Cronobacter spp.

BRIAN P. BLACKWOOD¹ and CATHERINE J. HUNTER¹

¹Ann and Robert H. Lurie Children’s Hospital of Chicago, Chicago, IL 60611

ABSTRACT The Cronobacter group of pathogens, associated with severe and potentially life-threatening diseases, until recently were classified as a single species, Enterobacter sakazakii. The group was reclassified in 2007 into the genus Cronobacter as a member of the Enterobacteriaceae. This chapter outlines the history behind the epidemiology, analyzes how our understanding of these bacteria has evolved, and highlights the clinical significance the Cronobacter spp. have for neonatal and elderly patient populations and treatment of the associated infections.

INTRODUCTION

The genus Cronobacter consists of a group of opportunistic Gram-negative pathogens associated with severe and potentially life-threatening medical diseases. Until fairly recently, the Cronobacter genus was thought to be a single species known as Enterobacter sakazakii (1–3). However, in 2007, the genus Cronobacter was formally named as a member of the Enterobacteriaceae family (4). Through new identification techniques and genomic sequencing, we have since learned a great deal about the bacteria that make up this genus (5, 6). Now, 10 individual species have been identified (2, 7, 8). These bacteria have been found in a wide range of environments, including water, food, and soil (9). In the clinical setting, Cronobacter spp. have been isolated in feces, sputum, blood, bone marrow, and cerebrospinal fluid (6, 10).

These bacteria are associated with neonatal diseases, including necrotizing enterocolitis (NEC), meningitis, and sepsis (11). Additionally, in the elderly population, Cronobacter has been linked to cases of urosepsis and bacteremia (2). Despite a better understanding of this genus, the pathophysiology behind its infections is not well known, and few virulence factors have been identified.

This chapter outlines the history behind the epidemiology of the genus Cronobacter. We analyze how our understanding of these bacteria has changed over time. Additionally, we highlight the clinical significance these opportunistic bacteria have for both the neonatal and elderly patient populations as well as the treatment of the associated infections.

CLASSIFICATION, EPIDEMIOLOGY, AND ENVIRONMENTAL SOURCES

Cronobacter spp. are Gram-negative, non-spore-forming, motile rods (Fig. 1) (9). The initial Cronobacter infection was reported in 1961 by Urmenyi and Franklin (12). At that time, it was thought to be yellow-pigmented Enterobacter cloacae. It was not until 1980 that species status was given when the bacterium was named Enterobacter sakazakii (1). It was then another 28 years before Enterobacter sakazakii was accurately described as the genus Cronobacter, a member of the family Enterobacteriaceae (1–4).

Advances in culture and measuring techniques have allowed us to gain a better understanding of the Cronobacter spp. The use of 16S ribosomal rRNA genes, hsp60 sequencing, and polyphasic analysis was responsible for truly shedding light on the fact that Enterobacter sakazakii isolates were actually unique species
The natural environment of *Cronobacter* spp. is not completely clear, but species have been found in a variety of environments, including water, soil, and plants. Additionally, these bacteria have been detected in both raw and fresh animal and vegetable products. They have also been found in processed and prepared foods that include powdered milk substitutes, processed cheeses, meats, spices, and herbs (20–23). Cockroaches, flies, and rats have been identified as potential sources of contamination as well (24–26).

*Cronobacter* is not typically found in the mammalian intestinal tract, and there has to date been only one report of it being isolated from the human vagina (27). This makes vertical transmission unlikely. *Cronobacter*-contaminated powder infant formula (PIF), which led to clinical disease, became a popular topic of both scientific and lay conversation (22, 28). Outbreaks of neonatal infections, specifically NEC and meningitis, have been linked directly to contaminated PIF (Fig. 2) (29). Early studies of powdered breast milk substitutes found 141 breast milk substitutes from 35 different countries to be contaminated with *Cronobacter* spp. (30).

In more recent years, *Cronobacter* isolates have continued to be found in formula and weaning foods (24). *C. sakazakii* has demonstrated its remarkable tolerance to desiccation and temperature extremes, being able to withstand temperatures as high as 60°C and as low as 4°C (31–34). This thermoresistance has allowed *C. sakazakii* to survive standard manufacturing processes. The Food and Agriculture Organization and the World Health Organization have, as a result, produced specific recommendations for PIF to be reconstituted with hot water at a temperature of >70°C in order to help reduce bacterial load and infection rates (35–38).

### CLINICAL ASSOCIATIONS

There have been many cases reported of *Cronobacter*-related infections, and many of these were reported as outbreaks (3, 37–40). *Cronobacter* is associated with infections in neonates and infants, as well as infections in the elderly (2).

*Cronobacter* is most known clinically for its association with neonatal infections, including NEC, meningitis, and sepsis (39, 41, 42). There are estimated rates of *Cronobacter* neonatal infections in the United States of 1 per 100,000 infants, 8.7 to 9.4 per 100,000 low-birth-weight infants, and 1 per 10,660 very-low-birth-weight infants (2, 3, 37, 38, 43). In fact, the sentinel *Cronobacter* case in 1961 was a case of neonatal meningitis (1). Previously thought to be as high as 80%, the

---

**FIGURE 1** Image is taken at magnification of x100 with z-stack under fluorescein isothiocyanate, 4',6-diamidino-2-phenylindole (DAPI), and differential interference contrast channels. *Cronobacter sakazakii* has been cloned with green fluorescent protein expression and is shown in green; the nucleus is stained with DAPI and shown in blue. *C. sakazakii* can be seen to permeabilize the cell (Caco-2). Scale bar is 15 μm.
Cronobacter is also associated with neonatal meningitis. This disease process has an extremely poor clinical outcome, with an approximate mortality rate of 42% (2, 3). It usually manifests in infants that are born at full gestational age, and it occurs in the first few days of life (2, 44). Cronobacter-associated meningitis causes ventriculitis, brain abscesses, brain cysts, cerebral infarcts, and late development of hydrocephalus, requiring ventriculoperitoneal shunt placement (54–56). Those patients that survive the disease can suffer from mental and/or physical developmental delay and quadriplegia (2, 57).

While not to the same degree as in the neonates, Cronobacter infections also affect the elderly. In the United States, there are approximately 3.93 Cronobacter-related cases per 100,000 people (58). In adults, Cronobacter spp. have been associated with bacteremia, splenic abscesses, osteomyelitis, pneumonia, wound infections, conjunctivitis, and urinary tract infections (2, 59–61). Reports indicate that older and immunocompromised adults seem to be at an increased risk of Cronobacter infection (2, 59).

**PATHOGENESIS AND VIRULENCE**

The pathogenesis and virulence of Cronobacter-related infections are an area of active research (3, 62, 63). Genome sequencing has allowed for potential virulence factors to be identified (64). Of the 4,392 genes identified in Cronobacter sakazakii, 223 appear to be related to virulence (6). The encoded proteins have been shown to be associated specifically with acid transport, phosphotransferase systems, and pilus assembly as well as both toxin and antitoxin transport systems (3, 65).

One of the best-characterized virulence factors to date is outer membrane protein A (OmpA). This virulence factor is encoded by the *ompA* gene, and it has been noted to be required, not only by Cronobacter but also by *Escherichia coli* K1, for binding and invading the endothelial cells of the brain, leading to meningitis (66–68). Cronobacter has also been shown to produce an endotoxin-like compound which aids in its translocation across both the blood-brain barrier in the central nervous system and the intestinal epithelial barrier in the gut. Additionally, this toxin seems to work similarly to lipopolysaccharide in that it induces an inflammatory response secondary to Toll-like receptor 4 activation (69, 70).

With regard to oral inoculation, it appears as though Cronobacter adheres to the intestinal epithelial cells, causing breakdown of the intestinal epithelial barrier and producing an inflammatory response (3). The...
inflammatory cascade, inducible nitric oxide synthase, and interleukin-6, all activated by *Cronobacter*, then cause downstream effects, such as apoptosis (46, 62, 71). *Cronobacter* then breaks through the barrier, gaining access to the bloodstream and causing sepsis or meningitis in the susceptible host. Evidence suggests that *Cronobacter* virulence factors may also help resist complement-mediated killing and can even alter the host immune response, in effect allowing these bacteria to hide from the host’s immune system (71, 72).

**ANTIBIOTIC SUSCEPTIBILITY**

Antibiotic treatment is essential in the case of a patient with a confirmed *Cronobacter* infection. The traditional antibiotic regimen for *Cronobacter* spp. was ampicillin in combination with either gentamicin or chloramphenicol (57). However, like many bacteria in our current age, *Cronobacter* spp. have developed resistance to these antibiotics (57, 73). There is a discrepancy in the literature as to how *Cronobacter* resistance occurs and whether or not these bacteria produce beta-lactamases. There have been studies that report beta-lactamase production in nearly all identified *Cronobacter* spp., while others report finding no beta-lactamase production (57, 74, 75). To date, *Cronobacter* isolates have been found to be resistant to not only ampicillin but also most first- and second-generation cephalosporins (23, 57). As a result, it has been suggested that carbapenems or third-generation cephalosporins be used with an aminoglycoside or trimethoprim-sulfamethoxazole (57). This treatment has been relatively successful for *Cronobacter*-related infections, but antibiotic resistance appears to be increasing (57, 76).

**CONCLUSION**

In conclusion, the once-misnamed genus now known as *Cronobacter* represents a group of opportunistic Gram-negative bacteria associated with severe and potentially life-threatening infections. *Cronobacter*-related sepsis, meningitis, and NEC are terrible diseases of the neonate, with poor long-term prognoses. Additionally, these bacteria are associated with less severe, yet still debilitating, infections in the elderly and immunocompromised.

Although we have achieved a significant improvement in our understanding of *Cronobacter* since it was first discovered, we still do not completely understand the environment, pathogenesis, virulence, or treatment of the genus *Cronobacter*, and these represent areas of future investigation.

**REFERENCES**

7. Brady C, Cleenwerck I, Venter S, Coutinho T, De Vos P. 2013. Taxonomic evaluation of the genus *Enterobacter* based on multilocus sequence analysis (MLSA): proposal to reclassify *E. nimbypressuralis* and *E. amnigenus* into *Lelliottia* gen. nov. as *Lelliottia nimbypressuralis* comb. nov. and *Lelliottia amnigena* comb. nov., respectively, *E. gergoviae* and *E. pyrimus* into *Pluralibacter* gen. nov. as *Pluralibacter gergoviae* comb. nov. and *Pluralibacter pyrimus* comb. nov., respectively, *E. cowanii*, *E. radiciclinans*, *E. oryzae* and *E. arachidis* into *Kosakonia* gen. nov. as *Kosakonia cowanii* comb. nov., *Kosakonia radiciclinans* comb. nov., *Kosakonia oryzae* comb. nov. and *Kosakonia arachidis* comb. nov., respectively, and *E. turicensis*, *E. helveticus* and *E. pulvorum* into *Cronobacter* as *Cronobacter zurichensis* nom. nov., *Cronobacter helveticus* comb. nov. and *Cronobacter pulvorum* comb. nov., respectively, and emended description of the genera *Enterobacter* and *Cronobacter*. *Syst Appl Microbiol* 36:309–319.


