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Cronobacter sakazakii: Emerging Public Health Threat for Infants

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Abstract

Cronobacter sakazakii is a new pathogenic bacteria that is the leading cause of severe diseases that affect public health, mainly infants and neonates and immuno-compromised persons. This bacterium is an isolated cause of severe infections such as necrotizing enterocolitis, meningitis, and bacteremia, and case fatality ranging from 40 to 80%. The following cases are most vulnerable: Children under 3 because their immune system is poorly developed. Despite the relatively low case rates globally of C. sakazakii infections, evidence suggests that foodborne transmission, specifically through infant formula consumption, is a significant route of infection. The bacterium likes to be in dry places, which is why it is seen increasing in infant formula and powdered foods. Thus, the risk of getting infected is very high among the young. It is only in the recent past that researchers have been able to propose probable virulence factors in C. sakazakii, including outer membrane proteins and enzymes. Among these modes are the facilities that allow the bacterium to penetrate the gastrointestinal tract and the blood-brain barrier, which qualify the bacterium as lethal to the infant populations. Reducing contamination risks requires committed control measures that include better ways of manufacturing, sterilizing equipment, and educating caregivers. Disease surveillance and laboratory reporting on C. sakazakii cases should be stepped up to detect cases and control the disease. In conclusion, the primary strategies for minimizing the effects of C. sakazakii can be achieved through concerted efforts by the government, health departments, and the food processing sector to defend vulnerable groups.

Keywords;

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Introduction

Cronobacter sakazakii is a pathogenic bacterium that is lethal to human health and a leading cause of neonatal, infantile, or immunocompromised-mediated illness. This bacterium causes several infections: NE, bacteremia, and meningitis, among the most lethal diseases associated with C. sakazakii. The death rate that these infections cause can be very high, averaging from 40%/ 60% to 80%, depending on the progression of the disease and the health status of the patient. These infections are more common in pediatric patients below the age of three years and immunocompromised adults (Heperkan et al., 2017; Demirci et al., 2018). However, information on C. sakazakii-related diseases still needs to be made available, and poor surveillance data make it challenging to establish causes of the marked pathogenicity of the bacterium and put efficient control measures into practice. Worldwide, there are about 120 reported cases of exposure to C. sakazakii in infants and young children, according to the WHO; however, no doubt, these figures are far from reflecting the actual situation (Ivy et al., 2013; WHO/ FAO, 2019). Transmission of infection due to C. sakazakii occurring through food consumption remains under-reported in many cases. Thus, many unknowns exist regarding the bacterium's impact on global public health. Therefore, identifying foodborne disease outbreaks associated with C. sakazakii is challenging, especially in areas without surveillance mechanisms. This emphasizes the importance of active surveillance and the study of data concerning the epidemiology of C. sakazakii to promote strong population health practices. Detailed reports of C. sakazakii isolation from the foodborne disease outbreaks and the identification of the strains involved would assist in formulating new detection methods for the microorganism, treatment regimes, and later prevention measures. While helpful, foodborne disease surveillance systems exist in most countries and are usually inadequate for reporting C. sakazakii. The currently available information also shows that outbreaks associated with C. sakazakii pose a higher risk to babies, especially neonates.

The high-risk groups include neonates, infants, and people with weakened immunity, which has informed the need to continue preventing C. sakazakii infections, especially for these categories of people. Therefore, it is essential to avoid

foodborne transmission of C. sakazakii, especially since baby food is at a higher risk of contracting pathogenic bacteria in food (Heperkan et al. 2017). The bacterium has been reported to be prevalent in numerous food items, including but not limited to baby foods, particularly infant formula. C. sakazakii was found in these products, and it is most worrying to note that this bacterium can live in dry conditions and is very risky to infants who depend on formula feed. Other complementary foods are typically fed to the child in the first year of their life through an infant formula, which acts as a milk substitute. It is one of the most essential food sources for many babies, but dried or dehumidified infant formula is quite sensitive to bacterial shuttles such as C. sakazakii. This is because, in the dry powder form of the infant formula, the bacterium can survive and even multiply, creating favorable conditions for infections that can be deadly. This is further exercised by the fact that C. sakazakii possesses several virulence attributes, amongst them the ability to invade the gastrointestinal wall and the blood-brain barrier, causing life-threatening conditions such as necrotizing enterocolitis and meningitis. Such conditions seen are usually fatal, and given this, the contamination of infant formula as a significant public health menace (Cava Gümüş et al. 2017).

In light of this, manufacturers of foods directly linked to C. sakazakii infections, especially infant formula manufacturers, must adopt strict measures to prevent contamination. These measures should include proper cleaning and disinfection of equipment and utensils used in the processing and packaging infant food products, avoiding Zur Environmental deterioration likely to enhance bacterial growth, and proper storing conditions when processing the food products. In addition, constant monitoring of Independent Financial Caregivers for possible violence is critical, as is training Independent Financial Caregivers to uphold suitable food safety measures and standard hygiene practices that can go a long way in eradicating bacterial infections among vulnerable persons. Many regulatory agencies of different countries and international organizations share concerns over C. sakazakii in infant foods, particularly in formulas and cereals. These agencies have implemented measures and food safety measures such that these foods do not contain pathogenic bacteria such as C. sakazakii. But the rules have been implemented, and compliance and enforcement are other concerns, so the problem in the surveillance and reporting sides should also be considered further to ensure fresh and safer products for infants. It is well established that facilities of baby food processing are infected with C. sakazakii. The bacterium has been known to exist in similar settings and might be transferred by dust since it is in the dust from where it can cause illness. Dust filters, vacuum cleaners, and other tools used to prepare, pack, and store food items have been defined as possible links to contaminants. Here, Zimmermann et al. (2018) found that C. sakazakii is frequently isolated from dust filters and voids connected to packaging or bagging operations. This means that achieving and maintaining appropriate levels of contamination during processing and in the pre-processing stages of food production is very important in preventing harm to the health of vulnerable groups, particularly the use of infants. Hence, there is a need to put in place efficient contamination control at every step of the process, from receiving raw materials to packaging and storing food. Such measures should involve enhanced public health measures such as washing and disinfecting, bacterial checkups, and applying innovative tools to eradicate bacteria. In the same regard, there is a need to create awareness of C. sakazakii risks for personnel working in food manufacturing plants and train them on measures to avoid these risks. C. sakazakii is a potential hazard to public health, mainly to neonates and immunosuppressed patients. This bacterium's capacity to be present on dry media like powdered infant formula and baby foods and its pathogenic factors demonstrate how it threatens food security. To overcome the above-associated risks of C. sakazakii, some of the following strategies would be crucial: Comprehensive surveillance systems, Strict manufacturing practices, and Continuous research. Safeguarding the foods that babies consume is not only an issue of the legal requirements but a vital public health issue that needs immediate positive input from the producers, health departments, and even members of society.

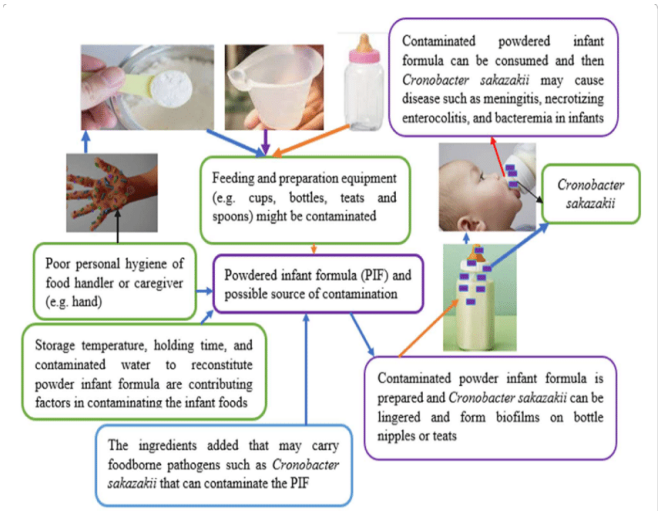

Figure 1: Mode of infection

2. The Pathogen – Cronobacter sakazakii

Cronobacter sakazakii is a bacterium that comes under the Enterobacteriaceae family. The key objective of this study was to. It is a true-negative stain bacterium that is straight, rod-shaped, can locomote via peritrichous flagella, and is facultatively anaerobic. This pathogen does not form spores and grows on solid media as brightly or weakly colored colonies depending on the used base (Ivsen et al., 2003). Cronobacter sakazakii also grows at a relatively low-temperature range between 6°C and a high-temperature range of 47°C. Data on the growth characteristics of the bacterium was obtained from work done by Forsythe, (2018). The six strains of this bacterium were most active at temperatures within the range of 37 and 43°C, and the single strain, which had a capsule, could grow at 47°C. However, the assessments also have revealed that the majority of pasteurization procedures required in the manufacture of powdered baby foods can heat-inactivate many strains of Cronobacter sakazakii even at ranges of temperature that the bacteria are known to withstand (Ivsen et al., 2004; Shaker et al., 2007; Al-Nabulsi et al., 2011; Jaradat et al., 2014 However, enrichment with micronutrients after pasteurization or during irrigation creates new sources of contamination with potential for further microbial growth (Nyati, 2018).

Cronobacter sakazakii is also growing because of its high tolerance to low pH in its habitat. Dancer et al., 2009 investigated the ability of more diverse C. sakazakii strains to grow in acidic environments. In the current study, all the strains were able to produce at pH 4.5; however, growth was slower at lower pH levels, with growth rates of 98.6%, 95.8%, and 79.2 % in medium with pH 4.3, 4.1, and 3.9, respectively. Similar observations are made by Edelson and Mammel (2006), who have shown that living cells reduced by less than one logarithmic unit over 5 pH measurements more than 5, and the viable cells at pH 3.0 were four logarithmic units for all strain types. Although C. sakazakii has been identified from acidic food samples with a pH value of 2.5, the bacterium could not survive to this level (Fakruddin et al., 2014). However, due to the weak gastric acidity in newborns, particularly premature children, the probability of infections from C. sakazakii increases since stomach acidity is relatively low enough to overcome the pathogen. Cross-contaminationCross-contamination of Cronobacter sakazakii in infant food products poses a significant threat to public health. Newborns, especially those with immature immune defense mechanisms, are easily affected by severe foodborne infections due to ingesting contaminated foods. The consumption of C. sakazakii contaminated food causes neonatal meningitis, necrotizing enterocolitis, and other severe infections in the baby, with mortality rates varying between 40–and 80% among the infected infants. Another way is that it contaminates other objects in the house, which may include kitchenware and equipment used to prepare formula feeds for the babies, for example, when not directly contaminated (Erkekoglu et al., 2009). Although infections by C. sakazakii are unfamiliar, its detection in PIf is worrisome because newborns are susceptible to infections (Ivsen & Forsythe, 2004).

Cronobacter sakazakii has been recovered from meat, vegetables, cheese, seeds, herbs, spices, and fruits, but cases of foodborne infection are rare (Ivsen & Forsythe, 2004; Lampel & Chen, 2009). The perspective in powdered infant formula is higher since infants have weak immune systems, and powdered formulas are often prepared in a less hygienic setting, leading

to microbial contamination (Ivsen & Forsythe, 2004; Almajed & Forsythe 2016). Of most concern with the bacterium is neonatal meningitis exposure, whereby cross-sectional studies have estimated that the principal route of contamination is through powdered infant formula (Nazaretroc & White & Farber, 1997, 1999). Analyzing the data of a study that focuses the C. sakazakii infection cases among newborns, infants, and young children from 1960 to 1999, it was observed that of the 32 patients for whom the type of infection was mentioned, 21 suffered from meningitis, 7 from bacteremia, 1 from urinary tract infection, 1 from diarrhea and 1 from the dermoid cyst (Hunter & Bean 2013). Furthermore, primary bacteremia due to C. sakazakii is a common problem in all ages but is particularly deadly in neonates and infants (Elkhawaga et al . 2020). For example, Blaser et al. (2002) described a child with sepsis whose symptoms had been observed for six days; C. sakazakii infection of blood, urine, cerebrospinal fluid, and purulent fluid was confirmed. This case also illustrates the anatomic tropism of Cronobacter sakazakii for the most vulnerable in our society.

Drudy al. (2006) revealed that C. sakazakii contamination could happen in the production of powdered baby food. In their study, they took swab samples from each factory and three samples in each factory location; C. sakazakii was isolated in eight out of nine powdered baby food factories; they also reported that the quantity of the bacterium ranged from ‘a few’ to ‘many’ depending on stage from floor surfaces, dried scraps found on the production line, vacuum cleaning bags. Vk2 also demonstrated that, in one factory, 28% of vacuum cleaning points, 5.3% of filling machines, and 8% of filling bands were contaminated by C. sakazakii. These results suggest that good hygiene and contamination prevention should be maintained during the production of infant food products. The pathogen Cronobacter sakazakii remains a critical danger to infant health owing to its versatility and association with severe infections, particularly in newborns. Therefore, it is important to adequately address the safety concerns of infant foods, especially powdered formulas, to minimize the effects of this pathogen (Zimmermann et al. 2014).

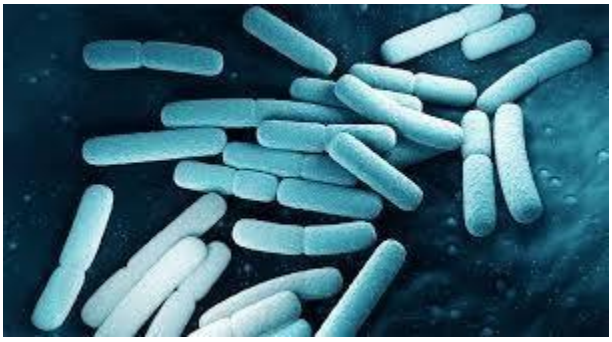

Figure 2:Cronobacter sakazakii

3. Infectious Dose of Cronobacter sakazakii in Food Samples

The threshold for the infective dose of Cronobacter sakazakii in the food samples remains another challenge with scarce epidemiological information, thus making it challenging for researchers to determine a precise infection level. However, studies on related pathogens, such as Neisseria meningitidis, Escherichia coli O157:H7, and Listeria monocytogenes, for similar pathogens it is while the estimated oral infective dose is approximately 1000 colony-forming units (CFUs). However, it is also important to note that the infectious dose of C. sakazakii may also differ under certain circumstances. Considering this, defining the specific level of the infectious dose for Cronobacter sakazakii remains a problem because the question is veiled in factors that affect the virulence of a microorganism (Parra-Flores et al. 2015). Environmental stress conditions, the immune status of the host, and the nature of food are very important factors in measuring this infective dose, and research presents C. sakazakii as a powerful pathogen in foods, especially in powdered INF FO. Thus, factors such as temperature and moisture content of foods and ozone variability can have a decisive influence on the infection potential of this pathogen. Another study by Forsythe (2018) determined the minimum infectious dose of C. sakazakii in powdered infant formula. The study entailed the identification of 0.36 kob (kilo organisms by one hundred grams) of C. sakazakii powdered formula. Its primary purpose was to define the amount of time and temperature necessary for the pathogen to overcome the level needed to cause an infection. The findings showed that under standard temperature settings, the powder should ideally be stored at 8 degrees Celsius for 9 days or at room temperature, about 20 degrees Celsius, for almost eighteen hours for the therapeutic dose that could result in infection. In this case, the researchers argued that microorganisms are not killed by water temperature used in food preparation and do not multiply in the stomach. Such assumptions are essential since the pathogenicity of C. sakazakii is directly linked to the ability of the bacteria to survive in food throughout the process of preparation and storage as well as in the human gastrointestinal tract. The bacteria does not multiply in the stomach, meaning its pathogenicity is more related to the quantity at the time of ingestion than the rate at which it multiplies once ingested (Nyati, 2018).

This study’s infection dose shows the consequences of incorrect handling and storage of the formula through the Forsythe (2018) study. For the threat to be minimized in

vulnerable groups such as infants, crucial measures of food safety will be required to counter the effects of the low-temperature threshold for survival and the short time needed at room temperature for the buildup of a dangerous quantity of C. sakazakii. Before this formulation can be established, more studies are required to identify the specific infectious dose for each food matrix under varying environmental circumstances. The 1000 CFU threshold of similar pathogens seems an appropriate reference point. However, C. sakazakii could still be dangerous even at low infective doses due to immunodeficient organs, premature infants, the elderly, and those with chronic diseases. Children, notably neonates, whose immune systems are still immature, may develop severe infections due to C. sakazakii even if exposed to low doses of the bacteria. However, because this pathogen can change its behavior under stress conditions, such as in the areas of temperature, humidity, and other microorganisms, foods that could have survived initially become dangerous if not properly stored or handled. Another factor that makes the control of C. sakazakii difficult, especially in food manufacturing environments, is that it forms biofilms on surfaces and is resistant to desiccation, especially in foods associated with the production of infant formulas. The infectious dose of Cronobacter sakazakii in food samples depends on factors that must be addressed in the risk assessment of the disease. As no cut-off value has been stipulated due to the considerable variation in environmental and host factors, low concentrations of the pathogen in food are shown to be prohibitive and tolerated, with infants being the most vulnerable group. Future studies should be conducted to clarify more characteristics in various types of food commodities and to extend efficient measures on the threats of C. sakazakii in food safety.

3.1 Disease mechanisms and factors that increase pathogenicity

Cronobacter sakazakii is a newly defined species of the Cronobacter genus, and the pathogenicity of organisms is relatively unknown (Ye et al., 2015). However, further improvements in DNA-based methods have helped identify more about the virulence factors and pathogenicity of C. sakazakii using 16S rRNA methods (Eshwar et al., 2016). Several research papers have pointed out at least seven O-serogroups and eleven proteolytic enzymes in the pathogen, all pointer to the pathogen's virulence (Erkekoğlu et al. 2009). The main virulence factors include outer membrane proteins such as OmpA and OmpX, which help the bacteria adhere to the gastrointestinal tract and may help the bacteria penetrate the blood-brain barrier (Ye et al., 2015). Zinc-metalloprotease (zpx) and Cronobacter plasminogen activator (Cpa) are two essential virulence factors for pathogenicity in an animal model. The zpx protein induces cell deformation and rounding, which is one way tissue damage occurs. However, Cpa prevents serum from killing bacteria, stimulates plasmin formation, and inactivates α 2-anti plasmin so that the pathogen is favored within the host. Hence, knowledge of these virulence factors can effectively help set up qualification criteria for pathogenic strains from nonpathogenic ones. Kim et al. 2015 discussed the genetic features of prospective virulence genes in C. sakazakii, shown in the table below. These virulence factors are present in related genes, each having or reflecting a particular function in the pathogen's capacity to infect and make an individual sick.

Table 1: Virulence Factors of Cronobacter sakazakii

Factors	Genes	Potential Role
Outer membrane proteins (OMPs)	OmpX, OmpA	Involved in the basolateral invasion of enterocyte-like human epithelial cells (Kim et al. 2010).
Enterotoxin	Not known	A heat-stable toxin produced by the pathogen.
Outer membrane protease	cpa	Provides resistance against the bactericidal action of serum, activates plasminogen, and deactivates α2-AP.
Iron acquisition system	iuc	Encodes a siderophore-mediated iron uptake system, essential for iron transport and regulation.
Efflux system	ibeB	Encodes a cation efflux system that mediates resistance to copper and silver, aiding brain invasion.

4. Taxonomy

Cronobacter sakazakii is one of the 14 species classified in the Enterobacter genus that belongs to the family Enterobacteriaceae (Iversen et al 2007). This large and diverse group has recently attracted more attention, given the pathogenicity of some species within this family in recent years. Cronobacter sakazakii, Enterobacter Gergovia, and nine species that form the Enterobacter cloacae complex are major

representatives of the Enterobacter genus responsible for hospital-acquired infections in man. It may be based on the morphology and biochemical characteristics of the organisms; however, with increased emphasis on higher-level taxonomy from 1980, genes are used. This shift was attributed to molecular developments, which later revealed that the species were not defined well earlier. Genotypic taxonomy entails identifying species with slight genetic differences corresponding to high conservation and hypervariable sequences with nucleotide variations (Gill, 2018).

The 16S rRNA gene, common in the prokaryote genome, has been used extensively in molecular taxonomic analyses owing to the gene's conservative nature and phylogenetic relevance. Although the genetic system has come a long way in modern taxonomic practice, chemical systems, specifically chemotaxonomic methods regarding biochemical entities in microbial cells, are still fruitful. Some of the chemicals found in these microorganisms, such as mycolic acids in cell walls, have made it easy to identify mycobacterial species. New methods such as ribotyping and AFLP (Amplified Fragment Length Polymorphism) clarify the differentiation between bacterial species. According to Forsythe (2018), itAFLP is helpful in the molecular ecology of bacterial strains and has been used in species differentiations and sub-species levels. The type strain of Cronobacter sakazakii has also employed molecular methods, such as DNA-DNA hybridization and ribotyping, to study the potential genetic heterogeneity within these species. Forsythe, in his study, isolated 210 strains of C. sakazakii, the genes of which were analyzed to reveal that four major groups exist based on DNA homology. These strains had greater than 62% similarity, some having greater than 70% homology between them. Since the strains ATCC 29544T were shown to have a high degree of genetic relationship, DNA-DNA hybridization was performed on these strains, giving positive results that justify the classification of these strains within the same species. However, there is such genetic variation. Indeed, some strain's 16S rRNA gene sequences exhibit merely a 0.04% difference overall; certain strains have 99.6% homology (Forsythe, 2018).

5. Detection and Typing Methods: Conventional Bacteriological Culture

The initial method for isolating Cronobacter species was developed by Muytjens in 1988. This method prepared the foundation for further enhancement, for instance, a technique developed by the U.S. Food and Drug Administration (FDA) in 2002. This FDA method was developed for detecting and characterizing Cronobacter sakazakii from powdered infant formula (PIF), an important food product linked to this pathogen (Fei et al. 2015). ISO 2009 and the International Federation of Dairy Products finalized the process for identifying Cronobacter species from the milk-based powdered formula in 2006 by the protocol ISO/TS 22964 (Capla et al. 2022). Since then, this protocol has been of immense value in surveilling Cronobacter in foods, especially infant foods. However, in recent years, the U.S. FDA method has been reviewed. Most recently, it employed the molecular biochemical method of polymerase chain reaction (PCR) and newly developed chromogenic agar (Hyeon et al. 2020). These advancements have also assisted in enhancing the detection process for improved and quick outcomes. The detection protocol usually precedes the pre-enrichment of PIF samples, and it may start with a maximum incubation period of 18-24 hours to a minimum incubation period of 6 hours, depending on the detection protocol. This is followed by selective enrichment, in which isolates are cultured using selective agars (Du et al. 2015). Proper identification of bacteria and successful control of infections requires accurate differentiation of bacterial species. These selective media facilitate the growth of Cronobacter species, which are further confirmed by PCR or biochemical analysis. The new protocol of U.S.F.D.A has an extra enrichment stage, which leads to faster confirmation of the existence of Cronobacter sakazakii in the systems. This revision has steered down the total detection time by removing two days from the previous protocol. The method also includes culturing typical colonies from selective agars and amplification of the Cronobacter species using PCR-based or biochemical tests. Several selective agar media have been developed in isolation of Cronobacter in the food samples (Kumar, 2019).

The media include Leuschner-Bew Agar, Druggan-Forsythe-Iversen Agar, Oh-Kang Agar, ESPM Agar, and Hijrome Cronobacter spp. Selective medium. Other selective media that have also been used in the isolation of gram-negative bacteria, such as CRM (VRBA: purple-red gall agar), Mac Conkey, and desoxycholate agar, have also been established to be effective media in the isolation of Cronobacter in food samples where it may be present. However, some of the selective agars' ability to support the growth of all Cronobacter strains has been raised. Some selective media can also be less efficient in the isolation of all the subtypes of Cronobacter and other related bacteria, for instance, Enterobacter helveticus, Enterobacter tritici, and Enterobacter turicensis (Forsythe, 2018). Although these bacteria are unrelated to Cronobacter,

they are part of the same ecological group and can hamper isolation. To cope with these problems, additional improvements in selective media design have been sought. The development of this chromogenic medium has been revealed to help grow a short two-day culture method used for isolating Cronobacter in powdered infant form. The process requires a specific technique, such as using a selective media known as Cronobacter enrichment medium, which enhances the detection rate. The other method for increasing the detection sensitivity is cationic magnetic bead capture. Mullane et al. (2006) used this method to increase the sensitivity of Cronobacter from PIF. Nevertheless, the methods above and the credibility of commercially available Cronobacter identification kits still need to be improved due to documented false- -negatives and positives (Forsythe, 2018). Nevertheless, to this date, the Gen III Identification Kit is the only commercial kit capable of detecting Cronobacter and identifying six species of Cronobacter species (Jang et al. 2020). Altogether, important progress has been made in the last years in the detection of Cronobacter sakazakii in food, especially in PIF, but more work is still needed to enhance the specificity and sensitivity of the detection methods. Further development of selective media, molecular procedures, and detecting systems is needed for better identification and to prevent the risk of spreading among higher-risk groups.

6. Conventional and Molecular Based Detection Protocols

Molecular detection tools are essential in increasing knowledge of the epidemiology of Cronobacter sakazakii. These methods can find and identify a particular gene in the pathogen and are, in a way, more accurate and faster than the regular approach. The latter in turn, the majority of the current molecular copied methods derived from real-time PCR, several studies for more detailed detection of Cronobacter have been developed. These molecular processes help to determine all the seven recognized species of the Cronobater genus, with the help of mismatch-PCR. However, a significant area for improvement in this approach is that C. sakazakii and C. malonaticus cannot be differentiated; therefore, a second PCR is required to type the species. Yan et al. (2011) identified the necessity of PCR and sequence-based biological token validation to confirm Cronobacter species. The researchers' intention of this study was to identify possible virulence factors that could be used to distinguish Cronobacter species from other contaminants such as Salmonella spp. However, more validation is still under process regarding the efficacy of all these markers to differentiate between these entities. Molecular subtyping has become an essential method for defining the variability and virulence of Cronobacter species that reside in advertised habitats, particularly in PIF plants (Miranda et al. 2017). This subtyping method may be of immense benefit in enhancing the direction of the intervention approach to minimize the dwell of Cronobacter in such vulnerable settings. Another second-generation subtyping tool, Multi Locus Variable-Number Tandem Repeat Analysis (MLVA), was developed. While whole-genome MLVA is beneficial for genotypic and phenotypic subtyping of Cronobacter isolates, it provides higher resolution than other techniques. However, the standardized process of PFGE has yet to be available. Many international laboratory networks like PulseNet are in the process of validating this for detecting foodborne infection. Yan et al. recommended, in 2011, a list of molecular detection methods for C. sakazakii targeting powdered infant formula. As presented in Table 2, these protocols describe thorough procedures concerning the pre-enrichment, selective enrichment, isolation, and confirmation of Cronobacter in foods. These protocols can successfully identify C. sakazakii in infant formula and improve food safety and pathogen controls in production industries (Müller et al 2013) .

Table 2: Detection Protocols for Cronobacter in Powdered Infant Formula

Procedure	FDA (Original)	ISO/TS 22964	FDA (Revised)
Pre-enrichment	Mix 1:10 (w/v) sample in distilled water, incubate at 36°C	Mix 1:10 (w/v) of test sample in BPW, incubate at 37°C for 18 ± 2 hours	Mix 1:10 (w/v) of test sample in BPW, incubate at 36°C for 6 hours
Selective enrichment	Transfer 10 ml pre-enrichment to 90 ml EE broth, incubate at 36°C	Transfer 100 µl pre-enrichment to 10 ml mLST/vancomycin medium, incubate at 44°C for 24 ± 2 hours	Transfer 100 µl pre-enrichment to 10 ml mLST/vancomycin medium, incubate at 44°C for 24 ± 2 hours
Selection/Isolation	Streak and spread from EE stock onto VRBG agar, incubate at 36°C	Streak refined mLST/vancomycin medium onto chromogenic agar, incubate at 44°C for 24 ± 2 hours	Centrifuge 40 ml samples, resuspend in PBS, spread 100 µl on chromogenic media, incubate at 36°C

Procedure	FDA (Original)	ISO/TS 22964	FDA (Revised)
Confirmation	Pick five presumptive positive colonies and streak onto TSA, incubate at 25°C	Select five typical colonies, streak onto TSA agar, incubate at 25°C for 48 ± 4 hours	Select two typical colonies from each chromogenic medium, confirm with real-time PCR

7. Recent Approaches to Inhibit the Growth of Cronobacter sakazakii

New highlights in food protection have been aimed at identifying appropriate measures for preventing the growth and dissemination of Cronobacter sakazakii, which is considered a severe risk factor for public health, especially for children. The pathogen is closely related to severe infection, particularly in neonates, and has been identified in powdered infant formulas and other foods. Various strategies have been attempted to prevent pathogen growth in foods and processing environments. They sought to reduce the probability of contamination and guarantee food safety for babies and young children foods. Mainly discussed are the methods of natural antimicrobials, application of bacteriophages, modified atmosphere packaging, heat treatments, and new post- and pre-pasteurization additions.

7.1 Natural Antimicrobials

The most effective strategy to minimize its growth is the application of natural antimicrobials. The bacterial pathogen in issue is sensitive to natural compounds like the essential oils obtained from plants like thyme, oregano, and rosemary. For instance, powdered infant formulas and food matrices, in general, are sensitive to such contamination. These essential oils contain bioactive compounds like phenol, terpenes, and flavonoids, which have been reported to possess antimicrobial activity by interfering with bacterial cell membranes, proteins, and enzymes. For instance, thyme oil, which contains thymol, has been observed to inhibit the growth of C. sakazakii on different types of food surfaces. As above, carvacrol-containing oregano oil has also been effective against C. sakazakii by affecting the production of bacterial cell walls and modifying the membrane permeability. By interfering with these crucial cellular processes, these oils bring about the total death of bacterial cells, drastically reducing the pathogen count on food products. Applying these natural antimicrobials is efficient because they are commonly recognized as safe, effective against various organisms, and may be seen as recognized sculpture additives. But even so, difficulties persist in prescribing the requisite application dosage and delivery methods of these compounds such that they work effectively in creating the needful sensory impact on the food item in question without changing its essential organoleptic characteristics. Future studies should be done on concentrations and formulations for big-scale commercial uses, especially in the preservation of foods, and on questions of law about the compounds used in foods that infants may consume.

7.2 Bacteriophage Applications

The last method of different methods of C. sakazakii control in food products is the use of bacteriophages – viruses that are harmless to humans but lethal to bacteria. Biocontrol is gaining popularity mainly because of the targeted and selective action of phages that selectively kill pathogenic bacteria while sparing the beneficial microbiota in the food matrix (Zhang & Lee 2022). However, bacteriophages selectively target the bacterial pathogens without negatively affecting the particular food's normal flora, making them the most suited for controlling C. sakazakii. They function by finding an attachment site on the bacterial cell, then attach themselves, penetrate the bacterial cell, and use the bacteria cell machinery to make duplicates of themselves. This process results in the lysis, or destruction of the bacterial cell, freeing more phages to attack and destroy more bacteria. The difference in using phage therapy is clear because it often provides efficient means to minimize bacterial load on contamination, whereas, in the same instance, it does not have the same tendencies to develop bacterial resistance as ordinary antibiotics. Significant findings of present-day research have shown that various species of phage preparations can significantly decrease the presence of C. sakazakii in foods, including PIFs. Unlike conventional methods of processing food products, phage therapy may be used before the packaging of the products as it is friendly to the food products, much more efficient in handling contaminated foodstuffs, and precise in its application. In addition, since phages are bioorganic substances, they can be considered |safe| when utilized in food applications, under the condition that they are well described and their application is controlled. Yet, there are issues regarding some peculiarities of creating a commercial phage preparation: the selection of specific bacteriophages and the increased scale needed for biotechnological production.

7.3 Modified Atmosphere Packaging (MAP)

Another suitable method of controlling the growth of *C. sakazakii* in foods is Modified Atmosphere Packaging (MAP). In MAP, the type and concentration of gases in a food package are changed to a condition unfavorable to the growth

of spoilage organisms and the pathogenic bacteria C. sakazakii (Lehner & Stephan, 2004). MAP usually entails lowering oxygen levels in the package while elevating carbon dioxide or nitrogen levels to restrict bacterial respiration and, by extension, slow the growth of many aerobic pathogens. MAP is also helpful in curtailing the growth of *C. sakazakii* by lowering the oxygen level needed to develop this pathogen. Again, carbon dioxide inhibits the growth of many foodborne bacteria, including C. sakazakii, and plays the role of a bacteriostatic. Food products require time to get spoiled; due to this, nitrogen gas displaces oxygen, thus extending the durability of the products. MAP has proven efficacy in inhibiting the growth of *C. sakazakii* in dry and low-moisture foods – such as powdered baby foods and dried milk products (El-Sharoud et al. 2009; Ogihara et al. 2014). Another strength of MAP is that its application can increase the shelf life of these products, even if the taste and nutritional value of the food product are not compromised. There is, however, the reminder that MAP is not a magical bullet and must always be employed hand-in-hand with other food safety strategies. However, such an environment can significantly or entirely halter the growth of *C. sakazakii* but cannot eradicate it. Therefore, MAP is most effective when applied with at least one other pathogen control procedure, either heat treatment or natural antimicrobial agents, to avoid risks to the food product.

7.4 Heat Treatment Innovations

New heat treatment technologies applied in foods have also indicated the ability to control the growth of C. sakazakii in foods, especially in powdered foods and formulas for babies. Conventional heat treatments used in pasteurization have been proven to cause some issues in food quality and nutrient content. This has, therefore, given rise to efforts to find better heat treatments that can eliminate C. sakazakii to a clinically significant level without compromising on the innocuousness of food regarding taste, texture, and nutrient quality. Various methods can help achieve this; one is High-Pressure Processing (HPP), whereby high pressure is utilized instead of heat. This study revealed that employing HPP to decontaminate C. sakazakii from the powdered infant formula does not tamper with its taste, texture, or nutritional worth. This method exerts force on the food product, destroying and killing the microorganism's cells. It is more effective in heat-sensitive commodities, which have undergone pasteurization, resulting in profound changes in the quality of products. Another exciting innovation in heat treatment is microwave-assisted pasteurization. Pasteurization is a process of heat treatment wherein microwave energy is combined with traditional pasteurization to pasteurize the product more evenly and within a very short time. The different methods indicate that microwave pasteurization can efficiently eliminate C. sakazakii in powdered edible products such as infant formula without affecting the quality of foods. This heating is done at a high rate, meaning that the food stays very short at high temperatures, most nutrients are retained, and pathogens are deactivated.

7.5 enzymes used before and after the pasteurization process

Preliminary use of antimicrobial agents before and after pasteurization can even further check the growth of C. sakazakii in food products. These additives, whether of natural or synthetic origin, can, among other things, serve as an added line of protection against further contamination that may occur after pasteurization. The pre–pasteurization fortificants, on the other hand, are added to the food product before heat treatment to avoid contamination from microbes. Such preservatives may be antimicrobial peptides, organic acids, or plant extracts, which, besides having preservative properties, do not impact the sensory characteristics of the food. Meanwhile, post-pasteurization additives are used after the heat treatment process to avoid recontamination of an already treated product or the growth of a few bacteria that may remain in the food. It is noteworthy that applying these additives is critical for the safety of products not exposed to extra heat treatment after pasteurization, including those packaged in MAP. Some investigations have demonstrated that post-pasteurization inoculation of natural antimicrobial agents, like nisin, lauric acid, or plant essences, can essentially hinder the chances of C. sakazakii proliferation in food products. These agents operate through inhibition of bacterial cell wall synthesis or by inhibition of microbial metabolism; the pathogen is thus 'killed' before the food product is processed, even if it is already processed.

Conclusion

Cronobacter sakazakii continues to be identified as a critical and emerging threat to public health, especially for neonates, infants, and immunocompromised persons. It causes severe and often fatal diseases such as necrotizing enterocolitis, meningitis, and bacteremia and has a mortality of 40-80%. These infections mainly affect children below the age of three, implying a need to scale up surveillance, diagnosis, and prevention interventions to prevent them. Further identification of C. sakazakii in different food items, especially in fabricated foods, including infant formulae, underlines the emergent concept of food-borne transmission as a potent transmission

mode. However, a lot more has to be done with guarding surveillance and preventive measures for controlling the spread of the pathogen in the world by identifying the virulence factors and ability of the pathogen to survive in the climclimatesesent data on C. sakazakii infections are sparse and, because of the absence of detailed and systematic reporting worldwide, the scope of the problem posed by this pathogen is not well understood. Centers for Disease Control and Prevention reported 120 confirmed cases worldwide but argue that these numbers are significantly low given the current nature of the virus, given people are experiencing poverty and developing surveillance systems in combination with limited diagnostic tools in the developing world. These ongoing issues with surveillance and reporting make it extremely difficult to understand the epidemiology of the pathogen; thus, strengthening global surveillance is paramount. As it has been observed, one of the dark features of C. sakazakii is its virility in dry conditions in infant formula and baby foods. Therefore, the highlighted products are perfect habitats for the pathogen, which poses a danger to infants. Consequently, manufacturers of these infant foods must endeavor to minimize contamination risks by sterilizing equipment and maintaining the proper storage and transportation conditions. Likewise, improving the knowledge of caregivers and health professionals on food hygiene and safety issues will go a long way in reducing bacterial infections in at-risk population groups.

National and international regulatory forces have a key responsibility for protecting various foods, especially those meant for babies. These agencies must ensure proper safety measures for infant formula, cereals, and other foods that are accorded to infants and young kids. Although various principles and standard measures have already been adopted, compliance and legal enforcement still need to be improved. These regulations must be periodically updated since new risks related to C. sakazakii contamination Surface may appear. The adulteration of food production facilities, specifically regarding the contamination of infant food, has been previously discussed. It is evident from past studies that dust, debris, airborne, and surfaces at manufacturing companies act as ideal media through which C. sakazakii spreads. These results make it clear that contamination control should be applied in every stage of food processing, from receiving and storing raw materials to packaging food products. There is a need to clean and sanitize production environments to reduce the possibility of contamination using solutions as pathogens, such as sensing technologies. Furthermore, the health education of personnel in food processing and production plants has to be recommended to reduce incidences of Pathogens such as C. sakazakii. Thus, though remarkable advances were achieved in substantiating the pathogenicity of Cronobacter sakazakii and its potential impacts, it is still high time to prolong efforts to minimize the danger this menace poses to the community, especially the young ones. Surveillance, better diagnostic tools, and whether or not food production plants enforce strict measures to prevent Listeria contamination should be well understood as components of a sound Listeria control framework. Depending on the actual value of processed foods such as infant formula, etc., keeping a keen lookout for C. sakazakii contamination is essential. This is a dangerous pathogen, and all governmental bodies, health care agencies, food producers, and child and elderly caretakers must do all they can to prevent the spread of this disease and, therefore, ensure the welfare of children and the elderly.

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