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Review Article

Cronobacter spp., Review and Analysis of an Emerging Pathogen and its Presence in Food Services

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ABSTRACT

In recent years, there has been an increase in the incidence of several emerging pathogens, some of which were previously known. In this latter group, *Cronobacter spp.* has been recognized. It was conducted a narrative review, through the search of scientific literature in databases and search engines such as PubMed, Google Scholar, SciELO, using keywords such as *Cronobacter spp.*, Food Service, food safety, foodborne illness, individually or in combination, in English and Spanish languages, from the year 1980 to 2024. *Cronobacter spp.* belongs to the *Enterobacteriaceae* family, Gram-negative, and non-spore-forming. It has been associated with some cases of meningitis and necrotizing enterocolitis. Furthermore, a recent publication by the FDA (Food and Drug Administration) titled 'Demystifying *Cronobacter spp.* and Actions FDA is Taking to Keep the Food Supply Safe' alerted the American population about this little-known pathogen in households, but identified and controlled in the food industry. Therefore, it is necessary to conduct a detailed review of *Cronobacter spp.* and its growth conditions, as well as the precautions in large-scale food production. However, based on the studies and reviews conducted, *Cronobacter spp.* would not pose significant complications at the industrial production level, as long as proper care is taken in quality assurance processes. In other words, collective food services require standardized operational processes and microbiological surveillance procedures to keep this and other microorganisms under control.

Keywords: *Cronobacter spp*; Food services; Foodborne illness; Food safety

INTRODUCTION

In recent years, several emerging pathogens have surfaced: Some previously known have increased in incidence, others resulted from changes or mutations, and some have expanded into new geographic areas. Within this group, *Cronobacter spp.* has been recognized. A narrative review was conducted by searching scientific literature databases such as PubMed, Google Scholar, and Scielo using keywords including *Cronobacter spp.*, Food Service, food safety, foodborne illness, individually or in combination, in English and Spanish, from 1980 to 2024. The review focused on relevant studies, reviews, and highly cited articles. A total of 40 relevant articles were reviewed, organized into sections covering general context, identification, characterization, origin, and occurrences of *Cronobacter spp.* in collective food services and industrial production.

CRONOBACTER SPP.

Cronobacter spp. belongs to the family *Enterobacteriaceae*, Gram-negative, non-spore forming. It has been associated with cases of meningitis and necrotizing enterocolitis [1]. These diseases primarily affect infants fed with infant formula, underscoring the importance of reviewing the conditions under which *Cronobacter spp.* develops. However, these diseases are not exclusive to young infants, as they have also been characterized in older infants, infants at home, and hospitalized adults [2]. The main complications of *Cronobacter spp.* include mortality rates reported as high as 50% or more. While this figure has decreased in recent years, the infection can lead to morbidity, presenting as neurological deficits, especially in meningitis patients. Most occurrences of *Cronobacter spp.* And related events have been observed in infants, immunocompromised older individuals, and that receiving enteral nutrition support.

Additionally, a recent FDA publication titled "Demystifying Cronobacter and Actions FDA is Taking to Keep the Food Supply Safe" alerted the U.S. population about this little-known pathogen in households but identified and controlled in the food industry. This followed the withdrawal of Abbott formula products, leading

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to a national shortage of such products. According to the FDA report, *Cronobacter spp.* is present in household environments such as yards, kitchens, and living rooms. It can survive on countertop surfaces, food manufacturing equipment, and sinks. However, genetic data linking *Cronobacter spp.* to specific food sources that could potentially trigger pathology are lacking. While epidemiological knowledge of this microorganism and its virulence remains limited and under investigation, various factors necessitate monitoring of this microorganism, particularly concerning food safety.

CHARACTERIZATION OF THE MICROORGANISM

As previously mentioned, *Cronobacter spp.* is a Gram-negative bacillus of the *Enterobacteriaceae* family, motile, first identified in 1958 in England [3,4]. In 2010, Simon, et al. investigated a case of meningitis in preterm infants, finding *Cronobacter spp.* In their feces, blood, and in the prepared milk formula that caused the infection due to inadequate preparation and storage post-reconstitution. *Cronobacter spp.* ranges in size from 1 micrometer to 3 micrometers approximately, are a facultative anaerobe, non-spore forming, and generally flagellated and motile [5-7]. Identification of *Cronobacter spp.* under the microscope reveals colonies with a yellow pigment on Soy Trypticase Agar. These colonies can appear rough or smooth, but consistently exhibit a yellow color and viscous consistency. In the laboratory, the genus shows a negative result for oxidase and positive for catalase tests, reduces nitrate, utilizes citrate, hydrolyzes esculin and arginine, and tests positive for L-ornithine decarboxylation. It produces acid from D-glucose, sucrose, raffinose, melibiose, cellobiose, D-mannitol, D-mannose, L-rhamnose, L-arabinose, D-xylose, trehalose, galacturonate, and maltose. Additionally, it typically tests positive for acetoine production in the Voges-Proskauer reaction and negative for methyl red, indicating a 2,3-butanediol pathway rather than acid fermentation [8].

Bacterial growth of *Cronobacter spp.* occurs between 6°C and 45°C in Brain Heart Infusion broth with a pH range of 5 to 10. It has been reported that no strains grow below pH 4.5, with optimal growth observed in sodium chloride concentrations up to 7% (w/v), but not at 10%. *Cronobacter spp.* growth is also possible in sodium phosphate concentrations up to 100 nM, 5% sodium sulfate, and 20% ethylene glycol, while it does not grow in concentrations exceeding 20 nM sodium benzoate. Both temperature and pH are critical factors in controlling *Cronobacter spp.* growth in food production environments. Strict temperature controls (above 45°C and below 6°C, with a limit of 6°C) are necessary to mitigate its presence. The primary reservoir of *Cronobacter spp.* is hypothesized to be plant material, which is a significant contamination source. It can be isolated from a wide variety of foods including milk, cheese, nuts, meats, water, vegetables, rice, bread, tea, herbs, spices, and powdered infant formulas [5]. Surveillance studies have detected *Cronobacter spp.* in homes, livestock facilities, food factories, and infant formula production facilities [9,10].

Cronobacter spp. has been epidemiologically linked to infant formula contamination incidents. High-profile contamination episodes, such as the 2022 recall of Lyons Magnus products in the United States, underscore the importance of rigorous production and handling practices to prevent contamination (FDA, 2022). The bacterium exhibits capsular formation with a polysaccharide that aids in surviving desiccation and provides protection against disinfectants. It adheres readily to materials like silicone, latex, polycarbonate, and stainless steel, as observed in studies on biofilm formation [11]. Regarding osmotic and thermal tolerance, *Cronobacter spp.* shows adaptation to water stress compared to other *Enterobacteriaceae* members. It can survive in low water activity environments for extended periods, emphasizing the need for stringent control measures in food manufacturing and home preparation of powdered infant formulas (FAO/WHO, 2004, 2006).

In conclusion, understanding the characteristics and environmental adaptations of *Cronobacter spp.* is crucial for implementing effective control measures to ensure food safety, especially in vulnerable populations such as infants. According to these investigations, it was stated that *Cronobacter spp.* is more thermoresistant compared to other members of the *Enterobacteriaceae* family and can grow over a range of temperatures from 6°C-47°C, both on food contact surfaces in food service settings, as per FDA guidelines, and in food items such as powdered infant formulas. Initially, the study's D and Z values closely correlated with previous reports described in 1999 by Nazarowec-White and Farber [12]. Then in 2005, Kim and Park studied collection strains isolated in Korea. The results of their D values in saline were 12 minute-16 minute, 3 minute-5 minute, and 0.9 minute-1 minute at 52°C, 56°C, and 60°C respectively. Considering that the critical limits currently managed in food services correspond to temperature ranges between 5°C and 65°C, we can foresee a standardized operational response. Although *Cronobacter spp.* has been mainly associated with infections in infants, recent reports indicate a high risk for immunocompromised adult patients, particularly the elderly. Jiménez, et al., in 1982, first reported *Cronobacter spp.* isolated from an adult patient with bacteremia. An additional case involved 19 patients who exhibited *Cronobacter spp.* infection, presenting clinical symptoms including pneumonia, sepsis, plantar ulcers, wound infections, osteomyelitis, and splenic abscesses. All these reports suggest that older adults may be more susceptible to *Cronobacter spp.* infections [12,13].

SYMPTOMS OF *CRONOBACTER SPP.* INFECTION

The disease manifests differently depending on the individual and their age. For instance, in infants, especially those under two months old, the bacteria can enter the bloodstream or reach

the spinal cord, causing inflammation of the meninges, resulting in meningitis. The disease caused by *Cronobacter spp.* generally presents with fever, anorexia, general malaise, and in some cases, seizures. Infants with meningitis may develop serious and permanent brain problems, with studies showing that 4 out of 10 babies with meningitis could die. In the adult population, not immunocompromised, *Cronobacter spp.* could cause infections in wounds or exposed body sites, such as recent surgical scars. It can also cause urinary tract infections. In immunocompromised patients, elderly adults, or those who have suffer infections, *Cronobacter spp.* is likely to be found in the bloodstream [1,4].

IMPORTANCE IN THE FOOD INDUSTRY

The presence of *Cronobacter spp.* in the food industry has become significant due to the implications it could have as a pathogen [14]. As mentioned earlier, *Cronobacter spp.* has been identified in raw fresh, frozen, fermented, or cooked animal and vegetable products. It has been identified in dairy products such as artisanal cheeses, where cold chain transport and product maintenance have been neglected, deficient, or simply non-existent [15]. As we have reviewed previously, *Cronobacter spp.* can remain in a food container for a long time, as described by Caubilla-Barron et al. in 2007, who noted that the microorganism can persist for up to 2 years in infant formulas [16]. Primarily, *Cronobacter spp.* has been associated with infant beverages derived from powdered infant formulas and utensils used in formula preparation. Regarding these preparations, a series of studies have been conducted, and cases of *Cronobacter spp.* infection in children under 2 years old have been identified worldwide, with cases resulting in patient death. Although the incidence is low, it is important to note that these infections are rare, and not all infections have been associated with reconstituted milk consumption. In relation to this problem in the SEDILES, Bustos proposed relevant quality standards, which were in turn incorporated into the Chilean Food Sanitary Regulations (DS977/96 RSA). Bustos suggests final product temperatures of 0°C-5°C and maximum refrigeration for 24 hours, in addition to mentioning logical precautions regarding general food safety considerations when handling such services [17]. ISO (International Organization for Standardization) and the International Dairy Federation devised a protocol for the identification of *Cronobacter spp.* based on powdered milk, ISO/TS 22964, using pre-enriched buffered water PIF with samples at 37°C.

PATHOGENICITY AND VIRULENCE

The mechanisms contributing to the pathogenicity of *Cronobacter spp.* have been mainly shown in the context of neonatal meningitis. It was initially speculated that a possible infection route was the translocation of bacteria from cerebrospinal fluid blood through the choroid plexus, followed by bacterial invasion of nutrient-rich brain matter. Other studies have described that OmpA binds to fibronectin, facilitating the invasion of cerebral endothelial cells. It was demonstrated that OmpA expression affected the onset of meningitis in newborns, a study conducted in rats, where OmpA-positive *Cronobacter spp.* successfully crossed the intestinal barrier and multiplied in the blood, capable of crossing the blood-brain barrier, while OmpA-negative isolates of *Cronobacter spp.* Could not bind to the intestinal epithelium cells. In addition, a 100% mortality rate was observed in OmpA-positive newborn rats infected with *Cronobacter spp.*, and no pathological manifestations were observed in newborn rats infected with OmpA-negative *Cronobacter spp.* Several *in vitro* studies, conducted in smaller mammals, investigated the binding and invasion properties of *Cronobacter spp.*, where adherence to 2 epithelial cell lines, HEp-2 and Caco-2, and human brain microvascular endothelial cells were studied. The data showed increased adherence during the latter stages of bacterial growth, with a tenfold increase during this phase. Similarly, the attachment and invasion properties of seven *Cronobacter spp.* strains associated with an outbreak of necrotizing enterocolitis, bacteremia, and meningitis in a neonatal intensive care unit were investigated. It was identified in all Caco-2 cells within a period of 3 hours. These isolates could replicate and persist in macrophage cells for a period of 48 hours. Invasion studies using rat brain capillary endothelial cells demonstrated that a meningitis strain was considerably more invasive compared to other strains not related to meningitis. Attempts to bridge the gap of knowledge between *in vitro* assays and *in vivo* mechanical models remain in their infancy. It has been shown in *Cronobacter spp.* research that they can succeed if they adhere to and invade endothelial cells, although these observations are based on astrocyte culture, which better represents what happens at the blood-brain barrier *in vitro*. The infectious dose associated with *Cronobacter spp.* has not yet been identified, but it has been estimated by Iversen in 2004 that the infectious dose could be approximately 1000 CFU. FAO/WHO proposed a dose program of 10,000 CFU [18]. However, despite preliminary estimates, it is widely accepted that the dose-response relationship also depends on the host. Pagotto et al., in 2003, first described the production of enterotoxin in *Cronobacter spp.*, finding that toxin activity was higher at pH 6 and showed stability at 90°C for 30 minutes [19]. Bacterial adherence to surfaces and biofilm formation are known to contribute to resistance to antimicrobial treatments. Available nutrition and temperature are significant factors affecting biofilm formation. Iversen et al. investigated biofilm formation on various surfaces commonly associated with equipment and infant formula preparation surfaces, isolated on silicone, latex, polycarbonate, and stainless steel, where exopolysaccharide capsule production [18]. Dancer in 2009 investigated the influences of milk components on biofilm formation by *Cronobacter spp.* It was proposed that whey protein and casein are important, rather than lactose. Regarding skim milk, the authors also suggested that the most determining factor is the amount of nitrogen derived from proteins, so the presence of fats is not decisive compared to the presence of carbohydrates.

EFFICIENCY OF ANTIMICROBIAL AGENTS

Cronobacter spp. has demonstrated intermediate sensitivity to many classes of antimicrobials including acylureidopenicillins, aminoglycosides, ampicillin, antifolates, aztreonam, carbapenems, cephalosporins, chloramphenicol, nitrofurantoin, quinolones, tetracyclines, ticarcillin, and various beta-lactams. *Cronobacter spp.* are more sensitive compared to other members of the *Enterobacteriaceae* family to many antibiotics including aminoglycosides and carboxy-penicillins. Almost all *Enterobacteriaceae* species exhibit resistance to glycopeptides, rifampicin, lincosamides, fusidic acid, and streptogramins. This resistance phenotype can be attributed to the outer membrane acting as a barrier preventing the passage of these agents. *Cronobacter spp.* has also shown reduced sensitivity to oxacillin, benzylpenicillin, clindamycin, and some macrolides.

ALTERNATIVE CONTROL MEASURES

Alternative prevention strategies aimed at preventing the growth of *Cronobacter spp.* have been investigated primarily through biocontrol. *Cronobacter spp.* is an opportunistic pathogen, and one potential measure to ensure food and surface safety is the use of bacteriophages to prevent organism growth. The use of specific phages that exhibit lytic activity only against *Cronobacter spp.* cells is proposed, thereby eradicating bacteria from infant formulas without harming normal microbiota when consumed. The activity of antimicrobial peptides produced by Lactobacillus acidophilus against pathogens such as *Cronobacter spp.* has been evaluated, identifying two peptides with antimicrobial activity similar to isracidin, namely caseicins A and B. Since these peptides are derived from milk proteins, they could potentially play a bioprotective role in dairy-based food products. Recently, the activity of fermented sodium caseinate has been studied, reducing the number of *Cronobacter spp.* in reconstituted milk and formulas from 6 logs to 0 logs. In Chile, a country at the forefront of microbiological control in Latin America, alerts have been issued regarding the presence of *Cronobacter spp.* in milk formulas. A cross-sectional analytical study in Chile analyzed 72 cans of premature LP and 65 Stage 1 or initiation LP of 450 grams sold in pharmacies and supermarkets, following the sampling reference of Codex Alimentarius CAC/RPC 66 standard [20-21]. The study aimed to identify mesophilic aerobic microorganisms (RAM) and *Enterobacteriaceae*, with quantification of both groups performed using the Compendium of Methods for the Microbiological Examination of Foods. *Cronobacter spp.* isolation utilized the method described by Iversen, employing 25 grams of LP in 225 ml of buffered peptone water (BPW, Oxoid, England). The enriched EE Monsel broth (BD Difco Spark, MD, USA) was streaked onto DFI Chromogenic Agar (CM 1055, Oxoid Thermo Fisher, UK), with presumptive colonies (green or blue) purified on Trypticase Soy Agar (BD Difco, Sparks, MD, USA). The limit was 0.0009 to 316 MPN/g of LP with 95% confidence.

DISCUSSION

Results showed elevated RAM and presence of *Enterobacteriaceae* in infant formulas. The prevalence of *Cronobacter sakazakii* was 2.7%, with only 2 strains confirmed from batches different from those manufactured in Chile. For samples produced in Chile, the pathogen's prevalence was 9.5%. The identification of *Cronobacter spp.* in 2 lots of milk produced in Chile prompted study and oversight by national authorities and manufacturers. Although the likelihood of illness is estimated low in countries like the United States (1 in 100,000 newborns), this increases to 9.4 per 100,000 in children weighing less than 1500 g. WHO recommends basic care measures such as water temperature of 70°C during milk formula reconstitution, which should be specified on labels and reconstitution instructions to reduce *Cronobacter spp.* and other pathogen risks. Following the alert in Chile, potentially contaminated batches were recalled and the population alerted, reinforcing precautions in food preparation, especially for prepared infant formulas, to prevent infections by this microorganism. Following these incidents and recognizing the importance of production control in food manufacturing, particularly in infant formula production, *Cronobacter spp.* was included in Chile's Food Sanitary Regulation. Parameters include presence and control of this microorganism under Article 9. INFANT USE FOODS, 9.1 DEHYDRATED FORMULAS FOR CHILDREN UNDER 12 MONTHS. *Cronobacter spp.* in 10 grams of product is categorized as hazard class 14 (severe), class 2, with a sample size (n) of 30.

CONCLUSION

Cronobacter spp. is a recently classified genus of microorganism. However, several ongoing studies are needed to better understand this type of bacteria. Reliable detection and identification of *Cronobacter spp.* are essential in food production and hospital settings, particularly in NICUs. While molecular detection methods are faster compared to conventional phenotypic methods, it is crucial to train teams and operators to implement these protocols consistently. Efforts are needed in clinical settings, food services, and commercial food production environments to update detection system foundations, especially commercially, for quick and safe protocol adoption. In households, collective education is crucial to enhance hygiene measures in food handling. Identification and differentiation of *Cronobacter spp.* could be achieved using comparative genome techniques, transcriptomics, proteomics combined with bioinformatics, essential to unravel complex interactions between the pathogen and host and identify emerging *Cronobacter spp.* properties.

Little is known about *in vivo* virulence factors and the pathogenicity of *Cronobacter spp.*, critical in designing therapies to treat and control infections. Improved understanding and pathogenesis of *Cronobacter spp.*, including *in vitro* cell assays combined with smaller mammal studies, are necessary. Like many pathogenic organisms, *Cronobacter spp.* has developed a broader bacterial

population capable of surviving under specific conditions found in infant formula production plants. Therefore, ensuring food safety through risk assessment to identify and manage problems is essential. Food systems can only be effective when based on current and sensitive microbiological data. Hence, emphasis should be placed on communication and dissemination of food safety protocols for the industry, from policymakers and researchers to consumers.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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