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# Predominance of *Cronobacter* *sakazakii* Sequence Type 4 in Neonatal Infections

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A 7-loci (3,036 nt) multilocus sequence typing scheme was applied to 41 clinical isolates of *Cronobacter sakazakii*. Half (20/41) of the *C. sakazakii* strains were sequence type (ST) 4, and 9/12 meningitis isolates were ST4. *C. sakazakii* ST4 appears to be a highly stable clone with a high propensity for neonatal meningitis.

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*Cronobacter* is a genus within the family *Enterobacteriaceae* and was previously known as *Enterobacter sakazakii*. It is closely related to the genera *Enterobacter* and *Citrobacter*. *Cronobacter* spp. have been frequently isolated from the environment, plant material (wheat, rice, herbs, and spices), and various food products, including powdered infant formula (PIF). They have come to prominence because of their association with severe neonatal infections, which can be fatal (1–3). Our current knowledge of the virulence and epidemiology of this organism is limited. However, because neonates are frequently fed reconstituted PIF, this product has been the focus of attention for reducing infection risk to neonates because the number of exposure routes is limited (1,2).

Infections with *Cronobacter* spp. occur across all age groups, and most infections, albeit less severe, are in the adult population. However, neonates, particularly those of low birthweight, are the major identified group at risk, because the organism can cause meningitis, necrotizing enterocolitis (NEC), and sepsis in patients in neonatal intensive care units and has high mortality rate (1–3). Bowen and Braden (4) reviewed 46 cases of invasive (non-NEC) infant *Cronobacter* infections to define risk factors and provide guidance for prevention and treatment. Although these infections have been associated with intrinsically and extrinsically contaminated PIF, other environmental sources are possible and several non–infant formula–associated cases have been reported (5). *Cronobacter* spp. have been shown to invade human intestinal cells, replicate in macrophages,

and invade the blood–brain barrier (6). Kucerova et al. (7,8) used comparative genomic hybridization-based analysis to describe a range of virulence traits in *Cronobacter* spp., including iron acquisition mechanisms, fimbriae, and macrophage survival.

Recently, Baldwin et al. (9) constructed a comprehensive multilocus sequence typing (MLST) scheme for *Cronobacter* spp. based on 7 housekeeping genes (*atpD*, *fusA*, *glnS*, *gltB*, *gyrB*, *infB*, *ppsA*; 3,036 nt concatenated length). The MLST scheme currently has 66 defined sequence types covering all *Cronobacter* spp. ([www.pubMLST.org/cronobacter](http://www.pubMLST.org/cronobacter)). However, the scheme has not been applied for any epidemiologic purposes. Therefore, we investigated whether severity of infection by *Cronobacter* spp. is associated with particular genotype(s) by compiling patient details, isolation site, and clinical signs for clinical *C. sakazakii* isolates and comparing these with the sequence type (ST) profile of the isolates.

## The Study

Forty-one clinical *C. sakazakii* strains were included in the study. These strains were from 7 countries and had been isolated during 1953–2008. The strains included those of recent (1–3,10–12) and those of more historic interest (>25 years; 13–15). Strains used in this study, along with patient details and clinical signs, are shown in Table 1. Details of clinical signs were collated from information in the associated publication, or supplied by the strain provider (Centers for Disease Control and Prevention, Atlanta, GA, USA). Primers and conditions for amplification and sequencing of the 7 MLST genes *atpD* (390 bp), *fusA* (438 bp), *glnS* (363 bp), *gltB* (507 bp), *gyrB* (402 bp), *infB* (441 bp) and *ppsA* (495 bp) were as described (9). All sequences are available for download and independent analysis through open access at [www.pubMLST.org/cronobacter](http://www.pubMLST.org/cronobacter).

Comparative analysis with the online *Cronobacter* MLST database (covering isolates from all sources) showed that the clinical isolates were in 10 of 30 STs defined for *C. sakazakii* spp. However, the clinical strains were not evenly distributed across the STs. Of particular interest was that half (20/41) of the strains were ST4 (Table 2). The remaining strains were ST8 (7), ST1 (4), ST12 (3), ST3 (2), ST13, ST15, ST18, ST31, and ST41 (1 each). Of the 20 ST4 strains, 10 were from neonates, 7 from infants, and 1 from a child; 2 had no patient details. Similarly, most (9/12) isolates from meningitis cases were ST4 strains; 7 were isolated from cerebrospinal fluid and the others from blood and the trachea. The remaining ST4 strains were from bacteremia cases (1), NEC (2), and undefined infection (1), with 6 from unknown sources. ST4 was the main ST associated with neonates (10/18); this ST has been reported by Baldwin et al. (9) for the high incidence of PIF isolates.

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The ST4 clinical strains were from 6 countries (the Netherlands, France, United States, New Zealand, Czech Republic, and Canada) and had been isolated during 1977–2008 (Table 1). Of the 30 strains with known patient details, only 1 isolate (ST1) was from an adult patient. To date, all other isolates from adults have been identified as *C. malonaticus* (S. Joseph, unpub. data).

## Conclusions

The 7 housekeeping genes for MLST analysis are not virulence related, but a large proportion of severe neonatal infections were caused by a single sequence type. Whether this is caused by survival characteristics increasing persistence under desiccated conditions, and hence neonatal exposure or particular virulence capabilities, is uncertain.

Table 1. Strains used in study of *Cronobacter sakazakii* genotypes and disease severity and clinical details derived from original case histories\*

| Strain      | Patient type/age (EGA)† | Clinical signs/outcome                  | Isolation site       | Year | Country     | ST | Reference |
|-------------|-------------------------|---|----------------------|------|-------------|----|-----------|
| 553         | Neonate/1 d             | UNK                                     | UNK                  | 1977 | Netherlands | 4  | (15)      |
| 557         | Neonate/5 d             | UNK                                     | UNK                  | 1979 | Netherlands | 4  | (15)      |
| 693         | Neonate/13 d (41 wk)    | Asymptomatic                            | Feces                | 1994 | France      | 13 | (3)       |
| 695         | Neonate/15 d (32 wk)    | Fatal NEC II                            | Trachea              | 1994 | France      | 4  | (3,6)     |
| 701         | Neonate/28 d (28 wk)    | Fatal NEC III                           | Peritoneal fluid     | 1994 | France      | 4  | (3,6)     |
| 709         | Neonate/18 d (29 wk)    | Septicemia                              | Trachea              | 1994 | France      | 4  | (3,6)     |
| 767         | Neonate/19 d (31 wk)    | Fatal meningitis                        | Trachea              | 1994 | France      | 4  | (3,6)     |
| 721         | Neonate/2 wk            | Meningitis                              | CSF                  | 2003 | USA         | 4  |           |
| 978         | Neonate/<1 wk           | UNK                                     | Enteral feeding tube | 2007 | UK          | 3  | (12)      |
| 696         | Neonate/17 d (32 wk)    | NEC II                                  | Feces                | 1994 | France      | 12 | (3,6)     |
| 984         | Neonate/3–4 wk          | UNK                                     | Enteral feeding tube | 2007 | UK          | 3  | (12)      |
| 690         | Neonate/27 d (31 wk)    | Asymptomatic                            | Feces                | 1994 | France      | 12 | (3)       |
| 1218        | Neonate/<1 mo (30 wk)   | Fatal meningitis                        | CSF                  | 2001 | USA         | 1  |           |
| 1219        | Neonate/<1 mo (36 wk)   | Fatal meningitis                        | CSF                  | 2002 | USA         | 4  |           |
| 1221        | Neonate/<1 mo           | Meningitis, adverse neurologic outcome  | CSF                  | 2003 | USA         | 4  |           |
| 1225        | Neonate/<1 mo (35 wk)   | Fatal meningitis                        | Blood                | 2007 | USA         | 4  |           |
| 1231        | Neonate (33 wk)         | Fatal neurologic damage                 | Feces                | 2004 | New Zealand | 4  | (2)       |
| HPB 3290    | Neonate (33 wk)         | Meningitis                              | CSF                  | 2001 | USA         | 1  | (1)       |
| 1249        | Neonate                 | Fatal infection                         | UNK                  | 2009 | UK          | 31 |           |
| 1220        | Infant/6 wk (37 wk)     | Brain abscess, nonfatal                 | CSF                  | 2003 | USA         | 4  |           |
| 1223        | Infant/6 wk (31 wk)     | UNK, in ICU                             | Blood                | 2004 | USA         | 4  |           |
| 1240        | Infant/7 wk             | Fatal meningitis                        | CSF                  | 2008 | USA         | 4  | (11)      |
| 1242        | Infant/7 wk             | Fatal meningitis                        | Brain                | 2008 | USA         | 4  | (11)      |
| 1241        | Infant/7 mo             | Sudden infant death syndrome            | Blood                | 2008 | USA         | 1  | (11)      |
| 1222        | Infant/8 mo             | Fever, recovered                        | Blood                | 2003 | USA         | 4  |           |
| 1224        | Infant/10 mo            | Fever, severe combined immunodeficiency | Blood                | 2004 | USA         | 4  |           |
| HPB 2856    | Child/6 y               | UNK                                     | UNK                  | 2002 | Canada      | 15 | (10)      |
| ATCC 29544  | Child                   | UNK                                     | Throat               | 1980 | USA         | 8  | (13)      |
| 20          | Child/6 y               | UNK                                     | Feces                | 2004 | Czech Rep   | 4  |           |
| 12          | Adult/74 y              | UNK                                     | Feces                | 2004 | Czech Rep   | 1  |           |
| CDC 0743–75 | UNK                     | Foot wound                              | Wound                | 1975 | USA         | 41 | (13)      |
| CDC 407–77  | UNK                     | UNK                                     | Sputum               | 1977 | USA         | 8  | (13)      |
| CDC 996–77  | UNK                     | UNK                                     | Spinal fluid         | 1977 | USA         | 8  | (13)      |
| NCTC 9238   | UNK                     | UNK                                     | Abdomen pus          | 1953 | UK          | 18 | (15)      |
| HPB 2852    | UNK                     | UNK                                     | UNK                  | 1990 | Canada      | 8  | (10)      |
| HPB 2853    | UNK                     | UNK                                     | UNK                  | 1990 | Canada      | 4  | (10)      |
| 511         | UNK                     | UNK                                     | UNK                  | 1983 | Czech Rep   | 8  | (14)      |
| 513         | UNK                     | UNK                                     | UNK                  | 1983 | Czech Rep   | 8  | (14)      |
| 520         | UNK                     | UNK                                     | UNK                  | 1983 | Czech Rep   | 12 | (14)      |
| 526         | UNK                     | UNK                                     | UNK                  | 1983 | Czech Rep   | 8  | (14)      |
| 558         | UNK                     | UNK                                     | UNK                  | 1983 | Netherlands | 4  | (15)      |

\*EGA, estimated gestational age; ST, sequence type; UNK, unknown; NEC, necrotizing enterocolitis; CSF, cerebrospinal fluid; ICU, intensive care unit; Czech Rep, Czech Republic; CDC, Centers for Disease Control and Prevention.

†Numbers in parenthesis are estimated gestational age. Values <37 weeks are considered premature.

Table 2. Summary of *Cronobacter sakazakii* sequence types and source details from study of *C. sakazakii* genotypes and disease severity\*

| ST    | No. infections | Patient details |         |       |       |     | Clinical signs |            |     |           |              |     |
|-------|----------------|-----------------|---------|-------|-------|-----|----------------|------------|-----|-----------|--------------|-----|
|       |                | Neonate†        | Infant‡ | Child | Adult | UNK | Meningitis     | Bacteremia | NEC | Infection | Asymptomatic | UNK |
| 1     | 4              | 2               | 1       |       | 1     |     | 2              | 1          |     |           |              | 1   |
| 3     | 2              | 2               |         |       |       |     |                |            |     |           |              | 2   |
| 4     | 20             | 10              | 7       | 1     |       | 2   | 9              | 1          | 2   | 2         |              | 6   |
| 8     | 7              |                 |         | 1     |       | 6   | 1              |            |     |           |              | 6   |
| 12    | 3              | 2               |         |       |       | 1   |                |            | 1   |           | 1            | 1   |
| 13    | 1              | 1               |         |       |       |     |                |            |     |           | 1            |     |
| 15    | 1              |                 |         | 1     |       |     |                |            |     |           |              | 1   |
| 18    | 1              |                 |         |       |       | 1   |                |            |     | 1         |              |     |
| 31    | 1              | 1               |         |       |       |     |                |            |     | 1         |              |     |
| 41    | 1              |                 |         |       |       | 1   |                |            |     | 1         |              |     |
| Total | 41             | 18              | 8       | 3     | 1     | 11  | 12             | 2          | 3   | 5         | 2            | 17  |

\*ST, sequence type; UNK, unknown; NEC, necrotizing enterocolitis.

†Age <28 d.

‡Age 28–364 d.

It is plausible that different age groups are exposed to different genotypes of *C. sakazakii* according to their diet and lifestyle. *C. sakazakii* ST4 appears to be a stable clone because strains have been isolated from 7 countries for >50 years. The earliest (1951) nonclinical isolate was from a can of dried milk (13). Whether this clonal nature occurs in other *Cronobacter* spp. awaits future investigation.

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