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Contamination of powdered infant formula by *Enterobacter sakazakii* and other pathogens

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CONTENTS

Summmary

Contamination of powdered infant formula by *Enterobacter sakazakii* and other pathogens will be considered in the following text with a description of the bacteria and their route of contamination as well as their pathological sequels together with preventive measures. *E.sakazakii* is a gram negative rod within the family Enterobacteriaceae. *E. sakazakii* in powdered infant formula has been implicated in outbreaks with sepsis, and infections of the central nervous system causing meningitis, cysts, brain abscess and necrotizing enterocolitis. Mortality has been reported to be as high as 50% but has decreased to less than 20% in the recent years. For those who survive subsequent developmental delay and hydrocephalus is a well recognized sequels. *Enterobacter* species are biochemically similar to *Klebsiella,* but unlike *Klebsiella,* it has has been found to be more resistant to osmotic and dry stress. Although exact virulence mechanisms are unknown, it is known that a small percentage of *E.sakazakii* cells can survive for extended periods in dehydrated powdered infant formula. While the organism has been detected in different types of food, only powdered infant formula has been linked to outbreaks of diseases. Disease caused by *E. sakazakii* in infants has been associated with the consumption of commercially prepared non-sterile infant formula, and contamination has been linked back to either the infant formula itself or formula preparation equipment like the blenders. The organism has not been found in drinking water sources used to prepare the formula. There is no evidence for person-person or more general environmentally transmissions. Development and distribution of educational documents related to powdered infant formula to caregivers of infants in the home, day care and health-care facilities and health-care professionals for infants should be encouraged.

1.0 INTRODUCTION

Contamination of powdered infant formulas by *Enterobacter sakazakii* and other known pathogens, including *Salmonella,* are an increasingly recognized entity with well documented morbidity and mortality. This problem is especially recognized as an nosocomial infection, primarily affecting the premature immunocompromized low birth-weight infant at the intensive care unit. To provide preventive measures, it is of a great importance to be aware of the risk of contamination from the factory production, through the reconstitution, storing and finally the feeding of the infant.

2.0 *ENTEROBACTER SAKAZAKII* AND OTHER PATHOGENS

The newborn infant has a sterile gastrointestinal tract that is quickly colonized through oral ingestion. Where appropriate, powdered infant formula milk (PIF) is offered in place of breast milk and it will influences the development of the gut flora. [10] Specific microbes commonly tested for in PIF are *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, Enterobacteriaceae and *Salmonella*. [11] Salmonella has been a cause of infection and illness in infants, including severe disease which can lead to serious developmental sequels and death. [26] *Enterobacter sakazakii* is a pathogen of increasing medical concern, due to it being implicated in cases of meningitis, sepsis, and necrotizing enterocolitis associated with the consumption of contaminated powdered infant milk formula. [24] *E. sakazakii* is isolated from plant food and food ingredients like cereal, fruit and vegetables, legume products, herbs and spices as well as from animal food sources like milk, meat and fish and products made from these foods. The spectrum of *E. sakazakii*-contaminated food covers both raw and processed food. The kind of processing of *E. sakazakii*-contaminated food is not restricted to dry products. Fresh, frozen, ready-to-eat, fermented and cooked food products as well as beverages and water suitable for the preparation of food, can be found to be contaminated by *E. sakazakii.* [9] Historically, outbreaks associated with Salmonella-contaminated milk products were recognized as early as the 1950's in the United Kingdom and Bulgaria. In the 1960's and 1970's there were also a number of outbreaks related to Salmonella in various powdered milk products. Between 1985 and 2005 at least 6 outbreaks of salmonellosis, involving as many as 250 infants, have been associated with powdered infant formula (PIF). In 2005, in France, an outbreak affecting more than 100 infants was associated with PIF contaminated with *Salmonella Agona.* [26] The first two known cases of meningitis caused by

E. sakazakii occured in 1961. Subsequently, cases of meningitis, septicemia, and necrotizing enterocolitis due to *E. sakazakii* have been reported worldwide. Although most documented cases involve infants, reports describe infections in adults as well. [27] In 2004, PIF was microbiologically linked to two *E. sakazakii* outbreaks, in New Zealand and in France. The French outbreak involved nine cases, and resulted in the death of two infants. While eight of the cases were in premature infants of low birth weight one case was in an infant born at 37 weeks and weighing 3.25 kg. The outbreak involved five hospitals, and a review of practices in the hospitals revealed that one hospital was not following recommended procedures for the preparation, handling and storage of feeding bottles, and four were storing reconstituted formula for more than 24 hours in domestic-type refrigerators, with no temperature control or traceability. Limited information was available on the numbers of *E. sakazakii* organisms that ill patients were exposed to in any of the various outbreaks. It is therefore not possible to develop a dose-response curve for *E. Sakazakii*. However, it is possible that a small number of cells present in PIF could cause illness. This risk increases rapidly when bacteria in the reconstituted formula are allowed to multiply, such as by holding at inappropriate temperatures for an extended period. In the United States of America, an incidence rate of 1 per 100 000 infants for *E. sakazakii* infection has been reported. This incidence rate increases to 9.4 per 100 000 in infants of very low birth weight, defined as less than 1.5 kg. [8]

2.1 Categorisation of the bacteriae

Category "A" Organisms – Clear Evidence Of Causality

Enterobacter sakazakii and *Salmonella enterica* are in category "A" because both are well established causes of illness in infants (e.g. systemic infection, necrotizing enterocolitis [NEC] and severe diarrhoea), and they have been found in powdered infant formula. Contaminated powdered infant formula has been convincingly shown, both epidemiologically and microbiologically, to be the vehicle and source of infection in infants. The presence of *E. sakazakii* in powdered infant formula, and its association with illness in infants, is more likely than other Enterobacteriaceae or other *Enterobacter* species to be detected, because of the paucity of other vehicles or modes of transmission for *E. sakazakii* in this age group, and because it is facilitated by the use of molecular fingerprinting detection techniques. In other words, there may in fact be more instances of powdered infant formula-borne infection with Enterobacteriaceae than with *E. sakazakii*, but the former elude detection. Although there are

clearly some differences in the microbial ecology of *S. enterica* and *E. sakazakii*, many of the risk-reduction strategies aimed at controlling *E. sakazakii* are also likely to control other *Enterobateriaceae*, especially other *Enterobacter* species. [8]

Category "B" Organisms –Causality Plausible, But Not Yet Demonstrated

Other Enterobacteriaceae are in category "B" because they are well-established causes of illness in infants (e.g. systemic infection, NEC and severe diarrhoea) and have been found in powdered infant formula, but contaminated powdered infant formula has not been convincingly shown, either epidemiologically or microbiological, to be the vehicle and source of infection in infants. These organisms include, for example: *Pantoea agglomerans* and *Escherichia vulneris* (both formally known as *Enterobacter agglomerans*), *Hafnia alvei*, *Klebsiella pneumoniae, Citrobacter koseri, C. freundii, Klebsiella oxytoca* and *Enterobacter cloacae*. These organisms are increasing in importance as neonatal pathogens and, being Enterobacteriaceae, known to be present in low levels in powdered infant formula, are potential candidates as powdered infant formula-borne pathogens. For example, infant formula has been implicated as the vehicle of infection in an outbreak of *C. freundii* infection. In this event, however, it was not shown how the feed became contaminated. [8]

Category "C" Organisms – Causality Less Plausible Or Not Yet Demonstrated

Other microorganisms are in category "C", either because, despite causing illness in infants (e.g. systemic infection, NEC and severe diarrhoea), they have not been identified in powdered infant formula, or, although having been identified in powdered infant formula, they have not been implicated as causing such illness in infants. These organisms include *Bacillus cereus, Clostridium difficile, C. perfringens, C. botulinum, Staphylococcus aureus* and *Listeria monocytogenes. Bacillus cereus*, a spore-forming gram-positive rod commonly found in the environment, is an acknowledged enteropathogen. Enterotoxigenic *B. cereus* has been isolated from reconstituted milk-based. Although one confirmed common source outbreak associated with infant formula has been reported in Chile, no evidence of intrinsic contamination of the infant formula with *B. cereus* was provided. Thus, a causal association between powdered infant formula and *B. cereus* infection was not demonstrated. *Clostridium difficile* is a frequent colonizer of newborns, usually without clinical manifestations. One

study, sparked by the finding of stools positive for *C. difficile* in two infants dying of sudden infant death syndrome (SIDS), showed significantly greater colonization of newborns fed on formula than breast fed infants. However, no direct link with powdered infant formula was established. [8]

2.2 Morbidity and Mortality

Mortality has been reported to be as high as 50% but has decreased to less than 20% in recent years. Reported case-mortality meningitis rates vary from 40 to 80% among infected infants, with the majority of those who survive *Enterobacter*-associated meningitis (94%) developing an irreversible neurological sequel. [19] Survival of Enterobacter sakazakii in powdered infant formula as affected by composition, water activity, and temperature and an increase in the parameters cause an increase in the rate of death. [11]

2.3. Detection

Enterobacteriaceae direct count test determines whether heat-treatment has been adequate, or if post-treatment contamination has occurred. However, this group of microbes has been hard to specifically define over the years, and the test does not allow for the recovery of bacterial cells which may be injured during processing (i.e. dehydration) and need resuscitation before they can grow on selective agars. The current 'Enterobacteriaceae' direct count test therefore does not guarantee the absence of enteric pathogens. Although *Enterobacter sakazakii* is a member of the Enterobacteriaceae, the 'Enterobacteriacae' and 'coliform' direct count test does not always detect the organism in PIF samples. [10] RAPD may be applied as a useful and reliable tool for direct comparison *of E. sakazakii* isolates providing traceability through the infant formula food chain. [6] Current media designed for the isolation and presumptive identification of *E. sakazakii* do not support the growth of all currently known *E. sakazakii* phenotypes; therefore, improvements in the proposed methods are desirable. [13] Quick and sensitive methods to detect low-level contamination sporadically present in IMF preparations would positively contribute towards risk reduction across the infant formula food chain. [19] The high contamination level *of E. sakazakii* suggests the need for monitoring hygienic

conditions in the manufacturing plant and to assess the prevalence of *E. sakazakii* in powdered infant milk formulas. [24]

3.0 RISK FACTORS FOR CONTAMINATION OF POWDERED INFANT FORMULA

Risk for contamination and subsequent infection depends on manufacturing and the number of bacteria present in the product, preparing the product, and handling after preparation. A small number of clinical outbreaks have been epidemiologically linked to IMF contaminated post-pasteurization during manufacture and/or mishandled when reconstituted. [6] *E. sakazakii* and several other Enterobacteriaceae are opportunistic pathogens and improper preparation and conservation of these products could result in a health risk for infants. [7] The underlying patient characteristics (e.g. immunosuppression, prematurity, or low birth weight) plays an important role. Because powdered formula is not sterile and can provide a good medium for growth, prolonged periods of storage or administration at room temperature might amplify the amount of bacteria already present. [12] So far, other food than PIF is not known to cause E. sakazakii-infections. The scarce information about the ecology of *E. sakazakii* and the uncertainty concerning the source of infection in children and adults warrant a summary of the current knowledge about the presence of this opportunistic microorganism in food other than infant formula.[9] Experimental studies suggest that *E. sakazakii* contamination of PIF is at low levels.

Basically there are three routes by which *Enterobacter sakazakii* can enter infant formula:

a) through the raw material used for producing the formula;

b) through contamination of the formula or other dry ingredients after pasteurization; and

c) through contamination of the formula as it is being reconstituted by the caregiver just prior to feeding. It is important to point out that not only PIF, but other food sources, have been described as the potential source of *E. sakazakii* infections in infants. However, these food sources have been neither epidemiologically nor microbiologically confirmed as the source of infection.

3.1 Manufacturing related risk factors

Environmental monitoring of Enterobacteriaceae provides baseline levels and therefore allows the tracking of changes over time. Lipopolysaccharide (LPS) is a heat stable endotoxin that persists during the processing of powdered infant formula milk (PIFM). Upon ingestion it may increase the permeability of the neonatal intestinal epithelium and consequently bacterial translocation from the gut. It is plausible that the risk of neonatal bacteraemia and endotoxemia, especially in neonates with immature innate immune systems, may be raised due to ingestion of PIFM with high endotoxin levels. [25] The likelihood of the presence and the level of microorganisms in the finished product will depend on their level in the processing environment. Enterobacteriaceae, including *E. sakazakii*, are part of the normal flora in any type of processing environment, including food factories and homes. In the case of processing environments, experience and data have shown that it is indeed possible to drastically reduce the levels present in high hygiene areas. On the other hand, it is not currently possible to completely eliminate this group of microorganisms from the environment. Thus, they are consistently present at low levels in processing environments and may sporadically gain access into the processing line and product.

3.2 Hospital and institutional related risk factors

Moist areas and liquid environments must be regularly checked for pathogenic microorganisms. Instead of using heated water to sterilize infant formula, dry air sterilization should be used. [3] Survival of *Enterobacter sakazakii* in powdered infant formula is affected by composition, water activity, and temperature. [11] Enterobacter sakazakii has been reported to form biofilms, but environmental conditions affecting attachment to biofilm formation on abiotic surfaces have not been described. [16] Nutrient availability plays a major role in processes leading to biofilm formation on the surfaces of these inert materials. These observations emphasize the importance of temperature control in reconstituted infant formula preparation and storage areas in preventing attachment and biofilm formation by *E. sakazakii*. [16] Intrinsic microbiological contamination of powdered milk formula can be a possible contributive factor in the development of NEC, a condition encountered almost exclusively in formula-fed premature infants.

3.3 Home related risk factors

Reconstituting, storing and feeding dried infant formula newborns and infants can be contaminated while being fed. [3] Contamination has been linked back to either the infant formula itself or formula preparation equipment (e.g. blenders). Many other outbreaks have occurred without identified hygienic lapses during formula preparation. The organism has not been found in drinking water sources used to prepare the formula. There is no evidence for person-person or more general environmental transmission.

3.4 Commercially related risk factors

The literature is filled with an ever increasing number of breast milk studies, unfortunately many of which are funded by the formula milk industry. In order to increase the market share, the formula milk industry looks for new ingredients to add to its milk formula. In doing so, they may create physiological imbalances: either toxicity due to excess or lack due to deficiency of essential ingredients. Formula-fed infants are at risk for neonatal hypocalcemic tetany. This is due to the high phosphate load in formula and lower calcium retention in newborns. New types of formulas which contain 45% palmolein can lead to decreased absorption of calcium. In general, the breast-fed infant is at little risk of either a deficiency or an excess of trace elements. Formula fed infants are at a higher risk for ingestion of lead, aluminum and other heavy metals and this may affect the growing brain adversely.There is high level of manganese in soya formula, its absorption and retention are high during infancy. Great advances in knowledge about mineral interactions and bio availability have occurred in the last decade. It is now recognized that the ability of breast milk substitutes to provide adequate levels of nutrients cannot be predicted from their compositional analysis alone and that growth by itself is not a sufficiently sensitive indicator of all possible adverse outcomes due to deficiency or excess. The mineral content of Na, K, Ca, Mg, C1 and P was determined in different batches of 5 preterm formulae.Chromium is well documented as an essential element for humans. Trivalent chromium, the main chemical form found in foods, is essential for maintaining normal glucose metabolism. [21] Linolenic acid has been added very recently which babies can only partially convert to DHA (docosahexaenoic acid), an essential omega-3 fatty acid, necessary for brain development. Recently it has been shown that commercial infant formulae containing long chain polyunsaturated fatty acids do not raise the plasma level of long chain polyunsaturated fatty acids to desired levels. [18] Taurine, essential for myelination of the central nervous system was absent from formulae till 1984. Even when

essential amino acids are added to formulae milk, some amino acid levels were low in plasma, whereas some amino acids were found to be high in the plasma of the infant.Nucleotides are present in human milk in large quantities and five nucleotides have recently been added to formula milk. Many studies suggest enchanced immunity with nucleotides in breast milk. However certain ingredients in formula may modify the action and absorption of nucleotides. Large amount of nucleotides can damage the neonatal kidney.

3.5 Population at risk

Although *E. sakazakii* has caused illness in all age groups, certain groups are likely to experience higher disease rates. Rate of invasive *E. sakazakii* infection to be 1 per 100 000 infants whereas the rate among low-birt hweight neonates was 8.7 per 100 000. Similarly, the annual incidence of invasive *E. sakazakii* infection was estimated at 9.4 per 100 000 infants of very low birth weight.The incidence worldwide of illness is not known for older pediatric or adult populations. Children more than 12 months and adults are assumed to be at lower risk than infants for invasive infection with *E. Sakazakii*. Premature infants tend to develop infection at a later median age –35 days of life – than infants who develop meningitis. Bacteraemia cases have occurred as late as age 10 months in an immunosuppressed infant, and 8 months in a previously healthy infant. Epidemiological and microbiological aspects of *Enterobacter sakazakii* meningitis during the first few weeks of life and may be more likely to develop meningitis rather than isolated bacteraemia if exposed to *E. sakazakii* during this time. Consequently, although there appear to be two distinct infant risk groups – namely, premature infants developing isolated bacteraemia after 1 month of age and term infants developing *E. sakazakii* has caused invasive infection in all age groups infants appear to be the group at particular risk, with neonates and infants under 2 months at greatest risk. [14]

4.0 PREVENTIVE MEASURES

Powered infant formula is not a sterile product, even if it has been manufactured to meet current hygienic standards. This means that it may occasionally contain pathogens that can cause serious illness. *E. sakazakii* and *Salmonella enterica* are the pathogens of most concern

in PIF. Severe illness and sometimes death in infants has been attributed to PIF that has been contaminated with *E. sakazakii* or *Salmonella*, at either the manufacturing or preparation stage. Because the manufacturing of commercially sterile PIF is not feasible using current processing technology, there is a potential risk of infection to infants through consumption of PIF. This risk is increased when prepared, handled or stored incorrectly. During the preparation of PIF, inappropriate handling practices can exacerbate the problem.

In 2005, the World Health Assembly (WHA) requested the WHO to develop such guidelines in order to minimize the risk to infants. These present guidelines are considered to be a generic document that will provide guidance and support for countries and governments. When adapted at the country level, conditions (i.e. climatic and socioeconomic differences, etc.) within the country should be reflected. Individual countries should outline minimum training requirements for parents, caregivers, and staff in hospitals and day-care centres. [8]

4.1 Manufacturing, control of production

It is recommended to minimize entry of the microorganisms and avoid their multiplication, such as the exclusion of water from the processing environment to the extent possible and feasible. The most effective means of achieving the latter is considered to be the implementation of systematic dry-cleaning. Support research that allows further evaluation of the effectiveness of Enterobacteriaceae as an indicator organism pointing to conditions in the manufacturing environment or final product that have increased potential for harbouring *E. sakazakii* or *Salmonella*. [14]Current prevention methods appear to be insufficient to ensure that PIF are free of E. sakazakii. Application of specific bacteriophages may provide a means for efficient prevention of *E. sakazakii* infection through reconstituted infant formula. [17] It can be reasonably anticipated that a reduction in the levels of the Enterobacteriaceae in the environment will correspondingly lead to lower levels of Enterobacteriaceae (including *E. sakazakii*) in the finished product. Tracking the levels of the Enterobacteriaceae in the plant environment is a useful means verifying effectiveness of the hygienic procedures applied and also allows undertaking corrective actions in a timely manner. Reduction of these microorganisms in the production environment is achieved through the combination of two measures minimizing their entry into high-hygiene zones; and preventing proliferation of those that are already present.

4.2 Preventive measures at hospitals and nursing homes

General requirements for feeding include that each institution should establish guidelines for the preparation and handling of powdered infant formula and the implementation of the guidelines should be monitored. Personnel should be trained according to the guidelines and there should be traceability of the PIF prepared in care settings there should be a clean dedicated area for preparation and storage of PIF. The use of sterilized liquid milk formula in neonatal care could prevent problems with intrinsic and extrinsic contamination of powdered milk formula.

4.3 Procedures at home

PIF is not a sterile product and can pose a risk to infants particularly if it is prepared and handled inappropriately. Correct preparation and handling reduces the risk of illness. Where available, commercially sterile ready-to-feed liquid infant formula should be used for infants at greatest risk. Reconstituted PIF provides an ideal environment for the growth of harmful bacteria. Even if present in powdered formula at very low levels, inappropriate preparation and handling of feeds provides ideal conditions for the growth of harmful bacteria, which greatly increases the risk of infection. However, the risk can be reduced if feeds are prepared and handled correctly. The recommendations below outline the best practice for the safe preparation, storage and handling of PIF in the home in order to reduce the risk of infection with *E. sakazakii*. These recommendations are also appropriate for reducing the risk of infection with *Salmonella*. It is recommended health care professionals ensure that parents and caregivers are instructed in the safe preparation, storage and handling of PIF.

4.4 Guidelines for the best practice

4.4.1 Cleaning and sterilizing feeding and preparation equipment

Outbreaks of *E. sakazakii* infection have been attributed to equipment used for preparing feeds [11]. *E. sakazakii* is widespread in the environment and has been shown to attach and grow (form 'biofilms') on surfaces commonly used in infant feeding equipment, such as latex,

silicon and stainless steel. It is therefore important that all infant feeding and preparation equipment (e.g. feeding cups, bottles, rings and teats) has been thoroughly cleaned and sterilized before use, since the formation of biofilms on such equipment may result in reservoirs of infection that can continually contaminate feeds. [13]

1. Hands should always be washed thoroughly with soap and water before cleaning and sterilizing feeding and preparation equipment.

2. Cleaning: wash feeding and preparation equipment (e.g. cups, bottles, teats and spoons) thoroughly in hot soapy water. Where feeding bottles are used, clean bottle and teat brushes should be used to scrub inside and outside of bottles and teats to ensure that all remaining feed is removed.

3. After washing the feeding and preparation equipment, rinse thoroughly in safe water.

4. Sterilizing: if using a commercial home sterilizer (e.g. electric or microwave steam sterilizer, or chemical sterilizer), follow manufacturer's instructions. Feeding and preparation equipment can also be sterilized by boiling: a. fill a large pan with water and completely submerge all washed feeding and preparation equipment, ensuring there are no trapped air bubbles; b. cover the pan with a lid and bring to a rolling boil, making sure the pan does not boil dry; and c. keep the pan covered until the feeding and preparation equipment is needed.

5. Hands should be washed thoroughly with soap and water before removing feeding and preparation equipment from a sterilizer or pan. The use of sterilized kitchen tongs for handling sterilized feeding and preparation equipment is recommended.

6. To prevent recontamination, it is best to remove feeding and preparation equipment just before it is to be used. If equipment is removed from the sterilizer and not used immediately, it should be covered and stored in a clean place. Feeding bottles can be fully assembled to prevent the inside of the sterilized bottle and the inside and outside of the teat from becoming contaminated. [28]

4.4.2 **Preparing a feed using powdered infant formula**

It is best to make PIF fresh for each feed and to consume immediately, as reconstituted PIF provides ideal conditions for the growth of harmful bacteria. The steps below outline the

safest way to prepare individual feeds of PIF in bottles or in feeding cups for immediate consumption:

1. Clean and disinfect a surface on the bottle or feeding cup and then wash the hands with soap and water, and dry using a clean cloth or a single-use napkin.

2. Boil a sufficient volume of safe water. If using an automatic kettle, wait until the kettle switches off; otherwise make sure that the water comes to a rolling boil. Note: bottled water is not sterile and must be boiled before use. Microwaves should never be used in the preparation of PIF as uneven heating may result in 'hot spots' that can scald the infant's mouth.

3. Taking care to avoid scalds, pour the appropriate amount of boiled water that has been allowed to cool to no less than 70 °C, into a cleaned and sterilized feeding cup or bottle. To achieve this temperature, the water should be left for no more than 30 minutes after boiling.

4. To the water, add the exact amount of formula as instructed on the label. Adding more or less powder than instructed could make infants ill:

a. If using bottles: assemble the cleaned and sterilized parts of the bottle according to the manufacturer's instructions. Shake or swirl gently until the contents are mixed thoroughly, taking care to avoid scalds.

b. If using feeding cups: mix thoroughly by stirring with a cleaned and sterilized spoon, taking care to avoid scalds.

5. Immediately after preparation, quickly cool feeds to feeding temperature by holding the bottle or feeding cup under running tap water, or by placing in a container of cold or iced water. Ensure that the level of the cooling water is below the top of the feeding cup or the lid of the bottle.

6. Dry the outside of the feeding cup or bottle with a clean or disposable cloth.

7. Because very hot water has been used to prepare the feed, it is essential that the feeding temperature is checked before feeding in order to avoid scalding the infant's mouth. If necessary, continue cooling as outlined in step 5.

8. Discard any feed that has not been consumed within two hours. [28]

4.4.3 **Preparing feeds in advance for later use**

It is best to make PIF fresh for each feed and to consume immediately, as reconstituted PIF provides ideal conditions for the growth of harmful bacteria. For practical reasons, however, feeds may need to be prepared in advance. The steps below outline the safest way to prepare and store feeds for later use.1 If refrigeration is not available, feeds should be prepared fresh and consumed immediately rather than prepared in advance for later use. If using feeding cups, a batch of formula should be prepared in a clean, sterile jar that is no larger than 1 liter, with a lid. The prepared PIF can be refrigerated and dispensed into cups as needed.2Place cooled feeds in a refrigerator. The temperature of the refrigerator should be no higher than 5 °C. 3. Feeds can be stored in the refrigerator for up to 24 hours. [28]

4.4.4 **Re-warming stored feeds**

Remove stored feed from the refrigerator just before it is needed. Then re-warm for no more than 15 minutes. To ensure that the feed heats evenly, periodically shake the covered jar or container. Microwave ovens should never be used to re-warm a feed as uneven heating may result in 'hot spots' that can scald the infant's mouth. Check feeding temperature in order to avoid scalding the infant's mouth. Discard any re-warmed feed that has not been consumed within two hours.Because of the potential for growth of harmful bacteria during transport, feeds should first be cooled to no more than 5 °C in a refrigerator and then transported. Prepare the feed and place in the refrigerator and ensure feed is cold before transporting. Do not remove feed from the refrigerator until immediately before transporting. Transport feed in a cool bag with ice packs. Feeds transported in a cool bag should be used within two hours as cool bags do not always keep foods adequately chilled. Re-warm at the destination. If you reach the destination within two hours, feeds transported in a cool bag can be placed in a refrigerator and held for up to 24 hours from the time of preparation. Alternatively, if you are going out for the day, individual portions of PIF can be transported in washed and sterilized containers. At the destination, hot water no less than 70 °C can be used to prepare the feed, using washed and sterilized feeding and preparation equipment.

4.4.5 **Good hygienic practice**

Poor hygiene has been reported as the probable cause of some *E. sakazakii* outbreaks [10] The person preparing the feed should clean and disinfect the preparation surface and wash hands

with soap and water before preparing a feed. This is because harmful bacteria can be carried on hands and can also be present on surfaces. Washing hands and cleaning and disinfecting surfaces reduces the risk of feeds becoming contaminated during preparation. Hands must also be washed after using the toilet and after diaper changing because harmful bacteria, including *E. sakazakii* [6], have been found in the urine and stools of infants. These bacteria can easily be carried on the hands and contaminate feed during its preparation.

4.4.6 **Temperature of water for reconstitution**

Risk is dramatically reduced when PIF is reconstituted with water that is no less than 70 °C, as this temperature will kill any *E. sakazakii* in the powder. This level of risk reduction holds even if feeding times are extended (i.e. up to two hours), and even if ambient room temperature reaches 35 °C. Consequently, reconstituting PIF with water no less than 70 °C dramatically reduces the risk to all infants, even slow feeding infants and infants in warm climates where refrigeration may not be readily available (e.g. developing countries). When PIF is prepared with water cooler than 70 °C, it does not reach a high enough temperature to completely inactivate *E. sakazakii* present in the powder. This is a concern for two reasons: a) a small number of cells may cause illness, therefore it is important that cells present in the PIF are destroyed; and b) the potential for surviving cells to multiply in the reconstituted formula. This risk is increased when the reconstituted formula is held for extended periods above refrigeration temperature. [14] Concerns have been raised over the use of very hot water for reconstituting PIF, but risk of *E. sakazakii* is only dramatically reduced when water at a temperature of no less than 70 °C is used. Currently, the instructions on many PIF products lead to PIF being reconstituted with water that is around 50 °C. But, reconstitution with 50 °C water generally results in the greatest increase in risk, unless the reconstituted formula is consumed immediately. Under no circumstances is risk reduced when PIF is reconstituted with 50 °C water. Manufacturer's instructions should be reviewed in the light of the findings of the risk assessment [8].

4.4.7 **Storage of prepared feeds**

Because PIF may contain harmful bacteria, it is best to prepare it fresh for each feed. However, in practical terms this is not always possible. Feeds may need to be prepared in advance, e.g. for the crèche, baby sitter, or if you are going out for the day. In these circumstances, feeds should be prepared using water no less than 70 °C, cooled quickly

immediately after preparation, and stored in the refrigerator (at 5 °C or colder) for no more than 24 hours. Feed stored in the refrigerator should be used within 24 hours of preparation. Even if water no less than 70 °C was used to reconstitute PIF, spoilage bacteria may have survived that can grow at refrigeration temperatures and can cause feeds to spoil. The quality of reconstituted PIF may also deteriorate on prolonged storage. Feeds should be cooled quickly before placing into the refrigerator, as hot feeds will increase the refrigerator's temperature. Feeds can be rapidly cooled by placing under cold running water or in a bowl of cold water. [28]

4.4.8 **Transporting feeds**

Transporting prepared feeds poses a risk as it increases the time from preparation to consumption, providing the opportunity for growth of harmful bacteria. Because of this potential for growth, feeds that need to be transported should be quickly cooled and refrigerated until they are cold before transport. In order to minimize growth of harmful bacterial, cooled feeds should only be removed from the refrigerator at the last minute and transported in a cool bag. At the destination, feeds can be re-warmed for feeding. Feeds held in a cool bag should be used within two hours. Alternatively, if transported feeds are returned to a refrigerator within two hours, they can be stored for up to 24 hours from preparation. By following these steps, feeds will be kept cool, which will slow down or prevent the growth of harmful bacteria.

4.4.9 **Holding and feeding times**

Minimizing the time from preparation to consumption is an effective measure for controlling the risk of infection with *E. sakazakii*. Prepared feed should be discarded after two hours, unless it has been stored in the refrigerator since. Leftover feed should never be saved for later, or added to a freshly prepared feed, as harmful bacteria may have had the chance to grow during the feeding period. It is recommended that formula is not held at room temperature for more than two hours, even if water at no less than 70 °C is used to reconstitute PIF. This is because the feed may have become contaminated during preparation, or harmful bacteria may have been introduced into the cup or feeding bottle from the infant's mouth. Also, hot water (70 °C) may have activated bacterial spores of harmful bacteria in the formula. Holding prepared feeds above refrigeration temperature for extended periods provides the opportunity for such bacteria to grow. [28]

4.5 Breast feeding

For infants, human milk is considered a food superior to formula food. Breast fed infants in the United States have lower rates of morbidity from infectious disease. [4] There is a pressing need to obtain additional information on what effect breast feeding has on public health impact *E.sakazakii* has in developing countries. [20] Besides having a nutritional advantage, it also has long-term benefits for metabolism and for disease prevention in later life. It also helps in protecting against infections through specific and nonspecific immune factors . A case control study showed that breast-feeding decreased the risk of sporadic salmonellosis in infants. However, breast milk has also been implicated as the source of several viral and bacterial infections in neonates include serotype Kottbus, was initially suggested to have a specific predilection for colonizing the human mammary gland. In the following years, several reports showed that other sero-types, including *Salmonella enterica* serotype *Typhimurium, Salmonellaenterica* serotype. *Typhimurium* definite type 104 and *Salmonella enterica* serotype *Senftenberg,* can also be transmitted via breast milk and other salmonellae serotypes that might be transmitted via this vesicle. Isolation of the first serotype Panama from milk collected aseptically strongly suggested that the mother is the carrier who excreted this pathogen into breast milk rather than the possibility that the milk was contaminated during collection. The excretion of *Salmonella* organisms from the breast asymptomatic is not unique. However, the source from and period within which the mother became colonized with this organism could not be traced and remain unknown. The *Salmonella* organisms could enter into the mammary duct via external contamination. [22] There is a pressing need to obtain additional information on what breasffeeding has on public health impact *E.sakazakii* has in developing countries. [20]Infants partially breast fed (breast milk along with animal milk or infant formula) or not breast fed have a significantly higher risk of hospitalization and death as compared to infants predominantly breast fed (breast milk and water) or exclusively breast fed.

5.0 CONCLUSION

The powder form of infant formulas offers some advantages compared to the liquid form in particular for its lower costs. It has been available and consumed by infants for more than 50

years and constitutes a substantial part of the infant formula that is used worldwide. The bacteria have been found in samples from newly opened sealed cans. It is important to note that powdered infant is not a sterile product and may contain low levels of pathogenic bacterium, and that contamination with *Enterobacter sakazakii* and other pathogens has been a cause of infection, illness and death in infants. However infections in children and immunocompromised adults has also been reported. It is not feasible, using current processing technology, to eliminate completely the potential for microbial contamination, and even though the incidence of *E. sakazakii* infections in infants appears to be low, the consequences can be severe. Reported fatality rates of *E. sakazakii* infections in infants vary considerably with rates as high as 50 percent. Infections from *E.sakazakii* have been documented as both sporadic cases and outbreaks, especially in the neonatal intensive care settings. The group at particular risk is infants and neonates, who are immunocompromised. There is a need to control the safety of infant formula by applying control measures during production, and to take into consideration the range of microorganisms of concern and to monitor their presence by appropriate microbiological methods. Manufacturers should identify any steps in their operations which are critical to the safety of powdered products used for infants by implementing effective control procedures at those steps, and monitor control procedures to ensure their continuing effectiveness; together with review control procedures periodically. There is also a need to identify further and to define high risk infant populations. Specific guidance for hospitals, day-care centres, food handlers, and caregivers are important preventive measures. Health education programs should cover general food hygiene. Specific information and recommendations on the labeling of the infant formulas regarding the preparation is crucial to provide further safety. In developing countries the availability of boiling water and refrigerators for keeping bottles with reconstituted milk will considerably reduce the incidence of contamination. Neonates and infant is so susceptible to infections that powdered infant formula requires a high level of microbiological quality control during production, distribution and usage. It is important to ensure that the product is prepared using good hygienic procedures, along with rapid cooling, and minimization of the time between preparation and consumption to reduce the risk of *E. sakazakii* infection. Despite the publicized outbreaks and product recalls, infant deaths caused by *E. sakazakii* infection is fortunately rarely reported.

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