaron tres casos de infecciones neonatales, dos con desenlace fatal. Dos estuvieron asociados a *Cronobacter malonaticus* y uno a *Cronobacter sakazakii*. Estos son los primeros aislamientos clínicos de *Cronobacter* spp. notificados hasta el momento en Argentina. El objetivo de este trabajo fue caracterizar y subtipificar los aislamientos clínicos de *Cronobacter* spp. y establecer la relación genética entre dichos aislamientos y cepas de origen alimentario, previamente identificadas en el país. El análisis de los patrones de electroforesis en campo pulsado mostró una alta relación genética entre los aislamientos de *C. malonaticus* de dos de los pacientes. Los patrones de campo pulsado provenientes de las muestras clínicas resultaron diferentes a los patrones presentes en la Base de Datos Nacional de *Cronobacter* spp., de aislamientos de alimentos.

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Cronobacter spp., formerly *Enterobacter sakazakii*, are members of the *Enterobacteriaceae* family. These organisms were originally identified as yellow-pigmented *Enterobacter cloacae*. In 1980, they were classified as a new species, *Enterobacter sakazakii*. More recently, further phenotypic and molecular studies led to their reclassification into a new genus named *Cronobacter*, which comprises the type species *Cronobacter sakazakii* and six other species, *Cronobacter malonaticus*, *Cronobacter turicensis*, *Cronobacter muytjensii*, *Cronobacter dublinensis*, *Cronobacter condimenti* and *Cronobacter universalis*⁷⁻⁹.

Cronobacter species are opportunistic pathogens that have been associated with severe infections such as septicemia, meningitis and necrotizing enterocolitis mainly in neonates and immunocompromised infants^{7,8}. Severe neurological complications such as hydrocephalus, quadriplegia, brain abscesses and developmental delay have been observed in surviving patients.

Cronobacter spp. is widely spread in the environment and has been found in a variety of food, being the powdered infant formula (PIF) the main infection source described⁷. Furthermore, PIF contamination has been demonstrated, both as intrinsic to the product as well as during its preparation for consumption. Therefore, cleaning equipment, hospital environment and proceedings must be taken into consideration for surveillance and control of this pathogen⁵.

In Argentina, from 2005 through 2008, the mentioned pathogen was found in three different brands of imported PIF manufactured by companies from three different countries¹⁵. However, *Cronobacter* spp. infections have not been documented in our country so far.

In January 2009, a case of meningitis and septicemia in a preterm neonate twin was associated with *Cronobacter* spp. infection isolated from blood culture and cerebrospinal fluid (neonate A) at "Hospital Universitario Austral, Universidad Austral", Buenos Aires, Argentina. In March 2009, a second case of septicemia, necrotizing enterocolitis with intestinal perforation in a preterm, neonate twin

having low birth weight was registered in the same hospital (neonate B). This case presented co-infection by *Cronobacter* sp., *Enterobacter cloacae*, coagulase-negative staphylococci and *Enterococcus faecalis* (isolated from peritoneal liquid). Both patients had been fed with fluid and breast milk. An antibiotic treatment with vancomycin and amikacin was administered; however, both patients died. Fluid milk batches given to the neonates at the time of the infection were cultured in BACTEC bottles (Becton Dickinson, Franklin Lakes, NJ, USA) for 5 days but no pathogens were recovered.

To investigate possible environmental sources, swab samples were collected from the counter, tap, sink, floor, refrigerator, breast pump and trolley found in the feeders within neonatal intensive care units during the week following the patients' diagnosis. These swabs, together with one breast milk sample (from neonate A's mother) were cultured in blood agar and chocolate agar (bioMérieux, Marcy l'Etoile, France). At the same time, these samples were cultured in brain heart infusion broth and thioglycolate broth (Britania, Buenos Aires, Argentina), and after enrichment, the cultures were transferred to solid agar plates for isolation of *Cronobacter* spp. This microorganism could not be recovered from any of the samples tested.

In September 2010, a third case of septicemia in a preterm neonate was associated with *Cronobacter* spp. infection, isolated from blood culture, at the same hospital (neonate C). The patient had been fed with breast milk through enteral feeding tubes. An antibiotic treatment with meropenem and amikacin was administered for fourteen days and the patient recovered. Clinical characteristics of the three cases described above are presented in Table 1.

The working protocol was approved by the Institutional Review Board at Universidad Austral (Comité de Evaluación Institucional de la Universidad Austral).

The objectives of this work were to characterize and subtype the clinical isolates of *Cronobacter* spp. from the neonate patients and to establish the genetic relationship

Figure 1. A

Dice (Opt:1.00%) (Tol:1.5%-1.5%) (H>0.0% S>0.0%) p.D.%-98.3%
 PFGE-XbaI PFGE-XbaI



Figure 1. B

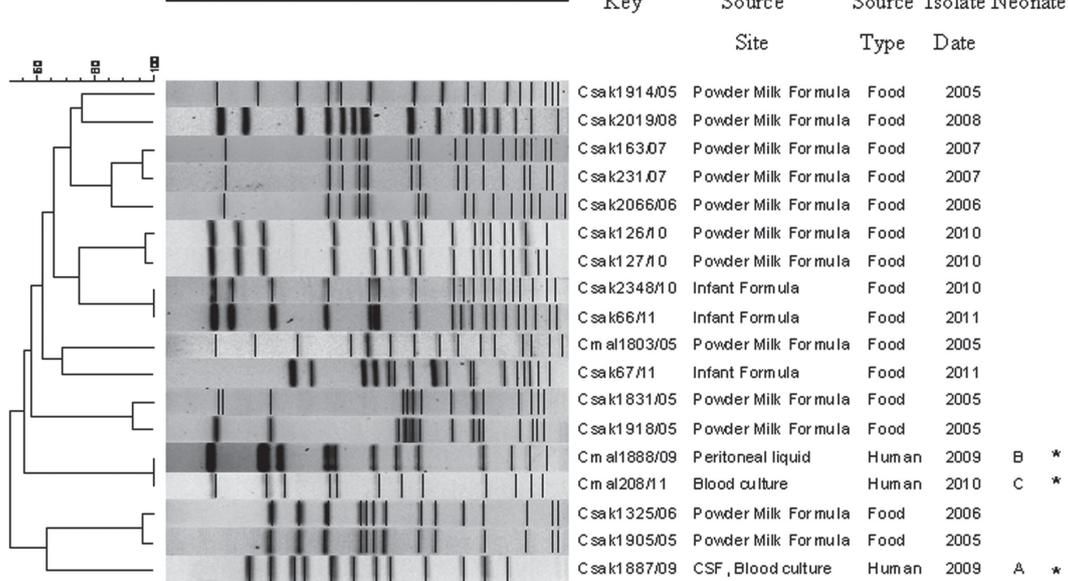
Dice (Opt:1.00%) (Tol:1.5%-1.5%) (H>0.0% S>0.0%) p.D.%-98.3%
 PFGE-SpeI PFGE-SpeI



Cma1: *Cronobacter malonaticus*

Figure 1 Pulsed-field gel electrophoresis (PFGE) dendrogram of the genetic relationship of the clinical isolates of *Cronobacter malonaticus*. A) *XbaI*-PFGE dendrogram. B) *SpeI*-PFGE dendrogram.

Dice (Opt:1.50%) (Tol:1.5%-1.5%) (H>0.0% S>0.0%) p.D.%-98.3%
 PFGE-XbaI PFGE-XbaI



* Clinical isolates

Csak: *Cronobacter sakazakii*

Cma1: *Cronobacter malonaticus*

CSF: cerebrospinal fluid

Figure 2 *XbaI* Pulsed-field gel electrophoresis (PFGE) dendrogram showing the genetic relationship of the clinical isolates and a selection of isolates recovered from imported powdered infant formula and infant formula of *Cronobacter sakazakii* and *Cronobacter malonaticus*.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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