codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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RISK PROFILE OF *ENTEROBACTER SAKAZAKII* IN POWDERED INFANT FORMULA

Prepared by the United States of America and Canada

Introduction

The United States and Canada request that an additional item be considered during the 35th Session of CCFH under Agenda Item # 12, Other Business and Future Work.

During the 24th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU), the subject of pathogens in infant formulas was raised. It was recommended that CCNFSDU submit a request asking CCFH to revise the Recommended International Code of Hygienic Practice for Foods for Infants and Children (CAC/RCP) 21-1979). The requested revisions would address, among other things, concerns with pathogens in infant formula, including *Enterobacter sakazakii*. Delegates and Observers at the 24th Session of CCNFSDU overwhelmingly supported this recommendation (Alinorm 03/26A, paragraphs 132-134).

With regard to CCFH, the Committee at its 33rd session generally recognized the necessity of revising the Code for Egg and Egg Products and the Code for Foods for Infants and Children (Alinorm 01/13A, paragraph 150). Work has been initiated for the revision of the Code for Egg and Egg Products, however, no work has yet been initiated for the revision of the Code for Foods for Infant and Children.

Background

Enterobacter sakazakii has been associated with a variety of severe and life-threatening conditions including meningitis, bacteremia, and necrotizing enterocolitis, especially in neonates and infants. The organism is a gram-negative rod within the family Enterobacteriaceae, genus *Enterobacter*. It was called "yellow-pigmented *Enterobacter cloacae* until 1980 when it was renamed *Enterobacter sakazakii*. The first two known cases of meningitis were reported in 1961 by Urmenyi et al.(1). Subsequently, cases of meningitis, septicemia, and necrotizing enterocolitis due to *E. sakazakii* have been reported worldwide. While the overall frequency of *E. sakazakii* infections appears to be low, the consequences can be dire. Although most reported cases have involved infants, reports have

described infections in children and adults as well (2). Overall, reported case-fatality rates have varied considerably with rates as high as 50 percent in some instances. Infections from *E. sakazakii* have occurred as both sporadic cases and as outbreaks. It is the latter that has lead to the link with powdered infant formula, especially in the context of neonatal intensive care settings (3-6).

Scope and Rationale

A number of outbreaks that have resulted in serious adverse health consequences and death underscore the need to better manage the risk of *E. sakazakii* in powdered infant formula. While it is not clear if powdered infant formula is the only source of *E. sakazakii* which leads to infection in infants and neonates, it is one source that is well documented and in need of an appropriate risk management strategy. There are many gaps in our knowledge of *E. sakazakii* including a better understanding of the at-risk population, the exposure vehicle, the infectious dose, host factors contributing to susceptibility and the molecular pathogenesis of the organism itself. However, *E. sakazakii* is known to be present in a proportion of powdered infant formula, such formula has been epidemiologically linked with illness in neonates, and the disease may be life threatening. That alone is enough to seriously consider appropriate strategies to reduce this documented risk.

Pathogen-Food Commodity Combination(s) of Concern

Pathogen of concern: *Enterobacter sakazakii*

Description of the food or food product and/or condition of its use with which problems due to this pathogen have been associated:

Powdered infant formula is the food item that has been linked with *E. sakazakii* infections. In 1988 Muytjens et al (7) reported recovery of Enterobacteriacae from >50% of 141 dry infant formula powder products from 35 countries. *E. sakazakii* was recovered from 20 (14%). All were in compliance with Codex Alimentarius since the concentration of the organisms did not exceed 1 colony-forming unit/gram dry powder. More recently, Nazarowec-White and Farber (8) studied the incidence of *E. sakazakii* in powdered infant formula obtained from five different manufacturers that sell at the retail level on the Canadian market. A total of 120 samples (cans) from different lots manufactured on different days were obtained and evaluated. *E. sakazakii* was cultured from 8 cans of product with levels in positive samples averaging 0.36 CFU/100g. There have been a number of outbreaks of neonatal *E. sakazakii* infection attributed to powdered infant formula in which identical organisms were isolated from ill neonates and previously unopened containers of formula (see below).

Description of the Pathogen and Public Health Problem

The pathogen

Enterobacter sakazakii is a gram-negative rod within the family Enterobacteriaceae, genus *Enterobacter*. It was called "yellow-pigmented *Enterobacter cloacae* until 1980 when it was renamed *Enterobacter sakazakii*. Little is known about specific virulence mechanisms but the organism appears to have a propensity to infect the central nervous system to cause meningitis, cysts or brain abscess. Subsequent developmental delay and hydrocephalus is a well-recognized sequela (2). *Enterobacter sakazakii* has also been associated with necrotizing enterocolitis in at least one outbreak in Europe (4). In relation to thermal tolerance, Nazarowec-White and Farber (9) reported ten Canadian *E. sakazakii* strains (5 clinical and 5 food isolates) in which they determined the heat resistance at 52, 54, 56, 58 and 60 °C in reconstituted dried-infant formula. D-values of 54.8, 23.7, 10.3, 4.2 and 2.5 min were obtained for each temperature, respectively. The overall calculated z-value was 5.82 °C. They concluded that in a comparison with D-values of several members of the Enterobacteriaceae in dairy products, *E. sakazakii* appeared to be one of the most thermotolerant organisms. A notion supported by at least one report of the isolation of *E. sakazakii* from thermal springs (10).

The public health problem

E. sakazakii has been isolated from a variety of sterile sites including blood and cerebrospinal fluid in humans with clinical conditions consistent with Gram-negative infections. While *E. sakazakii* has caused disease in all age groups the majority are in infants less than 2 months old. There are approximately 50 cases reported in infants less than 60 days old. Data on these infants is incomplete but what is available indicates that approximately three quarters of them had a birth weight of <2500 g, and three quarters were premature being born at <37 weeks gestation. One of the key questions is the susceptibility of term infants. There have been cases reported in term infants, and while some have major congenital abnormalities (e.g. neural tube defects, Downs syndrome), others have no reported evidence of compromised host defence yet have been afflicted with *E. sakazakii* sepsis or meningitis (2).

Mortality rates from *E. sakazakii* infection have been reported to be >50% but this figure has declined to <20% in recent years. While the disease is usually responsive to antibiotic therapy, a number of authors have reported increasing antibiotic resistance that are commonly used for initial treatment of suspected *Enterobacter* infection. Reports have also been made of β-lactamases and cephalosporinase from *E. sakazakii* (11). Even in the context of sensitive organisms long-term neurological sequelae are well recognized (2, 12).

While the reservoir for *E. sakazakii* in many cases is unknown, a growing number of reports have suggested a role for powdered milk infant formula as a vehicle for infection (3-6). In several investigations of outbreaks of *E. sakazakii* infection that occurred among neonates in neonatal intensive care units, investigators were able to show that the strain of *E. sakazakii* recovered from the ill neonates was indistinguishable from the strain recovered from unopened cans of infant formula used to feed the neonates (4-6). These reports strongly suggest that the infant formula contaminated intrinsically with *E. sakazakii* served as the source of infection for the neonates that subsequently became ill.

In addition to laboratory evidence corroborating contaminated infant formula as a source of infections in outbreaks of illness, epidemiologic associations subsequently confirmed by the laboratory have been established as well. Three cases of neonatal infection caused by *Enterobacter sakazakii* are reported from Iceland (5, 12). These infections occurred during a 9-month period in 1986 and 1987. Two of the neonates, who were normal at birth, survived but were left with brain damage. The third, which had Down's syndrome and severe cardiac malformations, died. *E. sakazakii* was not isolated from any environmental sources in the neonatal wards or in the milk kitchen, but it was grown from several lots of the powdered-milk formula used in the hospital. The *E. sakazakii* strains isolated from the neonates were indistinguishable from 22 strains grown from the formula. A combination of typing methods (plasmid analysis, antibiograms, chromosomal restriction endonuclease analysis, ribotyping, and multilocus enzyme electrophoresis) were used to evaluate the isolates from each outbreak as to their relatedness. The typing results differed among outbreaks, but in each one, patient and formula isolates shared the same typing pattern. The only exceptions were disk antibiograms, which often varied among colonies selected from each of the isolates. Plasmid analysis, chromosomal restriction endonuclease analysis, ribotyping, and multilocus enzyme electrophoresis all were effective as epidemiological typing methods for *E. sakazakii*, especially when used in combination. By using this typing scheme, the authors confirmed that *E. sakazakii* from intrinsically contaminated dried infant formula was the source of neonatal infection (12).

Simmons et al (3) reported an outbreak of *E. sakazakii* infection and colonization in neonates related to an infant formula contaminated during the manufacturing process. The outbreak occurred in a 20-bed neonatal intensive care unit during a six-week period in 1988, and involved a total of four infants. Three infants had sepsis and three had bloody diarrhea; all patients responded to intravenous antibiotics and recovered without complications. The *E. sakazakii* isolated from the formula had the same plasmid and multilocus enzyme profile as those isolated from patients.

Van Acker et al (4) described an outbreak of necrotizing enterocolitis that occurred in a neonatal intensive care unit. A total of 12 neonates developed NEC in June-July 1998. For two of them, twin brothers, the NEC turned out to be fatal. *E. sakazakii* was isolated from a stomach aspirate, anal swab, and/or blood sample for 6 of the 12 neonates. A review of feeding procedures revealed that 10 of the 12 patients were fed orally with the same brand of powdered milk formula. *E. sakazakii* was isolated from the implicated prepared formula milk as well as from several unopened cans of a single batch. Molecular typing by arbitrarily primed PCR (AP-PCR) confirmed, although partially, strain similarity between milk and patient isolates. The investigators described what turned out to be an inadvertent re-challenge test involving the formula implicated as the cause of the outbreak. This occurred after a decision was made to discontinue the use of that particular formula in the neonatal intensive care unit on 10 July 1998 immediately after the investigators suspected a possible link between that formula, *E. sakazakii*, and development of necrotizing enterocolitis. However, because their initial cultures demonstrated the presence of *E. sakazakii* only in prepared milk and not in original powder, the formula was released again on 20 July 1998. One patient given the released formula developed symptoms of necrotizing enterocolitis on 23 July 1998, and *E. sakazakii* was isolated from her stomach aspirate and anal swab. At the same time, further cultures demonstrated the intrinsic contamination of the powdered milk with *E. sakazakii*, including a matching molecular profile of the *E. sakazakii* recovered from this new case and from the powder obtained from an unopened can. From then on, feeding with that formula was suspended and no further cases of necrotizing enterocolitis occurred.

The van Acker report (4) is also noteworthy because it demonstrated that relatively low levels of *E. sakazakii* were present in the samples of powdered milk formula that were implicated as the cause of the outbreak. For example, the manufacturer's microbiological quality control data for the batch of formula implicated in the outbreak showed that, of the five samples analyzed, one yielded 20 coliforms/g whereas in the other four samples fewer than 1 coliform/g was found. These results fulfilled the requirements of the Codex Alimentarius (a minimum of four of five control samples with <3 coliforms/g and a maximum of one of five control samples with >3 but $\leq$20 coliforms/g) (7) but not the requirements of Belgian law (i.e., < 1 coliform/g in all control samples) (4). After the incident, the product facility was upgraded, appropriate hygienic measures were taken, and more stringent release norms for dietetic specialties (<0.3 coliform/g, 0 *E. sakazakii* isolates/10 g) were applied by the manufacturer.

A more recent outbreak of colonization and infection with *E. sakazakii* occurred in a neonatal intensive unit in Tennessee in 2001 (6). In the Tennessee outbreak, the investigators demonstrated a statistically significant association between *E. sakazakii* colonization/infection and powdered milk formula ingestion. Specifically, in that study, the investigators showed that nine of the nine infants who were infected/colonized with *E. sakazakii* had been fed a specific formula product compared to 21 of 40 infants who were not infected/colonized with *E. sakazakii* (P =0.008).

The recovery of *E. sakazakii* from samples of powdered infant formula has been reported in at least one survey of commercially produced formulas. For example, in an examination of a total of 141 different powdered formulas obtained in 35 countries for the presence of members of the *Enterobacteriaceae*, Muytjens et al. (7) cultured *E. sakazakii* from 20 formulas (14% of the 141 formulas); the formulas from which *E. sakazakii* was recovered were available in 13 countries and made by a number of different manufacturers. It is important to note that all of the formula tested met the FAO (1977) recommendation for bacterial coliform count in powdered infant formula (less than 3 CFU/g). Interestingly, however, the investigators speculated that these powdered formulas had the potential to serve as a reservoir for future outbreaks. It has been suggested that the high thermal resistance of *Enterobacter* spp. in comparison to other members of the *Enterobacteriaceae* can possibly explain their high prevalence in powdered and prepared formula milk (9).

While the above examples have discussed intrinsic contamination of powdered infant formula with *E. sakazakii*, extrinsic contamination has also been associated with disease in infants. In one instance a blender used in rehydration and rehydrated formula were found to be contaminated with *E. sakazakii*

(13). In another instance a cracked contaminated blender used to prepare formula from dry powder was implicated in an outbreak that involved two infants. These included one case of sepsis and meningitis complicated by cerebral infarction, and one case of sepsis. In addition, three cases of intestinal colonization were identified (14, 15).

Food Production, Processing, Distribution and Consumption

Powdered infant formula, which is not a sterile product, is the food commodity of interest for this risk profile. *E. sakazakii* is considered to be an environmental organism, and as such is likely to be present in both manufacturing facilities as well as domestic situations. Molecular epidemiology has clearly demonstrated that *E. sakazakii* present in powdered formula has caused serious human illness. It is unclear at what stage in the manufacturing process the organisms get into powdered formula. In some instances however, the contamination appears to have arisen from equipment used to prepare the formula in milk kitchens.

Little is known about the growth rates of different *E. sakazakii* isolates in formula, but it is likely that they grow readily in formula held at room temperature for prolonged periods. Nazarowec-White and Farber (8) reported that minimum growth temperatures for *E. sakazakii* in culture media varied from 5.5°C to 8°C; however, no growth occurred at 4°C. These authors also reported that generation times for *E. sakazakii* at 10°C varied from 4.18 to 5.52 h. They concluded that due to its relatively short lag time and generation time, even low levels of *E. sakazakii* may pose a safety concern. Hence, improper storage of reconstituted powdered infant formula at ambient temperature e.g., on a bedside table for night feeding, may permit the growth of *E. sakazakii*. Lack of specific information related to infectious dose and rate of growth of the organism makes it difficult to determine the adverse health consequences of a specific level of contamination of powdered formula. Several approaches have been suggested to minimize the risk from using powdered infant formula. These include preparing only a small amount of reconstituted formula for each feeding to reduce the quantity and time that formula is held at room temperature for consumption. Minimizing the holding time, whether at room temperature or while under refrigeration, before a reconstituted formula is fed. Minimizing the "hang-time" (i.e., the amount of time a formula is at room temperature in the feeding bag and accompanying lines during enteral tube feeding). Some have suggested using heated water to reconstitute the formula, but this has raised questions regarding safety to the infant (or handler if boiling water is used), destruction of nutrients and unknowns regarding thermal tolerance of the organism.

There is a paucity of data regarding the microbial ecology of *E. sakazakii.* Muytjens and Kollee reported that they were not able to isolate the organism from surface water, soil, mud, rotting wood, grain, bird dung, rodents, domestic animals, cattle or raw cow's milk (16). So the precise environmental niche for *E. sakazakii* remains unclear.

Other Risk Profile Elements

Published reports of *E. sakazakii* infections are largely from developed nations, and even there a significant level of underreporting of infections is likely. The lack of reports from developing countries is likely due to lack of recognition of the problem rather than there being no illness. Arguably the problem may be even greater in developing countries where cleaning and maintaining equipment poses a greater problem both in manufacturing facilities as well as hospitals. Also the numbers of susceptible infants is likely to be greater in developing countries.

Risk Assessment Needs and Questions

The key needs in relation to the risk posed by the presence of *E. sakazakii* in infant formula are as follows:

- Are there susceptible populations to *E. sakazakii* from powdered formula, and if so what are those populations?
- What is an acceptable level of *E. sakazakii* contamination of powdered infant formula? Does this vary depending on the age or immune status of the consumer?

- What are the appropriate risk management strategies to control *E. sakazakii* in manufacturing facilities, in the hospital or at home?

At the current time there are insufficient scientific knowledge to perform a quantitative risk assessment. However, this emerging food safety public health concern would benefit from a formal evaluation of the risks associated with this pathogen-product pair including a consideration of available control measures and their likely effectiveness for improving public health.

Available Information and Major Knowledge Gaps

Information regarding *E. sakazakii* is limited to a relatively small number of case reports of sporadic cases and outbreaks. Currently there is no active surveillance for *E. sakazakii*. There has been no risk assessment undertaken for *E. sakazakii* in powdered infant formula. While there are many major knowledge gaps, some of which have already been mentioned, the following are some of the more important ones:

- What are the susceptible infant populations to *E. sakazakii* in infant formula?
- What are the differences in virulence, thermal tolerance, growth kinetics between different *E. sakazakii* isolates.
- What is the infectious dose of *E. sakazakii* and how does this vary between susceptible populations?

Conclusions

E. sakazakii is an emerging infection that has clearly been linked with the consumption of contaminated powdered infant formula. The illness caused by *E. sakazakii* is often severe and life threatening with significant long-term sequelae in those who recover, particularly if the infection involves the central nervous system. The risk of potentially fatal infections appears to be highest for neonates in hospital settings, especially if low birth weight and, or immunocompromised. While the risk may diminish for older infants, reports in the literature indicate that there is still some degree of risk to this older population from consumption of powder formula containing *E. sakazakii*. Other than patient susceptibility, factors about which little is known that may contribute to the risk include the level of contamination in the formula, thermal stability, rate of bacterial growth, infectious dose and virulence of the pathogen. Powdered formula is not a sterile product and risk management strategies have to be developed in order address the presence of *E. sakazakii* in this product.

Recommended Risk Management Actions

Considering the current state of knowledge related to this emerging foodborne pathogen, it is recommended that the Codex Committee on Food Hygiene undertake the following risk management activities.

- CCFH designate the Code for Foods for Infants and Children as the next code of practice that should be updated and request permission from the Codex Alimentarius Commission to initiate this new work. CCFH establish a working group to draft the revised code of practice for consideration at the next CCFH meeting. Revision of the Code should take into full account the issue of *E. sakazakii* in dried infant formula.
- CCFH should request that FAO/WHO undertake an expert consultation to articulate the current state of scientific knowledge related to *E. sakazakii* and identify and evaluate potential risk reduction strategies. To the extent possible, the consultation should address impact of this emerging pathogen within a risk analysis framework, including the areas identified in the sections on Risk Assessment Needs and Available Information; however a detailed quantitative microbial risk assessment is not mandatory at the current time.
- CCFH should encourage member nations and international health agencies to increase both their surveillance and research activities related to this microorganism.

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