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## ***Enterobacter sakazakii*\***

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### **Introduction**

*Enterobacter sakazakii* is a Gram-negative, motile and non-sporing rod belonging to the family of the Enterobacteriaceae. Up to 1980 it has been described as “yellow pigmented” *Enterobacter cloacae*. However, based on DNA-DNA hybridization studies as well as on its biochemical characteristics it has been recognized as an individual species and then named after the Japanese microbiologist, Professor R. Sakazaki (1).

Recent molecular studies performed by *Lehner et al.* (2) have shown that different isolates of *E. sakazakii* could be further differentiated and assigned to distinct genetic clusters. This was confirmed in similar studies performed by *Iversen et al.* (3) in which important genetic heterogeneity has been shown amongst different isolates of *E. sakazakii*. Further taxonomic investigations in this field are certainly warranted in the future.

### **Epidemiology**

*E. sakazakii* can cause rare but severe neonatal diseases such as bloody diarrhoea, septicemia, necrotizing enterocolitis (NEC) or, most frequently, meningitis. In reports of recent cases, the occurrence of intestinal colonization of babies without any symptoms have been described as well (4, 5). The first case attributed to a yellow pigmented *E. cloacae* was described by *Urmenyi* and *Franklin* (6). Since then, about 40–50 cases involving either individual or several babies (up to 15) for a total of about 100 patients have been reported and described. Summaries of most of these cases have been published by different authors such as *Lai* (7), *Iversen* and *Forsythe* (8) or *Gurtler et al.* (9). The fatality rate has been reported to be as high as 50%–100%, but a decreasing trend has been recorded over the past years (10). However, in several cases where babies have survived the infection, chronic neurological disorders have been reported.

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No quantitative determinations of the ingested *E. sakazakii* have been performed in any of the reported cases. The dose-response curve is therefore not known. However, as discussed in the FAO/WHO report (10) and by *Havelaar* and *Zwietering* (11), the risk of infection increases dramatically with increasing numbers of cells, as is observed in case of growth in reconstituted products stored at room temperature. The number of publications on the virulence is scarce (12) but several research projects are currently being conducted. New results and insight may become available in the near future.

According to the published case studies, the majority of the babies affected by *E. sakazakii* can be considered as premature babies born before week 37/38 of the gestation. Another important element relates to the onset of illness – the large majority of infections being reported within the first days after birth and up to 18–30 days. Few cases have been reported for older babies up to a few weeks and exceptionally to about 6 months. The reports and studies related to the latter cases indicate, however, the occurrence of underlying diseases, heavy surgery or even mixed infections. Cases involving adults have been associated with infections (eye, urinary tract, diabetic wounds, etc.) but do not seem to be linked with foods. Indeed in a preliminary draft report issued by the New Zealand Food Safety Authority (13), it has been considered that other dairy products do not represent an issue for the general population.

### **Routes of contamination**

In a number of case studies and publications, the source and route(s) of contamination have not been investigated. In others contaminated infant formulae have been identified as the source of *E. sakazakii* (14, 15, 16, 17, 18). Quantitative determinations of *E. sakazakii* performed on powdered infant formulae by different authors revealed an incidence ranging between 3 and 14 % of analyzed cans and low levels of the organism ranging between 0.36 to 66 cfu per 100 g (19, 20, 21, 22) have been determined.

It is however interesting to note that in some of these cases, mishandling and abuse of reconstituted bottles have been shown to be likely (e.g. 14). In several cases extrinsic contamination through contaminated utensils such as mixers, brushes or bottles used to prepare the formulae, of catheters or the incubator have been shown to be the likely causes of contamination, thus highlighting issues and deficiencies in hygiene during preparation and handling (4, 20, 23, 24).

Recent publications have also shown the occurrence of infections in babies which had not been fed with powdered infant formulae but with breast milk (25) or sterile ready-to-feed formulae mixed with either starch or mother milk (10, 26). These cases as well as the detection of *E. sakazakii* in mother milk stored in milk banks (27) show that recontamination at hospital level cannot be excluded as source of infection.

While for numerous years, infant formulae has been considered to be the only source of *E. sakazakii*, recent publications have demonstrated that this organism is



not rare. It can be found in numerous foods and raw materials as well as in very diverse environments as shown in table 1. *E. sakazakii* has therefore to be considered as an ubiquitous microorganism.

Table 1

Overview of the occurrence of *E. sakazakii* (examples; list not exhaustive)

<i>Foods and raw materials</i>	<i>Environments</i>
Raw milk	Geriatric ward
Dairy products (e.g. cheese, caseinates)	Clinical specimens (from infections)
Mother milk (milkbank)	Households
Soya products	Different food processing environments
Meat products	Salmon and ostrich farms
Eggs and egg products	Soil
Vegetables, salad	Surface waters
Water	Waste water treatment plants
Shrimps	Insects
Fermented foods	

## Behavior

Following outbreaks in the mid 1990s research has been focused on the behavior of *E. sakazakii*. Although an increased heat-resistance has been postulated by some people at an early stage, it was shown by several authors that *E. sakazakii* is easily killed at pasteurization temperatures ( $>60^{\circ}\text{C}$ ). It would thus not survive normal processing conditions as applied by manufacturers (28, 29, 30). It was shown by *Nazarowec-White* and *Farber* (28) that some strains of *E. sakazakii* were exhibiting resistances to temperatures around  $50^{\circ}\text{C}$ , i.e. slightly higher than the one of other members of the Enterobacteriaceae. This characteristic along with a shorter lag phase and a more rapid growth rate at around  $40^{\circ}\text{C}$ , as observed by the same authors, could account for a competitive advantage after reconstitution of the powders and thus favour *E. sakazakii* over other Enterobacteriaceae if present at low levels.

*E. sakazakii* has been shown to be much more resistant to desiccation and thus osmotic shocks than other members of Enterobacteriaceae (29). This is in line with the ability to survive well over prolonged periods of time in finished products (31). These findings provide also a logic explanation to the ability of *E. sakazakii* to survive and persist in dry processing environments.

## Analytical methods

The primary, classical detection of *E. sakazakii* is based on detection methods for either coliforms or Enterobacteriaceae with subsequent biochemical identification of suspect colonies. It became quite early obvious that an increase in the incubation temperature from  $30^{\circ}\text{C}$  to  $37^{\circ}\text{C}$  or even  $42^{\circ}\text{C}$  had a positive effect on the selectivity of the enrichments. This is thus allowing for an easier and more effective detection of *E. sakazakii* amongst a competitive flora of other members of the

Enterobacteriaceae. This characteristic has been at the origin of further method developments and improvements as recently published by *Guillaume-Gentil et al.* (32). The recent commercialization of chromogenic media is a further improvement step and contributes in the easy identification of colonies of *E. sakazakii* on selective or elective plates after incubation. An ISO Technical Standard, based on these recent developments, is currently under preparation and would allow for an international standardization of the detection method.

Over the last years other more sophisticated molecular methods have been described (33, 34) and the automated and commercially available PCR BAX®-system has, for example, been approved as one of the reference methods in Canada.

## Legislation

Up to 2002 all existing national and international regulations or recommendations were based on limits for the relevant pathogen *Salmonella* (usually  $n=60$ ,  $c=0$ ,  $m=0$  (in 25 g)) and coliforms or Enterobacteriaceae. These groups have been used for years as hygiene indicators to demonstrate the adherence to Good Hygiene Practices (GHP). Limits of, for example  $n=5$ ,  $c=1$ ,  $m < 3$  and  $M=20$  (35) or absence in 1 g in certain national regulations have been in force during the last 15–20 years.

Following outbreaks related to infant formulae the US FDA initiated in 2001/2002 surveys of commercialized products applying requirements of absence of *E. sakazakii* in  $4 \times 333$  g, later of absence in 333 g. Based on the risk assessment published in the FAO/WHO report (10), the International Commission on Microbiological Specifications for Foods (ICMSF) proposed the preliminary criteria for Enterobacteriaceae and *E. sakazakii*:  $n=10$ ,  $c=0$ ,  $m=0$  (in 10 g) and  $n=30$ ,  $c=0$ ,  $m=0$  (in 10 g) respectively. These preliminary criteria have been included in the initial draft of the revised Code of Hygienic Practice for Food for Infants and Children (35) and may be further revisited as new information becomes available.

In the meantime, the European Commission has adopted the following criteria for powdered infant formulae: Enterobacteriaceae (as hygiene indicator) with the same limits as above and, in case of one or more positive samples, requirements for *Salmonella* ( $n=30$ ,  $c=0$ ,  $m=0$  (in 25 g)) and for *E. sakazakii* (as food safety indicator) with the same sampling plan as the one proposed by the ICMSF. These criteria will be introduced in January 2006 at European level.

## Control measures

As outlined in the FAO/WHO report (10) the issue of *E. sakazakii* can only be addressed through the implementation of a combination of control measures. One of them being the implementation of more stringent control measures during manufacture in order to fulfill the more stringent microbiological criteria, another one being the application of hygienic measures during preparation and handling up to consumption. Both are briefly discussed below, the others can be consulted in the above-mentioned report.



The manufacture of infant formulae can be subdivided in two very distinct parts, a wet and a dry part of the processing. In the wet part, raw materials such as fresh milk, liquid whey or dissolved dry ingredients are heat-treated (CCP) and then concentrated by evaporation before being spray-dried. The processing parameters (time and temperature) may depend on the manufacturer and upon the composition of the products but allow always to effectively control vegetative microorganisms such as *Salmonella* or *E. sakazakii* and destroy them in excess of 10–12 log units. The sections after the CCP and up to the dryer are closed hygienic systems submitted to regular cleaning in place procedures (CIP) including sanitation.

The major issues with respect to *Salmonella* and Enterobacteriaceae, including *E. sakazakii*, are encountered on the dry part of the process, i.e. from the dryer, through the cooling, intermediate storage steps, mixing of ingredients up to the filling in pouches or tins. To avoid or minimize post-process recontamination all these steps are located in high hygiene areas which are physically separated from the wet processing areas as well as from the rest of the factory.

*Salmonella* has been considered to be the most significant pathogen for years for these categories of products. The concept of high hygiene areas has been developed since the mid 1970s to control this pathogen following several outbreaks (36). These concepts encompass the zoning of the factory and the protection of processing areas and processing lines from the ingress of this pathogen. While physical separations of areas are important elements, attention must also be paid to the flows of personnel, of goods such as ingredients for dry mixing operations, of packaging material as well as fluids such as air.

An additional important element is the maintenance of dry conditions to avoid any multiplication of microorganisms within these high hygiene areas. This is best achieved by minimizing the use of water for cleaning operations, the implementation of specific areas where wet cleaning of pieces of equipment can be performed under controlled conditions and the application of dry cleaning procedures for the rest of the line.

The effectiveness of these measures can be measured through the environmental monitoring of *Salmonella* as well as of Enterobacteriaceae. This group of microorganisms has been used for decades as hygiene indicator and proven to be extremely useful to detect deviations such as presence of water or other poor hygienic practices. Experience has shown that it is possible to avoid the ingress and establishment of *Salmonella* and to keep high hygiene areas completely free of the pathogen. Under these circumstances it is also possible to guarantee its absence from finished products. Breakdown of the preventive measures may however lead to problems as shown in recent outbreaks (37, 38). However, in the case of Enterobacteriaceae, including *E. sakazakii*, experience has shown that it is only possible to minimize their presence but not to eradicate them completely. This requires of course well-established control measures as outlined above, but additional efforts in maintaining high hygiene levels is necessary, in particular it is important to eliminate completely

the presence water, e.g. condensation, leakage which may lead to multiplication of Enterobacteriaceae and *E. sakazakii* and to reinforce dry-cleaning procedures to avoid this.

The safety of powdered infant formulae cannot be ensured through stringent microbiological criteria alone. As shown in several of the case studies mentioned earlier, poor hygiene during preparation of the bottles and their storage under inappropriate conditions, leading to multiplication contribute to outbreaks. In order to minimize the risks it is important to adhere strictly to the recommendations of manufacturers who provide detailed information as to the preparation and handling and immediate consumption of reconstituted powders. In addition and to assist users, in particular in hospitals, recommendations and comments on hygienic practices have been issued by Public Health Authorities such as the US FDA or organizations such as the one of European Pediatricians (39).

## Summary

*Enterobacter sakazakii* has been at the origin of rare but severe cases of infections of premature and neonate babies. It is considered as an opportunistic pathogen and in this presentation the current knowledge on this microorganism is reviewed including control measures.

## Zusammenfassung

*Enterobacter sakazakii* ist als Erreger seltener aber schwerer Infektionen frühgeborener und neugeborener Babies bekannt. Dieser Erreger kann als opportunistischer Erreger bezeichnet werden und in diesem Vortrag werden die bestehenden Kenntnisse, inklusive Kontrollmassnahmen, dieses Erregers diskutiert.

## Résumé

*Enterobacter sakazakii* est connu comme agent d'infections rares mais sévères de bébés prématurés et de nouveaux-nés. Cet agent peut être considéré comme pathogène opportuniste et dans cette présentation sont discutées les connaissances actuelles de même que les mesures de maîtrise.

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