Biofilm formation, virulence factor, antibiotic and dry resistance of Enterobacter sakazakiiand its implication ininfant food contamination

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Abstract

Bacterial infections are an escalating public health threat that causes morbidity and mortality. Enterobacter sakazakii is an opportunistic foodborne pathogen that can contaminate a wide range of foods and causes different diseases like meningitis, necrotizing enterocolitis, and bacteremia in immuno-compromised, low weight individuals, neonates, infants, and elders. Enterobacter sakazakiiis expected to possess diversified virulence factors and have the ability to persist in extremely dried foods such as powdered infant formula(PIF) that can act as the main vectors of transmission. Therefore, to prevent infants from this bacterium it is better to use breastfeeding for the first 6 months of age. However, infants of HIV positive mothers, infants with very-low-birth-weight, infants born from working-class mothers, and infants of mothers who are taking medication are the main contributing factors that lower or hinder breastfeeding and in this case, they are forced to use powdered infant formula but these products are not sterile. Enterobacter sakazakii is also expected to imperil the health of infants who are found in emergency sites like a refugee because of the high consumption of powdered infant formula and lack of facilities. Infant food contamination by this bacterium is attributed to biofilm which are microbial communities attached to biotic or abiotic surfaces using a self-produced matrix of extracellular polymeric substances. Enterobacter sakazakii can form biofilms on food, food processing surfaces, and equipment that ushered cross-contamination. The contamination scenarios can be during manufacture, processing, handling, preparation, storing, and reconstitution. Enterobacter sakazakiialso showed multidrug resistance to some antibiotics. Biofilm formation accompanied by antibiotic resistance is worrisome for medication. Therefore, to prevent or reduce risk contamination infant food companies, health sectors, caregivers, aid providers, mothers, families, and other concerned bodies must be informed and alerted about this hidden bacterium. Thus, this review focuses on biofilm formation, virulence factors, antibiotic and dry resistance of Enterobacter sakazakii and its implication in infant food contamination.

keywords: Enterobacter sakazakii,infant foods, biofilms, antibiotic resistance, dry tolerance

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Introduction:

As a principle, every citizen of a country should get sufficient, affordable, safe and nutritive food [1]. However, outreaching these necessities for all is impossible due to different reasons such as food contamination by microbes or their toxins and chemicals which can imperil its safety[2,3, 4, 5].This is because food can reach us after passing so many stages in the food chain. In these long-chain foods can be contaminated during harvesting, manufacture, processing, packing, distribution, retailing, handling, preparation, storing, reconstitution and consumption[4].Thus, the safety of the food can't be maintained by a single person rather it is an issue of all individuals who are involved from farm to fork. Contaminated foods create a complicated health problem such as bacterial infections in humans[6], specifically the health of infants, young children, elderly and immuno-compromised individuals are scourged by bacterial infections [7]. That is why most hospitals are hosting many neonates due to bacterial infection along with other complaints[8].Although inspiring progress has been made on health sectors such as the discovery of antibiotics and the development of vaccines, bacterial infections are inevitable to date [9, 10, 11]. Thus, the bacterial infection becomes an escalating public health threat which causes morbidity and mortality worldwide [2,4], especially in developing countries where there is poor hygienic practice, lack of clean water and health facilities[8].

Among many food contaminants, Enterobacter sakazakii is the one which is a gram-negative, facultative anaerobic, motile with peritrichous flagella, rod-shaped and non-spore forming bacterium. Enterobacter sakazakii is one of the opportunistic pathogens that belong to the genus Enterobacter and Enterobacteriaceae family and becomes risky to infant health [12, 13].As we know bacterial infections can occur in all age groups of our society [`11], however, infants, elders, and immuno-compromised individuals are highly vulnerable age groups [14,15].The same is true for Enterobacter sakazakii that causes infections in all age groups, but it threatens infants and other susceptible individuals[12].Enterobacter sakazakii is the one that can contaminate a wide range of foods and

causes different disease like meningitis, necrotizing enterocolitis and bacteremia in immuno-compromised, low weight individuals, neonates and infants with a mortality rate of 40–80%[16,17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34,35, 36, 37]. For instance, in a neonatal intensive care unit Enterobacter sakazakii infections had occurred in 1994 in France and 17 neonates were infected and showed enterocolitis, septicemia, and meningitis with different prevalence [19].Enterobacter sakazakii is found in high-risk foods such as powdered infant formula, dried infant foods and can cause infections after consumption[38, 39] (Figure 2). Therefore, this bacterium can survive and persists in powdered infant formula and preparation equipment which have been linked to different complications among infants[40, 41] (Figure 2).Similarly the contamination scenarios can be during manufacture, processing, packing, handling, preparation, storing and reconstitution [4](Figure 2). Intrinsic contamination reports are coming up frequently that indicates the product is not sterile. In support of this notion, Cronobacter spp has been recovered from previously unopened tins of powdered infant formula, indicating intrinsic contamination [39]. One of the possible sources of contamination might be from the factories because of the ineffectiveness of methods to remove all microbes from powdered infant formula once and for all [42, 43] or maybe from the ingredients added that may carry foodborne pathogens such as Enterobacter sakazakii[44]. This is because various thermo-labile raw ingredients are added to PIF without heat treatment [45]. As the WHO report revealed a single contamination report in the food sector has the power to recall tones of food products from where they are found and leads to the economic crisis, tourism reduction and trade embargo and it has the power to darken country reputation as well[46]. This results in an economic impediment[7],knocking the door of individuals, families, communities, industries, and countries. Besides food safety, an exorbitant cost of food is worrisome for those who can't afford [47]. For instance, the prices of infant foods in developing nations is a headache for most mothers where there is no extra income except hand to mouth. Specifically, citizens who are wrestling with natural and man-made disasters are severely affected. As the WHO report indicated the cost of unsafe food is going far beyond human suffering[7].

Microbes have different strategies to fit with their environment and battle with the host immune system. For instance, Enterobacter sakazakii has several strategies such as osmotic and dry tolerance, tolerating a wide range of growth temperatures, biofilm formation, and antibiotic resistance are tremendous for the survival of this bacterium on hostile environments. Biofilms of pathogenic bacteria, such as Enterobacter sakazakii are problematic in the infant food industry. This is because the ability of this bacterium to produce biofilm is one of the principal factors for infant food contamination. Cronobacter sakazakii can form biofilms on the surfaces of silicone, polycarbonate, and stainless steel[48][Figure 3. Therefore, biofilm formation by Enterobacter sakazakii and other pathogens on enteral feeding tubes constitutes a risk factor for susceptible neonates [49][Figure 2].In addition to biofilm-forming, this bacterium is resistant to different antibiotics that worsen the medication. As reports revealed Cronobacter sakazakii is resistanta mpicillin,cefotaxime,cephalothin, andceftriaxone [36].

Enterobacter sakazakii possesses a weapon like virulence factors that aid in tissue adhesion, invasion and host cell injury[31,13]. It is also resistant to harsh environmental cues and stresses which contributes to being more armed and persist for prolonged periods[31]. Even if this foodborne pathogen is known in scientific communities, however mothers especially HIV positive mothers, caregivers, elders, pregnant women, employed mothers, employers, household workers, infant food companies and workers, aid providers, communities in refugee camps, food packing companies, baby bottle manufacturers and other concerned bodies must be alerted about this hidden bacterium including its ability to produce biofilms, antibiotic and dry resistance, and virulence factors. Therefore, the effects of Enterobacter sakzakii infection on those susceptible age groups can be minimized if all the above mentioned and others are collaborating. Thus, this review article focuses on biofilm formation and antibiotic resistance of Enterobacter sakazakii and its virulence factors which have a tremendous role in cross-contaminations of infant foods such as powder infant formula.

Susceptible and risky age groups

Infants, elders, pregnant women, people who have debilitated immune systems and poor individuals who cannot afford medication and nutrition are susceptible populations to foodborne diseases [4]. Age is one of the critical determinants for the immune system of infants because their immune systems are not fully developed which in turn leads susceptibility to infection [50].Enterobacter sakazakii is one of life-threatening bacterium for immuno-compromised, low weight individuals and premature infants whose age is less than 28 days [18, 25, 51, 52, 53, 54, 55, 56]. This bacterium is also a critical threat for infants of HIV-positive mothers since breastfeeding aggravates the viral transmission. Unless intervention is taken the virus can be transmitted from mother-to-child via breastfeeding [57]. Therefore, infants whose mothers with HIV are vulnerable age groups [58]. In this case, infants may use alternative feedings such as infant formula but these products are not sterile enough because of microbial cross-contamination like Enterobacter sakazakii[18, 39, 59, 60]. Generally immuno-compromised, low weight individuals, neonates and infants [16] and infants of HIV positive mothers are susceptible and risky age groups for this bacterium. As reports revealed reconstituted infant formula in neonatal intensive care unit was contaminated by Enterobacter sakazakii that causes infections and this alarms caregivers, industries, scientific communities, and other stakeholders to improve neonatal health care units [39, 51].Due to these reasons, it has been ranked by The International Commission for Microbiological Specification for Foods as a bacterium having "severe hazard for restricted populations, life-threatening or substantial chronic sequelae or presenting effects of long-duration" [61].

Breastfeeding and Enterobacter sakazakii

The fate and sustainability of each country depend on the shoulders of the present and upcoming generations like infants and children. Therefore, each generation should be healthy and better off than

us in every aspect of perpetuity. This can be achieved when every citizen is responsible and worry about the health status of infants and children. The principal and forefront infant food providers are mothers who feed their sterile breastfeeding. Breastfeeding is a precious gift given by either poor or rich mothers to their infants or children [62]. From a nutritive and health point of view, breastfeeding is the safest infant nutrition. However, this valuable gift may not be given or recommended to their children or infants due to different reasons, such as when mothers are infected with communicable diseases like HIV[42, 63, 64]. As we known mothers strive with different burdens within the family. In this case, the whole family will be also in trouble especially infants and children will face the problem with their mothers. Moreover, there are contributing factors that lower the rate or early cessation of breastfeeding like mothers might be informed not to breastfeed due different reasons, or the mother may take medication, low-birth-weight [42], infants born from working-class mothers or maybe knowledge or educational level of mothers. For instance, the employment of mothers, especially full-time employment, has a negative influence on the duration of breastfeeding [65].This is because mothers do not have enough time to nurse their infants so that they may use alternative feedings such as infant formula[66]. Unfortunately, this artificial feeding may open a road for other health-related complications unless safety measures are taken. This is because infant foods can be contaminated with Enterobacter sakazakii and other pathogens that can cause serious health problems and even death in newborns and children [67]. Therefore, it is recommended to feed breastfeeding for the first 6 months of age regarding mothers' health[68]. If the health status of mothers is good enough it is better to resume breastfeeding to prevent infants and children from Enterobacter sakazakii or other pathogens.

Infantfoods are scourged by Enterobacter sakazakii

Food contamination is a critical issue in food safety because of the transmission of opportunistic foodborne pathogens such as Cronobacter sakazakii[69]. Infant food contamination by this bacterium can exert pressure on food industries, families, infant health, mothers, health sectors and global markets. Although attention is given to powdered infant formula especially its contamination by Cronobacter sakazakii and its causative agents of life-threatening neonatal infections[56],in my opinion all concerned may nay not be awared about this bacterium especially in developing nations and emergency sites like refugee camps. Cronobacter sakazakii is a threat to infant, neonatal and other susceptible age groups resulted from utilizing contaminated powder infant formula, contaminated surfaces, or utensils such as spoons used for reconstitution and its outbreaks is linked to contamination of this product [54][Figure 2].Similarly, Cronobacter spp. were isolated from the manufacturing environment, intermediate powder, and finished products and from those isolates Cronobacter sakazakii was the most prevalent when compared to others[70]. This was also in agreement with a study conducted by Hochel et al. and Lee et al that indicated Cronobacter sakazakii was the most prevalent species recovered from food samples analyzed when compared to other Cronobacter spp. [25, 71]. Therefore, powdered infant formula is the greatest reservoirs of Cronobacter sakazakii. As reported by numbers of scholars this bacterium has been isolated from different samples such as clinical specimens, environmental, foodstuffs and food processing environments[34, 72].Cronobacter sakazakii has been isolated from milk, infant milk formula, cheese products, meat, fish, cereal, fruit herbs, spices, and vegetables, legume products, herbal teas, and environmental samples such as in dust and food preparation environments[12, 22, 39, 55, 70, 73, 74].Enterobacter sakazakii was also identified from the mouths of stroke patients[75]. Cronobacter sakazakii is the most frequently clinically isolated species of the Cronobacter genus[76]. Thus, it is an issue of immuno-compromised individuals. Cronobacter sakazakiiis ubiquitous[73], but it is profoundly linked with infant formula powders which are implicated in infant infections[77]. As reports revealed Cronobacter sakazakii is the cause of product recalling from the market. For instance, in Canada, United States, Europe, and Asia- Pacific region there was a recall of infant formula from the market because of contamination of the product with Cronobacter[78, 79]. Similarly, contamination of PIF with these opportunistic pathogens was quite common in German in the early 2000s[80]. This finding is tremendous evidence that powdered infant formula is not a sterile product that alarm all concerned bodies such as infant food companies, infant food aid providers and customers. Milk and meat products such as cheese, milk powder, fermented milk, sausage were also contaminated byEnterobacter sakazakii along with other bacteria such asStaphylococcus aureus, Escherichia coli O157: H7, Listeria monocytogens, Salmonella[81]. Besides Enterobacter sakazakii, infant formula powder samples had been contaminated by pathogens like Klebsiella pneumoniae, Klebsiella oxytoca, and Enterobacter cloacae[82]. As reported by Zhou et al, Enterobacter sakazakii and Klebsiella pneumoniae cause morbidity and mortality in infants after consuming contaminated powdered infant formula[83]. A study conducted by Cho et al revealed that Cronobacter sakazakii, Salmonella enterica, Staphylococcus aureus are major pathogens associated with infantile fatal diseases which can contaminate powdered infant formula from a different sources such as hands or skins of food handler or materials like spoons used for reconstituting [84][Figure 2].Salmonella enterica can contaminate powdered infant formula and grouped with Cronobacter sakazakii, under category “A” which causes illness in infants[59]. Cronobacter sakazakii is not the only problem for human beings but also other organisms such as chicks. As recent studies indicated Cronobacter sakazakii can also contaminate fertilized eggs which may result in weak chicks, poor chick growth increased mortality of embryos, lower hatch ability and increased early chick mortality [37]. Generally, even if this bacterium can contaminate various foodstuffs, it is an obscure danger in infant formulas and baby foods [85] that scourges infant health.

Infant feeding at refugee camp and threat of Enterobacter sakazakii on thier health

Human beings are living in an unpredicted environment where they forcibly displaced or lose their life within a moment either by natural

disaster or man-made problems. For instance, natural disasters like earthquakes, floods, typhoons, and tsunami pose a real challenge for communities[86].Similarly, man-made disasters such as war or internal conflict lead citizens to flee from their country without having anything at hand. In this dramatic and risky journey infants and young children [86], are the forefront vulnerable age groups exposed to food insecurity and foodborne diseases. During this internal instability infants, young children and mothers are wrestling with hunger, disease, anxiety, loneliness and other heartbreaking events like their day to day life is only restricted within refugee tents, where no sufficient access for health, food, clean water. education and other facilities such as electricity. At refugee camps, there is a high risk of malnutrition, illness, and death where children under two years of age who are not breastfed and in infants under six months of age who are not exclusively breastfed are severely affected [87]. Therefore, life at refugee is just like living at "hell" where everything is dark especially for those innocent infants and mothers who are punished without sins. Among many problems, food is one of the critical issues that infants are facing at the refugee camp. For instance, research conducted at Saharawi refugees, in Algeria revealed that there was poor breastfeeding practice which leads undernourishment of infants [88]. This leads to an interruption of breastfeeding and usage of inappropriate complementary feeding which increases the risks of illness, malnutrition, and mortality[86]. Therefore, the usage of commercial complementary food in humanitarian emergencies is an escalating issue alerting food policymakers and other concerned bodies[89]. For example, a refugee camp found in Greece was visited by Nurture Project International team members and they reported that 40 to 60% of mothers were formula feeding in the camps. In a situation where there is trouble and food insecurity mothers will agree and take anything that comes to their hand to save the life of their infant[90]. However, infant formula is not a sterile product and requires special handling infrastructures such as storage temperature, holding time and clean water to reconstitute the powder [91]. As the WHO reported infant illness and death has been attributed to PIF which has been contaminated with Enterobacter sakazakii or Salmonella, at either at the processing or during reconstitution [42][Figure 2]. However, in emergency sites, there is a scarcity of facilities such as storage equipment and temperature abuse allowed Entertobacter sakazakii to proliferate. For instance, using the formula in refugee camps is risky because the continuity of the supply is in question and women will dilute the milk with water that is again an issue of nutrition and health. Moreover, Bauer added that at a refugee camp in Greece even this nonsterile powder milk can't be heated properly which is as difficult as eating raw chicken[90]. That is why FAO/WHO expert committee recommended that powdered formula must be reconstituted at temperatures greater than 70°C, to inactivate intrinsic bacteria such as Enterobacter sakazakii and limit their growth[59]. Therefore, using infant formulas at refugee has an impact on infant health unless necessary safety measures are taken. Even if there is no specific previous research done on Enterobacter sakazakii at a refugee camp, it is believed that this bacterium is expected to be found in such risky sites because of high consumption of infant foods and lack of clean water to reconstitute and other facilities like a refrigerator for preservation of perishable foods. Bauer reported that the plethora of women gave birth in transit and refugee in Turkey or Greece, and mothers use a formula for their infants even some babies had never been breastfed[90]. Therefore, these infants and babies would at risk since powdered infant formulas can be contaminated by Enterobacter sakazakii[59].

Enterobacter sakazakii and its virulence factors

The effects of bacterial infections depend on health status, nutrition, hygiene, age and bacterial related virulence factors such as toxins, structures and enzymes [4]. Bacterial virulence factors have a decisive role in their pathogenicity. Some pathogenic microbes having either structural, enzymatic or toxic virulence factors can be considered as like a country having "nuclear weapons" which has the power to devastate their rival or enemy. That is why microbes possessing such kinds of weapons are deadly pathogens and infectious. Even if we have an array of "radar" like immune system, these deadly pathogens have the power to dismantle our immune system using virulence factors unless medical measures are taken. Although Cronobacter sakazakii is an opportunistic foodborne pathogen that can cause life-threatening meningitis and necrotizing enterocolitis in premature infants, much was not known about its virulence determinants [92]. As reports revealed Cronobacter sakazakii strains were able to adhere to cell lines, HT-29 and N1E-115 cells and majority of them demonstrated diversified virulence factors [13]. A study conducted by Parra-Flores et al. confirmed that Cronobacter sakazakii strains isolated from powdered infant formula were more invasive than other Enterobacter spp. which might be the attributes of virulence factors[36]. New and novel virulence factors known as labp have been discovered in Cronobacter sakazakii that promote the production of lipid A by using binding partner called LpxA [92]. Virulence genes that encode various proteins involved for motility, synthesis of iron-chelating such asEnterobactin, metabolism of sugurs like galactoseand mannoseare critical for for virulence [25]. Besides virulence factors of Enterobacter sakazakii, patient's susceptibility, age, level of contamination of food, tolerance to temperature, speed of growth, infectious dose and biofilm formation[31] are critical factors contributing for increasing infections[93]. Biophysical growth factors such as the formation of biofilms are critical for Enterobacter sakazakii that makes this bacterium to be a potential pathogen [31]. Microbes either causing infection or spoilage can be attached to biotic or abiotic surfaces which have a detrimental impact on the medical and food sectors. When they are attached on these surfaces they come together within a group of microbial communities known as biofilms. Microbes within a biofilm are "sewed" together by extracellular polymeric substances which act as a "thread" to sew them. Therefore, these surface-attached microbial communities can be a source of continual contamination of foods that has food safety and economic implications [94]. As it has been reported this bacterium can tolerate desiccation which might be the attributes of biofilm formation. The other critical issue that is accompanied by biofilm formation is antibiotic resistance of microbes that worsen the problem. Enterobacter sakazakii showed multidrug resistance to some antibiotics [95]. A study conducted by

Parra-Flores et al revealed that all isolated strains of Cronobacter sakazakii showed resistance to more than one antibiotic [36]. Biofilm formation and multiple antibiotic resistance were highly interconnected irrespective of the source, type, and environment of the isolates[96]. There was also a correlation between putative virulence factors and antibiotic resistance among the tested Cronobacter species[97]. Therefore, if microbes possess resistant genes along with biofilm formation it is also like an individual possessing "prohibited weapons" that threaten medical sectors.

Resistance of Enterobacter sakazakii to different environmental stresses

From a food preservation point of view, hyperosmolarity and desiccation are paramount to hinder food spoilers and foodborne pathogens. However, this food preservation technique is not applicable for all groups of microbes because some groups of microbes encode mechanisms to survive and withstand these stresses[98]. The prolonged persistence of pathogens in the desiccated state is important especially in contaminating dried foods that have low water activity [99, 72].Cronobacter sakazakii can survive in such kinds of dried habitats and other hostile environments for long periods [100, 101, 102, 103].Cronobacter spp. adapt to dry stress in relative to other Enterobacteriaceae family membrers. The ability of this bacterium to tolerate desiccation is a tremendous advantage to persist in dry environments, such as in milk powder factories, on food processing surfaces and food preparation utensils and so that it can be source of post-contamination for the final food product[104,105]. The ability of this bacterium to produce biofilm is paramount in resisting dry and other hostile environmental conditions. This is because biofilm by its nature has a "spongy" like structure that can absorb water and prevent bacteria from desiccation. Therefore, biofilm is resistant to adverse conditions such as antibiotics, desiccation, and immune defenses and are key contributors to many chronic infections [106]. As a general Enterobacter sakazakii is more resistant than other Enterobacteriaceae to various environmental cues and stresses, such as low water activity, pH, temperature and dryness [107]. Biofilm formation, possession of virulence factors, high resistance to elevated osmotic, low pH, heat, oxidation, and desiccation are critical features of Enterobacter sakazakii that help this bacterium to survive in hostile dried foods such as powdered infant formula and cause disease in infants and other susceptible age groups[32].Accessory genes, existingas sessile and planktonic life form, resistant genes, virulence and niche-specific genes enables Cronobacter sakazakii to adapt in diverse and hostile environments[25].

Biofilm formation by Enterobacter sakazakiiand its implication on contamination of foods

As previously defined biofilm is a microbial community that is attached to biotic or abiotic surfaces or interface onto each other and embedded in a self-produced matrix of extracellular polymeric substances [108]. Bacterial biofilm formation requires a series of stepwise processes accompanied by physiological and structural changes. This dynamical process comprises (1) initial attachment (2) irreversible attachment (3), microcolony formation, (d) maturation (e) dispersion [109] [Figure 1]. Biofilm formation steps have their own importance on food contamination point of view. As Jahid and Ha reported biofilms formation on food and food processing surfaces depends on the interactions of different cues such as physical, chemical, and biological processes, nutrient availability and types, temperature, molecular cross-talks, production of extracellular polymeric substance (EPS), maturation of biofilm, and dispersal steps[110].Microbial biofilm and quorum sensing (molecular cross-talk) are "two sides of the same coin" which are important for survival and increase virulence[111].As shown in figure 1 persistent cells are escaped from their "caves" and dispersed and seek new conducive environments for their next fate. Biofilm formation is cyclical, therefore, bacteria have the means to disrupt their biofilms and return to their free-form or planktonic lifestyle. For these cyclical events enzymes played a tremendous role in cleaving basic components of the biofilm matrix and thus weakening the biofilm architecture which allowed dispersion [112][figure 1]. As many research works revealed biofilm-related infections are an increasing health threat in medical sectors [113], which can cause chronic infections and antibiotic resistance [96]. Antimicrobial resistance and microbial biofilm formation by bacteria, in turn, lead to medical treatment failures [11]. Therefore, the ability of bacteria to form biofilm and antibiotic resistance has a critical role in increasing bacterial pathogenicity and the risk of death [10]. Biofilm-related infections are serious since microbes undergo physiologic and structural changes that are tolerant of hostile conditions such as antibiotics, sanitizing agents, and host immune systems [114]. The severity of this biofilm-related chronic infection is very high for those immuno-compromised individuals and infants whose immune system is not fully developed. Therefore, bacterial biofilms are a critical defense mechanism in their communities that enhance attachment and infection [115]. Microbial biofilms are also the chronic issue in food sectors since they are a potential source of food contamination [32] and this is because of surface-attached microbial community harbor pathogens and food spoilers [94, 102 116]. A plethora of bacteria including food spoilage bacteria and pathogens can have the ability to form biofilms on different surfaces such as food processing surfaces which could be a source of food contamination or spoilage[117]. Microbial adhesion to food processing surfaces and equipment is an issue of food safety because of food contamination[118]. Likewise, Cronobacter sakazakii is foodborne pathogen that can imperil food safety by forming biofilms on a number of different food processing surfaces [102]. Similarly, Cronobacter sakazakii forms a biofilm on the surfaces of equipment and processing environments which is an important source of persistent contamination in food samples. Biofilm of Cronobacter sakazakii on the surfaces of equipment and processing environments is an important source of persistent contamination in food samples[119][Figure 2]. This is because biofilm produce glue like structues that cement any particles as well as microbes to persist and resist for long periods. Microbes within this cave can hide and they are considered as "rebellion" groups against sanitizers. Cleaning and disinfecting would be more difficult when biofilm is produced in such inaccessible locations of a processing facility

and surfaces which leads food contamination[120]. Therefore, the ability to be attached to different surfaces, including rubber, silicon, polycarbonate, and stainless steel, is an evidence for the persistence of Enterobacter sakazakii on infant formula preparation equipment and in food-processing environments[77].

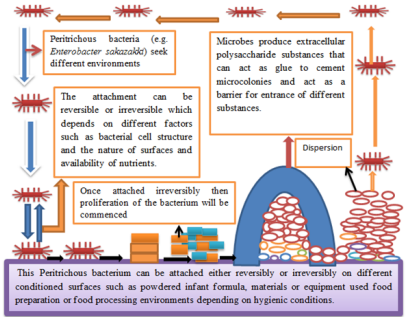

Figure 1. Schematic representation of biofilm formation by Enterobacter Sakazakii (own source)

As shown on figure 2, Enterobacter sakazakii can be attached on the surface of spoons, trays, tin, and other materials used for reconstituting artificial infant feeding so that it will be source of contamination. Therefore, poor sanitation practice as well as biofilm formation on food contact surfaces, materials, and food processing environments has its own role on outbreaks of foodborne disease [121]. As it has been reported Enterobacter sakazakii has the ability to attach and form biofilm on infant-feeding equipment and preparation areas such as latex, silicon, enteral feeding tubes and stainless steel [122, 123] [Figure 2,3]. For instance, cells of Enterobacter sakazakii attached to stainless steel or in biofilms formed on stainless steel are persistent that can survive for long periods[124]. In addition to forming biofilms on the surfaces of these materials, reconstituted infant milk formula (IMF) is also a conducive nutrient for biofilm formation which can be source of contamination and infection [125]. Therefore, microbial biofilms are an escalating issue in food industry and food saftey, since biofilm formation on raw materials or food contact surfaces could be a sources of food contamination with spoilers or pathogenic microorganisms[54]. The other critical scenario for contamination of powder infant formula along with biofilm formation is during production, reconstitution, addition of ingredients, handling or storage of reconstituted product [29][Figure 2]. As indicated on figure 2 extrinsic contamination can occur when contaminated utensils such as spoons, blenders, bottles, teats are used for reconstituting or feeding PIF, or contamination may occur from the preparation environment[42].Cronobacter sakazakii can cause infection via contaminated expressed breast milk, and it has been recovered from neonatal feeding tubes of neonates[36][Figure 2]. Therefore, we can infer that materials that we used for preparing infant feeds must be cleaned using appropriate methods, otherwise it will be source of contamination as depicted on figure 2. As the conducted by Hurrell et al Enterobactenaceae members were able to attach and grow on enteral feeding tubes as bacterial biofilms. From disinfection point of view Enterobacter sakazakii could produce biofilm and protect the cells from being affected by disinfectants. It is also more difficult to wash such bacteria off from the surface they attached with biofilm and the chance of cross-contamination could increase [125]. For instance, as the biofilm ages, clumps of cells will be shed which may survive passage through the neonate's stomach due to protection from the acidity [49] [Figure 1, 2].

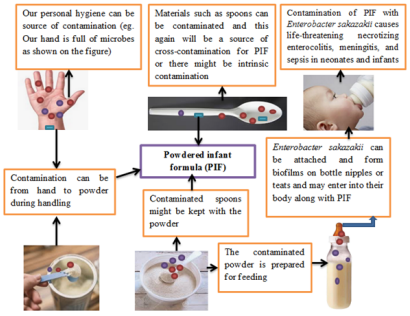

Figure 2. An example of possible source of bacterial cross-contamination [Source, 84with own modification]

In addition to producing biofilm, their ability to grow in a wide range of temperatures is also paramount. Enterobacter sakazakii was able to grow in PIF at a wide range of temperature between 6° and 45°C and its optimum temperature being 37° to 43°C [124]. During preparation, handling, feeding, and storage the powdered infant formula might be subjected to different temperatures that can either increase or decrease the concentration of Enterobacter Sakazakii[99].Therefore, knowing its optimum temperature is important to reduce their concentration that can be found either on the infant food or processing surfaces, and preparation equipment. Thus, the temperature of the water that we used to reconstitute or wash these materials must be beyond its optimum temperature to reduce or kill the attached bacterium. However, this recommendation may not be applied by mothers or caregivers in a refugee camp and other emergency sites because of lack of heat, clean water or other facilities that ushered them into further health complaints.

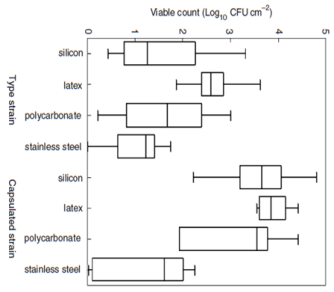

Fig. 3 Biofilm formation by Enterobacter sakazakii on infant-feeding equipment and work surfaces using infant formula milk as growing medium[124]

Antibiotic resistance of Enterobacter sakazakii

Antibiotic resistance is increasing with an alarming rate which poses a health hazard. If antibiotic resistance is increasing in such momentum really medication will be in trouble so that microbial infections will not be treated using antibiotics. Overdose utilization of antibiotics and spread of antibiotic resistant bacteria are the forefront factors for the emergence of resistance besides natural

resistance[126].Overdose utilization of antibiotics is just like "training bacteria" to develop resistant mechanisms from antbiotics and they will be fit for new antibiotics and challenge. Enterobacter sakazakii and Klebsiella pneumoniaeisolated from PIF showed multi-drug resistant and producedenzymes such as extended spectrum β-lactamase (ESBL) that can inactivate antibiotcs. Even if this threatens infants worldwide,theseverity is very high in developing nations where antibiotics were used wrongly[83].A similar report also revealed that extensive utilizationantibiotics will born multidrug-resistant bacteria that can devise their own mechanisms against new drug launched in the clinic [127]. Similarly, Enterobacter sakazakii is resistant to different antibiotics that worsen the medication. A study conducted by Kilonzo-Nthenge et al revealed that Enterobacter sakazakii showed multidrug resistance to penicillin, tetracycline, ciprofloxacin, and nalidixic acid[95].However, as antimicrobial susceptibility test conducted by Fei et al revealed that Cronobacter strains were senstive to most of the antibiotics and whereas some strains were resistance to amoxicillin-clavulanate, ampicillin, and cefazolin[128].In on the contrary to this report most Cronobacter spp. isolates were susceptible to ampicillin, tetracycline, and chloramphenicol[25]. As Kilonzo-Nthenge et al[95] reported Enterobacter sakazakii showed multidrug resistance to tetracycline. As so many research works indicated biofilm formation is accompanied by antibiotic resistance. Therefore, it can be said biofilm formation and antibiotic resistance are "two sides of the same coin". Biofim formation paves ways to antibiotic resistance. Physiological and structural change within biofilm enables microbes be resistant against most antibiotics. Structural barriers along with persist cells within biofilm plays a decisive role for antibiotic resistance [112].Enterobacter sakazakii possess functional genes that are responsible for biofilm formation which may have the potential to cause risks to food safety[102]. Biofilm forming bacteria is resistant to antibiotics when when compared to their planktonic counterparts due to the above mentioned advantages[96]. Biofilms generally microbial protect the cells from antimicrobial action and other hostile environmental conditions so that they can stay for long periods by adhering to biotic or abiotic surfaces. As general antimicrobial resistance along with biofilm formation has become an escalating health threat and food safety worldwide.

Conclusions

A bacterial infection is escalating public health that can cause mortality, morbidity and can impede economic development. Cronobacter sakazakii is one of the emerging and opportunistic pathogens linked with outbreaks of life-threatening necrotizing enterocolitis, meningitis, and sepsis in neonates, infants, and other susceptible age groups. Cronobacter sakazakii can contaminate a wide range of foods, especially powdered infant formulas are the main reservoir of this bacterium that scourged infant health. This bacterium has the potential to survive in dried environments for extended periods which allows it to persist in dried foods such as powdered infant formula and other foodstuffs.Biofilm is a group of microbial cells firmly attached to biotic and abiotic surfaces enclosed within a glue-like matrix which are serious health challenges and cause of economic losses. Dry tolerance is also assisted by biofilm formation. This is because biofilm are microbial communities that can produce cement like extracellular polymeric substance and these structures, in turn, are spongy which prevents cells from desiccation. Biofilm formation by Enterobacter sakazakii again paved the way for antibiotic resistance. This is because cells within biofilms are just like "rebellion" groups that hide inside "caves" so that antibiotics and host immune systems will not take any measures or actions. Biofilm formation, dry resistance, and antibiotic resistance are not the only strategy but it is also armed with diversified virulence factors that can dismantle the host immune system and protect its "sovereignty" from enemies such as host immune systems. Generally, Enterobacter sakazakii is an opportunistic pathogen that scourge infant healths and it is capable of forming a biofilm, resistant to antibiotics and dryness and other hostile environments.

Recommendation

Cronobacter sakazakii is the scourge of infant health that causes life-threatening necrotizing enterocolitis, meningitis, and sepsis in neonates, infants, and other susceptible age groups. Therefore, all responsible person who is involved from the very beginning of its production up to consumption must give attention for this bacterium that targets on specific segments of our populations who are the most susceptible age groups. Powdered infant formula and other infant foods are the main vectors for Cronobacter sakazakii. If the health status of mothers is good enough, then there is no substitute for breastfeeding in terms of safety and nutrition. Therefore, breastfeeding advocacy is necessary to scale up and resume breastfeeding. However, there are some barriers that lower or hinder the rates of breastfeeding such as workplace barriers and the health status of mothers. Due to these and other factors, most mothers are forced to wean early. Therefore, by considering the seriousness of the issue, the concerned bodies must fulfill facilities for mothers to nurse their infant at the workplace. Thus, it is better to allow a reasonable break time for mothers to nurse their babies at the workplace. Even though breastfeeding is the best food, there are occasions in which artificial feeding such as powdered infant formula is consumed. For instance, infants of HIV infected mothers are the most vulnerable age groups so that special care must be given to protect them from the virus as well as bacterial infection via contaminated PIF. Under such circumstance mothers and caregivers should be informed to follow up on the guidelines by prepared product manufacturer, WHO or FAO or while they are reconstituting, preparing, and storing. However, this is a critical challenge to apply these guidelines at emergency sites such as refugee camps where no access to facilities. In connection with, further investigation is needed about the prevalence and associated risk factors of Enterobacter sakazakii in such risky sites. Since this bacterium is capable of forming biofilms on infant food processing surfaces and food preparation utensils such as spoons, cans, trays and other equipment, then strict hygienic practice must be employed. The stakeholders such as mothers should be seriously alerted to clean the feeding utensils especially bottle nipples or teats must be cleaned and disinfected frequently along with handwashing

to prevent biofilm formation. If food particles are left on bottle nipples or teats, then it can be considered as preparing appropriate growth media for cultivating Enterobacter sakazaki.Additionally, the temperature of the water that is important for reconstituting the formula or washing these utensils must be sufficient enough to inactivate this bacterium. The current prevention methods appear to be insufficient to ensure that such foods are free of Enterobacter sakazakii. Therefore, the scientific community should device novel methods that can exclude this bacterium from infant foods without affecting the nutritive value of the food. Thus, this should be an assignment for all concerned bodies. Product recalling from the market will not solve the problem rather infant food companies and concerned bodies should formulate their protocols to check up and follow up any failures through food processing stages. Therefore, to detect any failures at the infant stages Good Manufacturing Practice (GMP) and Hazard Analysis and Critical Control Point (HACCP) and Cleaning-in-place (CIP) play pivotal roles that save the infant food companies from economic losses and health defects. The last but not the least is antibiotic resistance which is the major public health threat so that attention must be given on utilization of antibiotics. It is not to that much difficult to guess what will be our fate if all microbes develop resistance to antibitics. Therefore, awareness is mandatory especially we shouldn't use antibiotics without prescription. Therefore, if we use antibiotics without doctors' prescription, then microbes will be trained for challenges.

Data availability

The data or information used to write this review article is available from the corresponding author upon request.

Conflicts of Interest

I declare that there are no conflicts of interest in regard to the publication of this paper.

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