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Contamination of Powdered Infant Formula with *Enterobacter sakazakii* and *Salmonella*

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… like the stars, these ideals are hard to reach –
but they serve for navigation during the night.

"Ideals", The Oxford handbook
of Clinical Medicine

CONTENTS

ABSTRACT

Powdered infant formula is not a sterile product and it has been shown to be a common vehicle for the transmission of pathogens to a specific group of infants, particularly *Enterobacter sakazakii* and *Salmonella* which are implicated in several outbreaks causing meningitis, necrotizing enterocolitis, sepsis and salmonellosis, with a mortality rate around 20%. The International Commission on Microbiological Specifications for Food (ICMSF, 2002) classified *E. sakazakii* as "severe risk for a restricted population, representing a threat of death or chronic sequels of long duration". From the age distribution of the reported cases, it is deduced that the population at risk are the infants under one year old, especially premature and low-birth weight infants, as well as the immunocompromised and those born from HIV + mothers. The infant formula can become contaminated through the raw ingredients used in the production of the formula, through contamination of the formula after pasteurization or through contamination of the reconstituted formula used by the caregivers at the hospital or at home prior to feeding. From the powdered infant formulas, sources of contamination can accumulate in bottles and utensils used on the preparation of feeding bottles, facilitating the dissemination of the bacteria. Thus, although the occurrence of *E. sakazakii* in the powdered infant formula is low, its control depends on the application of strict hygienic conditions, especially the control of time/temperature of preparation, handling and storage of the reconstituted formula and also the continuous control of the production line since the product is not commercially sterile and is rich in nutrients which favours the multiplication of bacteria. Preventive measures have been undertaken in order to avoid the contamination of the formula and transmission of the pathogenic microorganisms to the groups at high risk. These measures start with the avoidance of the PIF itself through the promotion of exclusive breastfeeding up to the six month of age and from here up to the age of two years with the introduction of new foods to the diet with continuous breastfeeding. Because not all the infants are breastfed, measures that improve the safety of PIF and decrease the risk of infection transmission should be also implemented. These include specific guidelines for manufactory industries involved in the production of PIF and caregivers of infants, that if strictly followed help to reduce the fatality of the outcomes.

1. INTRODUCTION

The intestinal tract is a dynamic ecosystem, influenced by host, intrinsic and environmental factors (Makie et al., 1999). Variations in its microbiota keep a straight relationship with the alimentary changes observed by the infant and with the functional role of the gastrointestinal tract. Qualitative and quantitative changes in the infant may trigger bacterial diarrhoeal diseases, associated or not with the presence of enterotoxins, with consequent malabsorption of nutrients, loss of fluids, malnutrition and, in some instances, death.

In this way, infections occurring during the first year of life constitute one of the most important causes of high levels of morbidity and mortality among infants. The frequency and severity of these infections by gram negative bacteria, influenced by the immaturity of the immune system, are characteristics of this age group (Novak et al., 2001). When referring to the nutrition of such a special class of consumers, such as premature or low birth weight newborns, the attention must be intensified. Many of these babies are deprived of their natural nutrition, the colostrum and maternal milk, essential for the acquisition of immunological resistance, becoming even more susceptible to the contaminated food administered to supply these needs.

The infant formulas, based in dehydrated milk products, are the most used breast milk substitutes. Many studies have been done since the last decade, because of serious public health problems affecting premature newborns. Among the opportunistic microorganisms that have been implicated in this area, *Enterobacter sakazakii* and *Salmonella* are worth of mention for their high risk of contamination and infection.

Enterobacter sakazakii was, up to the decade of the 80s, known as yellow pigmented *Enterobacter cloacae.* It is transmitted through contaminated powdered infant formula and has been implicated in severe forms of neonatal meningitis and necrotizing enterecolitis.

Besides being the most common pathogen isolated from contaminated food in adults, *Salmonella* has also been associated with diarrhoeal disease in infants and children, and reconstituted powdered infant formula seems to be an important vehicle for transmission and infection.

Aware of the necessity to supply a safe nutrition to all the infants, the Food Agriculture Organization and World Health Organization (FAO/WHO) had convoked a meeting about the *E. sakazakii* and other microorganisms in the powdered infant formula in Geneva (Switzerland) in February 2004 (WHO, 2004). This meeting was sustained by a number of epidemiological episodes and by the microbiological questions related with the microorganisms present in the powdered infant formulas, the industrial practices involved in the production of these products, the variety of products in the global market and its preparation in care settings and in the home. According to the FAO/WHO, the *E. sakazakii* is an emergent opportunistic pathogen, and little is known about its ecology, taxonomy, virulence and other characteristics. This bacteria has been frequently isolated from the environment involved in the production of the powdered infant formulas, becoming a potential source of contamination in the post-pasteurization settings. The powdered infant formulas are not sterile and even low levels of contamination by *E. sakazakii* are considered a significant risk factor for its potential for multiplication during the preparation and time of conservation under certain conditions previous to the ingestion of the reconstituted formula (WHO, 2004).

The objective of this thesis is to summarize the current knowledge on the occurrence of *E. sakazakii* and Salmonella in the powdered infant formula, the microbiological characteristics of these etiological agents, methods of contamination, risk assessment and recommendations for safe preparation, storage and handling of powdered infant formulas.

2. Enterobacter sakazakii - Microbiology

Enterobacter sakazakii is recognized as an emergent pathogenic bacteria of alimentary origin and is not part of the normal human or animal gastrointestinal microbiota (Farber et al, 2004). As a member of the Enterobacteriacae family, E. sakazakii is described as a rod shaped, gram negative, non-spore forming, and facultative anaerobe with glucose and oxidase fermentation (Brenner et al, 2005).

Until 1980 this Enterobacteria was designated as a variant of the yellow-pigmented *Enterobacter cloacae* specie. Meanwhile, studies on DNA homology, biochemical characteristics, capacity of producing pigment (Farmer et al., 1980), incapacity of fermentation of D-sorbitol, and principally, the activity of a-glucosidase, indicated this species as unique and different from E. Cloacae. (Nazarowec-white; Farber, 1997).
It was then proposed the creation of a new species (Farmer III et al, 1980). The typical characteristics of the species are described as follows:

The natural reservoir of E. sakazakii is still unknown, but other members of the same genre are usually found in human and animal faeces, water and soils. (Sakazaki, 1974). Reports show that the microorganism can be isolated in several types of food and environment. (Iversen; Lane; Forsythe, 2004).

Generally, Enterobacter are considered opportunistic pathogens that rarely cause disease in healthy individuals. E. sakazakii, however, has been related to several sprees and sporadic cases of diseases in debilitated neonates. For this reason, this organism has been gaining much attention from the public health authorities in many countries (WHO, 2004).

Little is known about its ecology, taxonomy (WHO, 2004), and about its mechanism of virulence, but E. sakazakii seems to have a tendency for infecting the CNS causing meningitis, cysts or brain abscesses (Lai, 2001).

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2.1 Type strain

E. sakazakii is identified as the strain 29544 according to the American Type Culture Collection (ATCC). Originally it was identified as CDC 4562-70 (78-067947) (Farmer III et al., 1980).

2.2 DNA-DNA hybridization

The type strain shows 83 to 89% similarity with other strains of the same specie, but only 31 to 49% similarity with *E. cloacae strains* (Farmer III, et al., 1980).

2.3 Cell morphology

E. Sakazakii is a rod shaped bacteria with approximately 3 μm length and 1μm diameter, flagellated (Farmer III et al., 1980).

2.4 Nutritional and growth characteristics

This microorganism uses glucose or citrate as only sources of carbon and energy, not requiring vitamins, aminoacids or other organic factors of growth (Farmer III et al, 1980). It forms capsules of polysaccharide material, composed of glucuronic acid (29-32%), D-glucose (23-30%), D-galactose (19-24%), D-frutose (13-22%) and D-manose (0-8%). Being a facultative anaerobe, it may grow under an anaerobic atmosphere. All the strains develop at 25,36 and 45ºC, others at 6 and 47ºC and none at 4 or 50ºC (Farmer et al, 1980). The optimal growth temperature is at 37-43ºC, depending on the culture medium. The generation time at 37ºC varies from 14-29 min in different culture mediums and from 19-21 min in reconstituted infant formula. At 6 and 21ºC the generation time is of 13.7 and 1.7 h, respectively (in reconstituted infant formula), being able to multiply, even if slowly, under refrigeration (Iversen; Lane; Forsythe, 2004).

As a member of the Enterobacteriaceae family, *E. Sakazakii* may grow in all the selective media used for isolation and counting of Enterobacteriae, such as the MacConkey Agar, Violet Red Bile agar, Eosin-Methylin Blue agar and others (Iversen; Forsythe, 2003). All the strains multiply fast in Trypticase Soy agar (TSA) at 36ºC and form colonies of 2-3mm in diameter in 24h. At 25ºC, the colonies are generally 1-1.5mm in diameter after 24h and increase to 2-3mm, developing a shinning yellow

coloration in 48h. Among all the other species of Enterobacter, only *E. agglomerans* has similar characteristics. When immediately isolated, the strains present two or more morphological types of colonies: dry or mucoid, with imperfect borders, elastic and difficult to remove with the inoculation needle (type A) and smooth and easy to remove colonies (type B). Stock cultures made from type A colonies are easily converted to type B. Some strains also produce smooth colonies with weak pigmentation, hard to recognize as yellow (Farmer III et al, 1980).

2.5 Biochemical profile

The biochemical profile of *E. sakazakii* presents some characteristics typical of all the species belonging to the genre Enterobacter, such as positive citrate, Voges Proskauer (VP) and O-nitrophenyl-b-D-galactopyranoside (ONPG) tests, and negative H2S production and methyl red tests. As a member of the Enterobacteriaceae family it ferments glucose with production of gas at 35-37ºC, but it may not produce gas at 44,5ºC. It is strictly related to the E. cloacae, being differentiated only by its non-fermentation of sorbitol and the production of yellow pigment. However, one should have in mind that many other Enterobacteria may also form yellow-pigmented colonies, including *Escherichia hermannii*, *Escherichia vulneris*, *Enterobacter cowanii* and others.

E. Sakazakii strains test positive for α-glucosidase activity. Based on this biochemical characteristic, several culture media were created including Druggan-Forsythe-Iversen, Oh-Kang, and Leuscher-Baird-Donald-Cox agar which facilitate its identification (Drudy et al., 2006).

2.6 Thermal resistance

Nazarowec et Farber (1997) in a study about the thermal resistance of *E. sakazakii* in reconstituted dried-formula concluded that *E. sakazakii* seems to be more thermo-tolerant than many other Enterobacteriaceae in diary product. The results showed that Enterobacter spp. cultivated from infant formulas did not grow at temperatures below 5.5ºC but began to multiply at temperatures between 5.5ºC and 8ºC. Average generation times were only 5 hrs at 10ºC and only 40 min at 23 ºC. In contrast, studies by Nestlé Research Centre concluded that "*E. sakazakii* is not particularly thermo-resistant... it is well adapted to survive in dry

environments" (Breeuwer et al., 2003) and that "E. sakazakii does not survive such heat treatment" (Kandhai, 2004). However, according to IBFAN (IBFAN, 2005), "industry-sponsored research allows manufacturers to create the belief that the contamination of PIF by pathogens such as *E. sakazakii* at factory level is simply not their responsibility" and therefore results from their studies should not be over-estimated.

2.7 Detection of E. sakazakii using the BAX® system

E. sakazakii can be detected using the BAX® system. This system is an automated system for the detection of pathogenic bacteria in food, based on the DNA. It reduces the problem with contamination, since it contains in a single stable tablet all the reagents needed for the PCR, protected inside analysis tubes, besides reducing the potential for errors caused by the technician (Kushida, 2005).

It combines velocity and easiness of utilization with performance, supplying trustworthy results, in a fast and precise manner.

3. *Salmonella*

Salmonella sp is a genre belonging to the Enterobacteriaceae family. The majority of the species are motile by flagella, though some are immotile such as *S. Galinarum* and *S. Pulorum*. Most also reduce nitrates to nitrites and are tested positive for glucose fermentation, producing gas, but not for lactose and sucrose. They produce H2S, and are oxidase negative and catalase positive (ICMSF, 1998). The pH values and the optimal temperature for the multiplication of Salmonella are close to pH 7.0 and 35-75ºC, respectively.

The diseases caused by Salmonella are divided into three groups: typhoid fever (caused by *S. typhi*), enteric fever (caused by *S. paratyphi*) and the enterocolitis or salmonellosis (caused by the other Salmonellas).

The genre Salmonella is extremely heterogeneous, comprehending almost 2000 serotypes, from which some are pathogenic to the man. Being concomitantly encountered in the intestinal tract of animals, especially poultry and swine, the environmental sources of this microorganism include the water, soil, insects, kitchen and industrial surfaces, animal faeces as well as uncooked meat, poultry and seafood. Other food associated to Salmonella sp are the eggs, milk and its derivatives, sauces, salads, cake mix, desserts stuffed with cream, powdered gelatine and peanut and chocolate butter (Carvalho, 2003). In relation to milk derivatives, the contamination is almost always cause by raw or inadequately pasteurized milk. (Franco; Landgraf, 1996).

Salmonella sp is the principal agent of diseases transmitted through food. In the developed countries it occupies the first place. In the United States it corresponded to 54.5% of the outbreaks and 46.4% of the deaths by bacteria, in the period of 1993-1997 (Kushida, 2005).

The reconstituted PIF is probably a common vehicle of salmonellosis transmission in children, due to its important role in the infant diet. However, it is more probable to occur as a result of the preparation environment rather than from the production process.

The occurrence of outbreaks due to the intrinsic contamination of the PIF seems to be rare (WHO, 2004).

4. *Enterobacter sakazakii and Salmonella in Powdered Infant Formula* – Epidemiology

4.1 Populations at risk

According to FAO/WHO (2004), *E. sakazakii* has already provoked diseases in individuals of all age groups. However, the distribution of reported cases indicates infants less than one year old to be the principal group at risk. Among them, the risk of infection for E. sakazakii is high in preterm and low birth weight infants, in term infants during the first one to two months of life and in children with impaired immune defenses (Agostini et al., 2004). The stomach of the newborns, especially the premature ones, is less acidic than the stomach of the adults, which seems to be an important factor for the survival of E. sakazakii and for the development of infections.

Of particular importance for some developing countries is the amount of children born from HIV+ mothers, since these children should receive powdered infant formula and are therefore more susceptible to infection (WHO, 2004).

Infants are also the group at higher risk of Salmonella infection and invasive disease, including complications from diarrhoeal disease. (Jones TF et al., 2006).

4.2 Diseases

E. sakazakii is an occasional contaminant of PIF (Forsythe, S.J., 2005), which has been causing several outbreaks and sporadic cases of life-threatening forms of sepsis, neonatal meningitis and necrotizing enterocolitis. (Iversen; Forsythe, 2003).

The FAO/WHO expert meeting on *Enterobacter sakazakii* and other microorganisms in powdered infant formula (WHO, 2004) ended with the conclusion that intrinsic contamination of powdered infant formula with *E. sakazakii* and *Salmonella* has been considered a cause of infection and illness in infants, including severe disease which can lead to serious developmental sequelae and death (Dadhich, 2006).

The neonatal sepsis is characterized by systemic signs of infection along with positive haemoculture. However, according to the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (ACCP/SCCM), in 1992, the clinical concept of sepsis is one of a systemic response to infection, which is manifested by two or more clinical conditions: hypo or hyperthermia, tachypnea and tachycardia, alterations in the white cell count, not being necessary the presence of a positive hemoculture (Ceccon et al, 1999). The principal complication of the sepsis in the newborn is meningitis (Ceccon et al, 1999).

The signs and symptoms of meningitis caused by E. Sakazakii, such as fever and irritability (Willis; Robison, 1988), are clinically similar to the symptoms of meningitis caused by other gram negative bacteria. However, the frequency of complications and the lethality are more elevated (Lai, 2001). This similarity of symptoms may difficult the treatment.

The neonatal necrotizing enterocolitis (NEC) is characterized by intestinal necrosis and pneumatosis intestinalis (Acker et al., 2001)**.** Moreover, the triad of neonatal intestinal ischaemia, microbial colonization of the gut, and excess protein substrate in the intestinal lumen associated with oral formula feeding seems to be a prerequisite in the pathogenesis of NEC (Koshloske, 1984). According to Lucas & Cole (1990), NEC is 10 times more common in babies fed with infant formula, in comparison to those fed with breast milk, and both meningitis and NEC associated with ***E. sakazakii*** have mortality rate of 40-80% and 10-55% respectively.

Little is known about the specific virulence mechanisms of E. sakazakii, but the microorganism seems to have a predilection for the central nervous system, causing meningitis, cysts or abscesses. Developmental delay and hydrocephalus are well known consequences (WHO, 2004).

As said before, the incidence of salmonellosis is higher in infants than it is among other age groups, and infants are more likely to experience severe illness or death from salmonellosis, particularly if immunocompromised. A case-control study of the epidemiology of sporadic *Salmonella* infection in infants by Jones et al (2006), showed

that diarrhoea and fever was present in 92% of cases, with vomiting being present in 33%. Cases complicated by bacteraemia or meningitis were reported in 7%.

Many risk factors for salmonellosis in infants exist, the largest contributor being exposure to reptiles, followed closely by riding in a shopping cart next to meat or poultry, or to have consumed concentrated liquid infant formula during the 5-day exposure period (Jones et al., 2006).

Salmonellosis outbreaks have also been linked to PIFs. According to FAO/WHO (FAO/WHO, 2004), between 1985 and 2000, at least five outbreaks of salmonellosis involving approximately 150 infants were associated with PIF and involved unusual *Salmonella* serotypes. However, because in many regions of the world *Salmonella* serotyping is not routinely performed, these outbreaks due to contaminated PIFs are likely to be under-reported.

4.3. Reported cases

The two first cases of neonatal meningitis caused by *E. sakazakii* where reported by Urmenyi & Franklin in 1961 (S. Forsythe, 2005). The microorganism was at the time named yellow-pigmented *E. cloacae.* Since then, the number of neonatal infections has been increasing worldwide and, in many cases, the origin of the microorganism is still unknown.

Meanwhile, in a crescent number of reports, the powdered infant formulas have been established as a source and route of contamination and infection. Several investigations were able to demonstrate the statistical and microbiological association between the infection and the consumption of powdered infant formula, without evidence of infant-infant or environmental transmission (WHO, 2004). Following are described in chronologic order some cases related with the infection by *E. sakazakii*.

In Holland, eight cases of neonatal meningitis, two of which involving E. sakazakii, over a period of 6 years (1976 to 1982) were investigated retrospectively. Two patients had NEC and meningitis simultaneously. Besides the treatment, the mortality rate was

75%. In most of the cases, the route of transmission was through the vaginal canal (Muytjens et al, 1983).

Biering et al (1989), reported three cases of neonatal infection caused by E. sakazakii, at the Intensive Care Unit of the University Hospital of Iceland, Georgia (EUA) during 1986 to 1987. Two babies who were born normal survived the infection, although with cerebral lesions. The third baby born with Down syndrome died due to cardiac system malformations. The bacteria was isolated from the sample of powdered milk used by the hospital.

Lai (2001) reported 5 cases of nosocomial infection by E. sakazakii involving a child and four adults, occurring between January 1995 and December 1996 at the Medical Centre of Massachusetts University, USA. The cases involved hemorrhages (three cases) and respiratory infections (two cases), with three deaths, two of them proved has being caused by E. sakazakii.

Acker et al (2001) described an outbreak of NEC occurring in Brussels, Belgium, between June and July 1998, with 12 newborns affected and two deaths. *E. Sakazakii* was isolated from the stomach, blood and rectal swab of six of the twelve neonates. A revision of the procedure revealed that ten of the twelve patients were fed orally with the same brand of the powdered infant formula. *E. sakazakii* was isolated from the powdered formula (opened can and several others of the same lot) and also from the two feeding bottles prepared. Molecular typing by PCR confirmed, although only partially, the similarity between the strains present in the infant formula and those isolated from the patients. No other case was observed in the same hospital, after interrupting the use of the contaminated infant formula.

In France, four different hospitals identified cases of infection with E. sakazakii in premature babies between 25th October and 13 December 2004. Most of these babies had been fed on a powdered infant formula for lactose-intolerant babies. Two of them died from the infection and others became severely ill (IBFAN, 2005).

In Uguanda, May 2007, the Food Standards Agency had advised parents and carers not to feed the babies with a particular batch of “Baby Soya with Enkejje makes a Stout

Baby" powdered infant formula, which was shown to be contaminated with *E. sakazakii* (IBFAN, 2008).

The contamination of Bebivita starter milk I infant formula with deadly bacteria was alerted in June 2007. About 10,000-30,000 packets had been distributed to shops since March with an expiry date of 18.05.08 on each package. Three days after, Milupa announced that it had discovered life-threatening *E. sakazakii* species in Milumil I starter formula. It was also stated that if baby milks infected with *E. sakazakii* are not refrigerated hours after being prepared and before being give to the infant, there is a risk of life-threatening illness (IBFAN, 2008).

Salmonellosis outbreaks from PIF were also reported in several countries between 1985 and 2000. The serotypes of *Salmonella* implicated were: Ealing in UK, 1985 (Rowe et al., 1987); Tennessee in USA and Canada, 1993 (CDC, 1993); Virchow in Spain 1994 (Usera et al, 1996); Anatum in France 1996-7 (Threlfall et al., 1998) and London serotype in Republic of Korea, 2000 (Park et al., 2004).

In France, 104 infants developed *Salmonella agona* infections in France, between January and April 2005, with at least 38 of them being hospitalized.

However, in many regions of the world Salmonella serotyping is not routinely performed, and therefore identification of geographical outbreaks could be difficult. Also, since most cases of infant diarrhoea are caused by viral pathogens (Lieberman, 1994), stool cultures may not be collected routinely even in places with laboratory surveillance capacity. As a result, outbreaks of salmonellosis due to contaminated milk products are likely to be under-reported (FAO/WHO, 2004).

4.4. Contamination methods and sources

Intrinsic and extrinsic contamination are, according to INFOSAN (2005), the two main routes by which *E. sakazakii* can enter reconstituted infant formula. Intrinsic contamination refers to contaminated ingredients added after drying or from the processing environment after drying and before packing. Extrinsic contamination of the formula occurs during reconstitution and handling, e.g. through poorly cleaned utensils.

Thus, the sources of contaminated PIF can be said to be the powder itself, the equipment and possibly the personnel preparing the feed (Forsythe, 2005).

- **Powdered infant formula**

As mentioned previously, PIF is not a sterile product. Muytjen et al. (1983) reported the first association of E. sakazakii with contaminated PIF, by isolating the bacteria from prepared milk formula, a dish brush and a stirring spoon. Since then many other cases were reported by different authors. According to INFOSAN (2005), powdered infant formula is both the source and the vehicle of E. sakazakii induced illness in 50-80% of the cases.

A study by Muytjens et al (1998) in 141 samples of powdered infant formulas from 28 countries, showed that 52.5% of those samples were tested positive for the presence of members of the Entreobacteriaceae family. The most frequent species found where *E. agglomerans* (35 samples), *E. cloacae* (30 samples), *E. sakazakii* (20 samples) and *K. pneumoniae* (13 samples). The level of contamination for those microorganisms was less than 1UFC/100g in 78% of the samples and the highest level observed was of 92 UFC/100g for *E. cloacae* and 66UFC/100g for *E. sakazakii*.

Nazarowec-White and Farber (1997a) evaluated 120 samples of infant formulas from five different companies in Canada and detected *E. sakazakii* in 6.7% of the samples. The levels of E. sakazakii most frequently found in positive samples were 0.36UFC/100g.

According to FAO/WHO (2004), these data indicate a low level of contamination of the infant formulas by *E. sakazakii*, but still considered of high risk, because of the capacity of replication in the time interval between the preparation and the consumption of the reconstituted product. Based on the preliminary conclusions on the risk assessment analysis, the inclusion of a lethal step at the time of preparation and the reduction in the interval between the reconstitution and the consumption reduce the risk (FAO/WHO, 2004).

- **Personnel and equipment**

It is well accepted that poor hygienic practice has been a probable source of outbreaks (Forsythe, 2005).

Iversen and Forsythe (2003) also emphasised the importance of the hygiene of the equipment used for food production, such as, for example, liquefiers, spoons, etc. According to these authors, it is more probable that the presence of the microorganism in the equipment is the principal source of contamination of the feeding bottle and served portions, since the level of E. sakazakii contamination in the powdered product is low.

In fact, in the investigation of several outbreaks and sporadic cases, the microorganism was isolated from the liquifiers, flasks, dish brushes and other items used in the food production environment (Kandhai et al, 2004).

The organism is also found in the hospital environment. (Forsythe, 2005). Nazorewec-White and Farber (1999) showed that isolate obtained from one hospital over 11 years were indistinguishable. The organism has been isolated from contaminated dish brushes used for cleaning bottles (Smeets et al., 1998), doctor´s stethoscope (Farmer et al., 1980) and from nursery food preparation equipment, such as spoons and a blender (Simmons et al., 1989).

4.5 Mortality rate

According to FAO/WHO (2004), the mortality rate from E. sakazakii has been reported to be around 50% or more, but this level has been declining to less 20% in the past years. The sequelae are significant among affected children, principally those who developed meningitis or cerebritis. The infection generally responds well to the treatment with antibiotics.

5. Recommendations

5.1 Breastfeeding

The WHO/UNICEF Global Strategy for Infant and Young Child Feeding (2001) aims at improving the survival, growth and development of children during the first three years of life.

According to IBFAN, lack of breastfeeding during the first six months of life is an important risk factor for infant and childhood morbidity and mortality. It is therefore important to support breastfeeding and promote its benefits to infants and young children (WHO, 2006). The optimal infant feeding consists of exclusive breastfeeding for the first six months of life, followed by age-appropriate complementary feeding with continued breastfeeding from six to twenty-four months and beyond.

Hoosen Coovadia, a paediatrician at the University of KwaZulu-Natal and author of a recent study on formula feeding, when interviewed by the Washington post for an article about the anti-breastfeeding measures in Botswana, stated that "Everyone who has tried formula feeding . . . found that those who formula feed for the first six months really have problems …They get diarrhea. They get pneumonia. They get malnutrition. And they die." (IBFAN, 2008)

However, there are still situations where the child is unable to be breastfed. Breast milk may not be available, the mother may be unable to breastfeed, or may have made an informed decision not to do so. It is equally possible that breastfeeding is not appropriate, such as when the mother is taking medication that is contraindicated for breastfeeding or the mother is HIV-positive. Very low-birth-weight infants may not be able to be breastfed directly, and it may happen that the expressed breast milk is not available in sufficient quantities (WHO, 2006). Hospitals and social institutions are also possible contributors, representing obstacles for the initiation and continuation of

breastfeeding. (Cattaneo, 2004). In presence of any of these situations, an infant requires a suitable breast-milk substitute to support the nutritional needs, and in case a PIF is chosen, it must be produced and prepared according to applicable standards.

Despite of its important advantages, the current rates of prevalence and duration of breastfeeding are lower than what is recommended. (Cattaneo, 2005).

Many public health initiatives for the promotion of breastfeeding have been implemented which are based on the Global Strategy on Infant and Young Child Feeding. These include the International Code of Marketing of Breast-milk Substitutes, Innocenti Declaration on Protection, Promotion and Support of Breast Feeding and the WHO/UNICEF Baby Friendly Hospital Initiative (BFHI). The "French Initiative" and EURODIET project are examples of initiatives being taken in Europe.

Briefly, the International Code of Marketing of Breastmilk Substitutes aims at protecting and promoting breastfeeding by ensuring appropriate marketing and distribution of breastmilk substitutes. According to this code, there should be no advertising of breastmilk substitutes to the public or promotion of products among health care workers. This includes no free samples or gifts and no free or low-cost supplies on maternity wards and hospitals. The product information must be factual and scientific, and the informational materials must explain the benefits of breastfeeding, the health hazards associated with bottle feeding and the costs of using infant formula. Concerning the product itself, it should be of high quality (Codex Alimentarius Standards) and take account of the climatic and storage conditions of the country where they are used (IBFAN, 2005).

BFHI is a designation awarded by the WHO/UNICEF to hospitals worldwide, that promotes evidence based strategies concerning infant feeding. Its main goal is to increase the likelihood of informed decision regarding infant feeding and greater skills for mothers to initiate and sustain breastfeeding. Baby Friendly accreditation requires that maternity units implement all Ten Steps to Successful Breastfeeding (see Appendix I) and the International Code of Marketing of Breastmilk Substitutes.

5.2. Safe alternative feeding

Drudy et al (2005) stated that « *Infants should be exclusively breast-fed for the first six months of life, and those who are not should be provided with a suitable breast-milk substitute. Powdered Infant Formula is not a sterile product.* »

Infants who are not breastfed require a suitable breast-milk substitute. Because at present, no available technology is able to produce sterile PIF, the recommendations are being directed to the food industry to improve its safety. Recommendations and guidelines have been implemented in this sector, and it is estimated that if these measures are followed, the frequency of contamination and risk of infection will be significantly reduced (WHO, 2001).

Bacterial growth in reconstituted PIF is favourable when given adequate conditions of water availability, time and temperature. Once PIF is rehydrated, the only remaining barriers to bacterial growth and risk of infection are time and temperature. In regard to temperature, the minimum, optimum and maximum temperature for E. sakazakii are 6, 39 and 46 ºC respectively. Also important is the temperature range over which E. sakazakii can multiply. At 10ºC, which is slightly above refrigeration, it takes 14 h for E. sakazakii to double, while at room temperature this takes only 45 min (Forsythe, 2005).

Iversen; Forsythe (2003) linked five relevant measures to the control E. sakazakii in the dehydrated infant formulas. Of those five measures, four may be applied by the processing industries. These are:

1. Control of the initial population of E. sakazakii in the prime material.
2. Reduction in the contamination by the thermal treatment of the milk and the ingredients added to the formulas.
3. Prevention of contamination post-processing.
4. Application of microbiological criteria.
5. Supply of appropriate information and instructions for the preparation.

Farber (2004) drafted strategies aimed at reducing the occurrence of E. sakazakii in the food industry, being obligatory the monitoring of the factory environment, better hygienic practices and tests on the final product.

It is important to emphasize once again that the powdered infant formula, according to the actual patterns is not a sterile product and, occasionally, pathogens may be found. Even low levels of contamination with E. sakazakii in the PIF are considered significant risk factors, due to its potential of multiplication during the preparation and during the time between preparation and consumption.

According to WHO (2004) the infant food industry should be encouraged to:

a) Develop a greater variety of alternative formulas, commercially sterile, to the high risk groups.
b) Reduce the concentration and prevalence of E. sakazakii, both in the factory environment and in the PIF. In this regard, the industry should take into account the implementation of an effective programme of environmental monitoring and evaluate the presence of Enterobacteriaceae instead of coliforms, as indicator of the hygienic control of its products.

5.3. Information for caregivers

Physicians and other caregivers to infants must advocate breastfeeding as the preferred means of feeding infants (Drudy et al., 2005). In such cases where PIF is recommended as a breast milk substitute, caregivers, especially to those infants at high risk, should be alerted to the fact that PIF is not a sterile product (WHO, 2001). Many consumers are not aware that PIF may be contaminated with pathogens, which, even at extremely low levels may cause serious illnesses.

Prevention should start with information about and training in the safe preparation of PIF according to the present available guidelines. (WHO, 2006). Educational programmes targeted to caregivers of infants at home, day care and healthcare facilities, as well as labelling procedures should be available (see Appendix II) (INFOSAN).

If the only available option to serve the nutritional needs of an infant in particular is then the PIF, the FDA suggests that the following measures are taken so that the risks of infection are reduced (United States, 2002):

a) Reconstitution of the PIF with boiling water and subsequently refrigeration before use. It is important to recognize that there will be probably the loss of certain vitamins, such as thiamine or ascorbic acid.

b) Preparation of each meal of only a small volume of the reconstituted formula, in order to reduce the amount of time the formula remains at ambient temperature.

c) Decrease the waiting time between preparation and consumption of the PIF being it at ambient temperature or under refrigeration.

d) Reduction on the amount of time the prepared formula remains at ambient temperature, being it in the feeding bottle, glasses or pack for the nasogastric administration. This time should never exceed the four hours.

Particular guidelines for home and institutional settings are described as follows (Agostini et al, 2004):

Home settings

- PIF should be prepared fresh for each meal.
- Remnants should be discharged and not used on the following feed.
- PIF should never be kept warm in bottle heaters or thermoses. Better is to keep only warm water in the thermos and mix it with PIF at the time of feeding.

Institutional settings

- Written guidelines for preparation and handling of infant formula should be established for hospitals and other institutions and their implementation monitored.
- Sterile liquid formulas usage should be encouraged for healthy newborn infants in maternity units.
- If formula needs to be prepared in advanced, it should be kept at or below 4ºC and for no longer than thirty hours. Storage should be monitored. Re-warming of the formula should only be done immediately before feeding.
- Formula can only be kept at room temperature for a maximum of four hours.

With regard to the particular needs of developing countries, FAO/WHO should address the need to establish appropriate measures to minimize risk in the use of breast-milk substitutes for children at higher risk, such as infants of HIV-positive mothers, and low-birth weight infants. One should also have in mind that certain situations may difficult

the achievement of some of the current recommendations for the preparation and use of PIF, such as unavailability of refrigeration, cost of the fuel and others (INFOSAN; WHO, 2006).

Also important is to encourage all countries to investigate and report sources and vehicles of infection by E. sakazakii and Salmonella, including PIF. A possible approach could be the establishment of an international-based network. Research could also contribute for a better understanding of the ecology, taxonomy, virulence and other characteristics of E. sakazakii and in the future ways to reduce its levels in reconstituted PIF may be possible (INFOSAN). The use of internationally validated detection and molecular typing methods for *E. sakazakii* and other relevant microorganims (WHO, 2006) is of great value and should not be under-estimated.

5.4. Risk reduction strategies for salmonella

Preventive measures to prevent the contamination of dehydrated dairy products with Salmonella are not a recent topic. Guidelines have been published by the International Dairy Federation (IDF) and several other organizations.

According to FAO/WHO (FAO/WHO, 2004), current industry control measures are based on four principles: "(1) avoiding the entry of *Salmonella* into processing facilities and in particular in the zones from drying to filling, considered as high hygiene areas, (2) avoiding the multiplication of *Salmonella* in case of entry, (3) the hygienic design of high hygiene zones and the equipment located in such zones, and (4) the use of dry-mixed ingredients which are free of *Salmonella"*.
Of note is that the control measures are not specific for PIF but can be also applicable for other types of dried dairy products, milk powders, dry-mix ingredients or soya-based products, where *Salmonella* is considered the most significant hazard (WHO/FAO, 2004).

Preventing the entry of *Salmonella* into the high hygiene areas can be achieved through the practise of good hygiene practices, such as isolating the high hygiene areas from the rest of the factory either by walls or well-controlled access points for both personnel and for goods, supported by appropriate procedures to minimize the pathogen entry such as

clothing changes and even control of utilities by filtration of external air and creation of overpressure.

In case of entry, *Salmonella* multiplication can be avoided through the reduction or elimination of water in high hygiene areas, which would otherwise lead to an increase of *Salmonella* in the processing environment and thus an increase in the risk of recontamination of the product.

Also important is the design of the processing areas, such as walls, ceilings, floors and equipment, which should be design in such a way to maintain a high standard of cleanliness and to prevent or otherwise rapidly eradicate the bacteria in the premises (FAO/WHO, 2004).

The effectiveness of the preventive measures can be verified through testing programmes targeted against *Salmonella*, as outlined by ICMSF (ICMSF, 2002) and complemented by programmes targeting Enterobacteriaceae, which are also a commonly found in the processing environments. These programmes, if followed correctly will detect *Salmonella* at the earliest possible stage in the high hygiene areas and thus immediate eradication of the pathogen. It is then true that *Salmonella* may be virtually absent in processing environments, the risk of recontamination being extremely low (FAO/WHO, 2004).

Concerning the preparation and use of PIF by consumers, the meeting report about E. sakazakii and Salmonella contamination in powdered infant formula stated that "those scenarios which decreased the risk of E. sakazakii infection were also likely to decrease the risk of salmonellosis". As E. sakazakii, Salmonella may be also introduced in PIF during preparation and handling in hospital and home setting, which emphasizes once again the importance of hand-washing with soap and the careful cleaning of surfaces and equipment during preparation and handling of PIF.

Storage of reconstituted PIF should be at temperatures of less than 5ºC to prevent the growth of Salmonella during refrigeration. This could be a problem for many refrigerators at home which usually refrigerate at temperatures above the 10ºC and

therefore may be a hazard for growth of Salmonella in PIF being stored in those refrigerators.

6. Conclusion

Powdered infant formula is not sterile and conscience regarding the possibility of contamination should be in the mind of all caregivers, especially when concerning the nutrition of infants of the high risk group, i.e. preterm and low-birth weigh infants, the immunocompromised and those of HIV + mothers. The newborn infant is so susceptible to infections that PIF requires a high level of microbiological quality control during production, handling and storage (Forsythe, 2005). Thus, for the situations where breastfeeding is not a first choice, more feasible options are recommended such as the use of a commercially sterile liquid formula or inclusion of control measures for PIF that would minimize the risk of contamination. These control measures include the preparation of PIF using good hygienic procedures, minimization of the time between preparation and consumption and if stored, refrigeration at a temperature below 4ºC for no longer than thirty hours. These and other measures, including also guidelines for the manufacturing industries, were published by WHO after the WHO/FAO expert meeting on E. sakazakii and other microorganisms in PIF in Geneva in 2004, and if strictly followed should lead to a decrease in the risk of contamination and infection. Also important is continuing research on this problem and a better understanding of the contamination methods and pathogenesis of the bacteria, which could bring some light to the development of technologies able to produce a sterile formula.

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APPENDIX I

TEN STEPS TO SUCCESSFUL BREASTFEEDING

(Taken from the WHO/UNICEF Baby-Friendly Hospital Initiative (BFHI))

1. Have a written breastfeeding policy that is routinely communicated to all health care staff.
2. Train all health care staff in skills necessary to implement this policy.
3. Inform all pregnant women about the benefits and management of breastfeeding.
4. Help mothers initiate breastfeeding within one half-hour of birth.
5. Show mothers how to breastfeed and maintain lactation, even if they should be separated from their infants.
6. Give newborn infants no food or drink other than breast milk, unless medically indicated.
7. Practice rooming in - that is, allow mothers and infants to remain together 24 hours a day.
8. Encourage breastfeeding on demand.
9. Give no artificial teats or pacifiers (also called dummies or soothers) to breastfeeding infants.
10. Foster the establishment of breastfeeding support groups and refer mothers to them on discharge from the hospital or clinic.

Appendix II

Ten reasons for informative labelling of powdered infant formulas

(Taken from IBFAN www.ibfan.org, January 2005)

1. *E sakazakii* has been isolated from as many as 14% of samples tested for Enterobacteriaceae. Yet all were in compliance with Codex Alimentarius criteria for bacterial counts.
2. A number of outbreaks of neonatal *E. sakazakii* infections have traced the causative organism back to unopened containers of powdered formula.
3. *E. sakazakii* infections are associated with serious, life threatening conditions, mainly meningitis, necrotizing enterocolitis and bacteremia. Although all infants fed powdered formulas are at risk for *E. sakazakii* infections, neonates, low-birth weight, premature and immunocompromised infants are at even greater risk. Currently the extent of illness and death associated with *E sakazakii* infections in most parts of the world is unknown.
4. Neonatal infections caused by *E sakazakii* have a reported mortality rate ranging from 20% to 50%. The organism is increasingly more resistant to antibiotic treatment.
5. *E. sakazakii* is a highly heat-tolerant organism. Powdered infant formulas cannot be sterilized, as this would compromise the nutritional adequacy of the product. Thus heat treatment of the product during manufacturing is limited.
6. Infant formula is designed to be the sole source of food for infants for the first six months of life. The number of 500g or 450g tins required to exclusively formula feed infants for this duration would be 40 or 45 respectively. Up to 14% of these tins selected from supermarket and pharmacy shelves may be contaminated.
7. To reduce the risk of infection for formula fed infants when the product is reconstituted, stringent recommendations for preparation, handling and storage are essential. Factors that make adherence to such recommendations difficult and perhaps unrealistic include: normal infant feeding behaviours such as frequent feeding and night-time feeding, high rates of poverty and low levels of literacy as well as limited access to refrigeration and fuel.
8. In countries where refrigeration is not available and accessible for the majority of parents and caregivers, the risk of infection increases one thousand fold.
9. The majority of parents and caregivers share the misconception that powdered infant formulas are sterile and safe to use. Parents have the right to full information about infant feeding products, including the risks associated with the use of infant formula products. Such information must also be available on the labels of powdered infant formulas and must be clear, visible and understandable.
10. The mandate and responsibility of the World Health Assembly is to uphold the principle of the highest attainable standard of health as a fundamental human right. Hence the role of WHA is to show leadership and guidance to Codex and Member States. WHA must therefore take specific action to address the health consequences of contaminated formulas and recommend the safeguards that Member States have to put in place immediately to reduce the disease and mortality risks associated with this urgent public health concern.