Enterobacter sakazakii: epidemiology, clinical presentation, prevention and control

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Summary. The *Enterobacter sakazakii* is considered an emerging pathogen and has been recently connected to neonatal cases of necrotizing enterocolitis and meningitis due to use of contaminated powdered infant formula. However its presence is not limited to powdered infant formula; it can also be found in a broad range of foods and in water, in a variety of areas, including hospitals and houses. Due to the gravity of the infections attributed to *E. sakazakii*, it is necessary to introduce rigorous control measures to reduce the risks of contamination at various levels: industrial, to prevent from production to marketing the contamination of products; at a domestic level by reducing the risk of contamination, during preparation, handling, and storage, of reconstituted products; and legislative by establishing guidelines and recommendations issued by competent authorities, to guarantee the safety of infant food.

Key words: E. sakazakii, powdered infant formula, infections, infants, control measures.

Riassunto *(*Enterobacter sakazakii: *epidemiologia, manifestazioni cliniche e misure di controllo)*. Recentemente, l'*Enterobacter sakazakii*, considerato un patogeno emergente, è stato legato a casi di meningite ed enterocoliti necrotizzanti neonatali dovuti al consumo di latte in polvere per l'infanzia contaminato. La sua presenza non è comunque limitata al latte in polvere; esso può, infatti, ritrovarsi in un'ampia varietà di alimenti, acqua e ambienti inclusi ospedali e abitazioni. A causa della severità delle infezioni sostenute da *Enterobacter sakazakii* è necessaria una riduzione del rischio da esso rappresentato, intraprendendo una serie di rigorose misure di controllo, sia a livello industriale, per prevenire la contaminazione del prodotto lungo la filiera produttiva fino al consumatore, sia a livello domestico per ridurre la contaminazione del prodotto ricostituito durante la preparazione, manipolazione e conservazione; nonché a livello legislativo per stabilire linee guida e raccomandazioni, emanati da organi competenti, per garantire la sicurezza degli alimenti destinati all'infanzia.

Parole chiave: E. sakazakii, latte in polvere per l'infanzia, infezioni, neonati, misure di controllo.

INTRODUCTION

Enterobacter sakazakii (E. sakazakii) is a gram-negative rod belonging to the family of Enterobacteriaceae *(Table 1)*.

Until 1980 it has been considered a yellowish variant of *Enterobacter cloacae*, due to its peculiar characteristic of forming colonies ranging in colour from bright yellow to pale yellow. In recent years, it has been identified as a distinct species and researchers named it in honour of the Japanese bacteriologist Riichi Sakazaki who greatly contributed in understanding *Enterobacteriaceae* [1]. In a recent study a reclassification of this microorganism has been proposed, on the basis of the creation of the genus *Cronobacter*. This genus includes four species, two subspecies and one genomospecies. *Cronobacter sakazakii* is the type species of this proposed genus [2].

E. sakazakii is considered an ubiquitous microorganism: it has been in fact isolated from a great variety of sites [3]: foods, water and several areas, including houses and hospitals. A recent article reported the presence of *E. sakazakii* in a lot of maternal milk stored in a milk bank [4].

It is also considered an emerging opportunistic pathogen, responsible of cases of neonatal infections (sepsis, meningitis, necrotizing enterocolitis) related to use of contaminated infant milk formula, which can represent both the vehicle and the source of contamination. Infant milk formulas are preparations in which cow's milk or soy milk is used to substitute, modify and/or strengthen/fortify human milk. The essential requirements of infant formulae, in Europe, are reported in the Infant Formulae Directive [5]. Powered infant formula is basically a non-sterile product which can be, once rehydrated, a good medium for microorganisms. Its production process [6], in fact, unlike the one for liquid formulas, may be subject to bacterial contamination, lack-

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Table 1 | *Characteristic features of E. sakazakii*

General features	Gram negative rods, facultative anaerobic, no spore forming, mesophilic, yellow pigmented, osmotholerant, generally motile, peritrichous, provided of capsule Maximum temperature of growth: 41-45 °C Minimum temperature of growth: 5.5-8.0 °C No growth at 4 °C Distinguished into 16 bio groups
Generation times	40 min at 23 °C 4.18-5.52 h at 10 °C 75 min a 25 °C in reconstituted infant milk powder
Significant biochemical features	Catalase positive, oxidase negative, alfa-glucosidase positive, phosphoamidase negative
Habitat	Foods, environment, animals, biological liquid
Virulence factory	Enterotoxin
Antimicrobial susceptibility	Tetracycline, aminoglycosides, beta-lactam antibiotics, chinolone, antifolates, chloramphenicol, nitrofurantoine
Chemical compounds susceptibility	Chitosans, oligomers of chitosans, monocaprylin (caprylic acid ester)

ing in the required conditions guaranteeing a sterile end product.

A large part of *E. sakazakii* infections occur in hospitals, in the neonatal intensive care units. Keeping reconstituted formula at room temperature for long periods of time, in fact, increases the risk of infection. That is why improving training and hygiene in hospitals is dramatically important in avoiding *E. sakazakii* outbreaks.

The International Commission for Microbiological Specification for Foods [7], due to the seriousness of pathologies associated with *E. sakazakii*, defined it a "serious danger for a segment of the population, with chronic effects which may be long-lasting, capable of constituting a threat for life". As a consequence, *E. sakazakii* has been included among the most common pathogens such as *Listeria monocytogenes*, Type A *Clostridium botulinum* and *Cryptosporidium parvum* [3]. Infants are the type of population most seriously affected, particularly those hospitalized in neonatal intensive care units and fed with powdered formulas. For this reason, the Codex Committee on Food Hygiene of FAO/WHO, is considering a revision of the Recommended International Code of Hygienic Practices for Foods for Infants and Children, and the creation of a risk profile for *E. sakazakii* in infant milk formulas.

E. sakazakii is generally detected in foods, environment (*i.e.*, dust) and in clinical samples by using traditional and molecular biology methods *(Table 2)*.

EPIDEMIOLOGY

E. sakazakii may cause infections in all age groups and particularly in subjects from 0 to 12 months. Among this last population, premature born after less than 36 weeks, underweight infants, infants with immunodeficiency, infants whose mothers are HIV-positive and infants hospitalised in intensive care units are more at risk of infection. The reason is that they are usually fed with formulae, which are the most common vehicle of transmission of the microorganism.

Only 50% of infected adults suffer a severe course of illness (even if so far no cases of meningitis have been reported); in such cases the vehicle of transmission and the infectious dose are unknown.

The number of reported cases of infection is very low, but nevertheless it recently slightly increased. Nowadays the reported cases worldwide are less

Table 2 | *Methods for enumeration, detection and typing of E. sakazakii in food samples**

Current methods for				DNA-based methods for			
Enumeration	**Rif.**	**Detection**	**Rif.**	**Detection**	**Rif.**	**Typing**	**Rif.**
MPN	[8] [9]	Detection	[11]	BAX-PCR	[12]	Pulsed-field gel electrophoresis (PFGE)	[14] [15]
Colony - count	[10]			Real-time PCR	[13]	Random amplification of polymorphic DNA (RAPD-PCR)	[16]
						Ribotyping	[17]

**detection of* E. sakazakii *in environmental samples [18]; detection of* E. sakazakii *in biological samples [19]*

than 60 [20]; anyway, it needs to be noted that this number may be underestimated since not all clinical analysis laboratories carry out research on the pathogen and not all countries have a system for reporting diseases.

In the past years, mortality due to infections from *E. sakazakii* was higher than 50%; in recent years it has decreased, even though it is still quite high 20% [21].

By and large, cases so far reported can be divided into two groups: sporadic and epidemic [22].

The first two cases of meningitis related to *E. sakazakii*, where reported in 1961, when the microorganism was still called *E. cloacae* [23]. Since then, cases of infections related to *E. sakazakii* have been largely reported worldwide [24] with an increase of reported cases in recent years. This increase is probably due to a higher number of subjects at risk and to improved diagnostic ability [22].

Between October and December 2004, five cases of infections, two of which fatal, and five cases of intestinal colonization, were diagnosed in the paediatric units of five French hospitals, and attributed to *E. sakazakii*. Nine of the infants affected had been fed with the milk usually administered to premature infants. After these events, Ireland decided to prohibit the use of infant formula, although the lot had been sold only in France and in Algeria [25]. During the same year, in New Zealand, another case of *E. sakazakii* infection had a fatal outcome, the microorganism affected and killed a hospitalized infant fed with milk formula [26].

In July 2006, in a medical care centre in India, a two month infant developed nosocomial *E. sakazakii* bacteraemia. The vehicle of contamination was unclear and transmission from hospital environment cannot be excluded [27].

The most recent case of *E. sakazakii* infection has been reported in 2007 by the paediatric unit of a hospital in Bilbao, Spain [28], where a premature and underweight infant had contracted neonatal sepsis. The source of infection was unclear: the infant had not been fed by infant formula but with breast milk and a liquid formula for premature infants.

CLINICAL PRESENTATION

Enterobacteriaceae is the most important family of gram-negative bacteria. Bacteria of this family are responsible of infections affecting bile ducts, prostate, the urinary tract, and of the most common nosocomial infections. The main source of diffusion and transmission to sensitive sites is the intestine [29].

E. sakazakii, one of the bacteria of the Enterobacteriaceae family, is considered an invasive opportunistic pathogen responsible of sepsis, meningitis, and necrotizing enterocolitis (NEC), especially among newborns (premature, underweight, affected by immunodeficiency) and of nosocomial infections, especially in paediatric intensive care units where it causes almost 50% of infections. The most severe infection caused by *E. sakazakii* is meningitis. The pathway the pathogen follows to reach the cerebral spinal liquid and cause meningitis is unknown. It is widely assumed that the route of infection is the carotid plexus; paracellular and transcellular mechanisms are involved. Bacterial glycopeptides, endotoxins, proteases, and elastases seem to induce permeability of haematic and cerebral barriers opening the way to the pathogen. The most common pathological symptoms in advanced stages of meningitis, are: ventriculitis, cerebral abscess, formation of cysts, hydrocephalus, quadriplegia, retarded neural development and infarction. Patients who recover from the disease suffer from physical and mental retardation.

E. sakazakii, in the same way as *Pneumococcus*, *Haemophilus* and *Meningococcus*, which cause meningitis among children below 5, has a particular tropism for the central nervous system. On the basis of its tendency to cause cerebral lesions, it has been compared to *Citrobacter diversus*.

Neonatal necrotizing enterocolitis is characterized by necrosis and intestinal pneumatosis. The disease affects 13% of underweight infants.

Essential prerequisites for the pathogenesis of this disease seem to be: neonatal intestinal ischaemia, microbial colonization of the intestine and excess of proteins in the intestinal lumen associated with administration of infant formula. The presence of necrotizing enterocolitis among infants fed with artificial milk is ten times higher than among breastfed infants; this is probably due to the presence of class A protective immunoglobulins in maternal milk.

Necrotizing enterocolitis as well as meningitis cause high mortality. In particular, with respect to necrotizing enterocolitis, the percentage varies between 10% and 55%, while in respect to meningitis it varies between 40% and 80%.

Neonatal infections due to *E. sakazakii* could also be contracted directly from the mother at birth, but no cases of intestinal and genital infections have been reported among mothers of infected newborns. Cases of neonatal infections after a caesarean section have been reported [29]. Nevertheless, colonization of infants, especially if premature, with human and environmental bacteria is almost inevitable.

The signs and symptoms of the initial phase of *E. sakazakii* infection are: anorexia, irritability, jaundice, paleness, cyanosis, collapse, spasms and temperature instability [30].

PREVENTION AND CONTROL

In order to reduce the risk associated to consumption of contaminated formulas, correct information and education regarding good practices required during preparation and handling of this product are required.

It is unknown if specific techniques for preparation and preservation of rehydrated milk in hospitals and day care centres do actually exist.

USA competent authorities ensured that hospital units which prepare and handle powdered milk follow the guidelines issued by the American Dietetic Association [31]. These recommendations include using ready-to-feed formulas, complying with the rules for aseptic preparation and refrigeration at 2-3 °C of reconstituted milk for a lapse of time shorter than 4 hours. Recommendations also underline the importance of dedicating specific areas to the preparation, preservation and administration of milk, tasks that should be performed by specifically trained and qualified personnel.

Reducing risks connected to *E. sakazakii* is mandatory for all people involved: producers, parents, health professionals. This purpose can be fulfilled following several combined steps [6, 31] illustrated in Table 3.

CONCLUSIONS

E. sakazakii is an emerging pathogen, often transmitted through powdered milk and responsible for a series of infections, some of which with potential fatal outcomes, in a particular segment of the population.

Factors contributing to increase the risk of infection include: patient's susceptibility, level of contamination of food, tolerance to temperature, speed of growth, infectious dose and the virulence of the microorganism.

Recently, in order to face the risks connected to microbiologic contamination, producers have been obliged to adopt new control measures based on the HACCP system (Hazard Analysis Critical Control Point).

Table 3 | *Combined steps to reduce risks connected to E. sakazakii*

During production	At home	In hospital/nurseries
Monitor raw materials, specifically ingredients which do not require further thermal treatment before mixing	Use clean and disinfected containers	Follow practices of good hygiene in preparation areas
Reduce level of *Enterobacteriaceae* in areas used for production in order to prevent subsequent contamination	Prepare only food enough for the meal avoiding the preparation of following meals; if necessary limit the number of meals prepared in advance to 1-2	Produce guidelines related to preparation, handling, preservation and control procedures for the product, and make them available to trained personnel
Increase frequency of inspections on food production environments and on the end product	Avoid leaving unused reconstituted milk at room temperature	Have access to a room reserved for preparation, which has an area for stocking the product and to which only authorized personnel have access
Identify the sources of possible contamination and take corrective measures	Store in a refrigerator the reconstituted product	If a dedicated room is not available, select an area reserved for this purpose
Revise the instructions for milk preparation suggesting a higher water temperature, but not higher than 80 °C, for solubility (> 70 °C). A temperature too high would damage the nutritional characteristics of the product	Reduce as much as possible the lapse of time between the reconstitution of the formula and its use	Have access to utensils and equipment manufactured in order to be easily sanitized
		Sterilize all utensils used for preparation with thermal treatment (*e.g.*, washing in dishwashers) or with autoclave
		Whenever possible use disposable utensils
		Employ qualified and specialized personnel (*i.e.*, dieticians)
		Store the reconstituted product in a refrigerator
		Reduce as much as possible the lapse of time between reconstitution of the product and its use
		Avoid leaving reconstituted milk at room temperature if unused
		Appropriately seal all containers with remaining milk, place them in the refrigerator, noting down an expiration date
		Whenever possible, use milk in liquid form
		Enforce appropriate control measures which can assess potential hazards, identify critical control points (CCP), monitor non-conformities and necessary corrective actions, and register results

Nowadays in Italy, as well as in other European countries, new microbiological criteria that can be applied to foods, have been enforced in compliance with EEC Regulation n. 1441/2007 dated December 5, 2007 [32]. In particular, with respect to *E. sakazakii*, there must not be any trace of the microorganism in 10 grams of tested product taken from 30 samples. The products taken into consideration are powdered foods for infants and powdered dietetic foods for under 6 months infants having special medical needs.

An investigation on marketed infant powdered milk samples showed the presence of levels of contamination ranging from 0.36cfu/100g to 66.0cfu/100g [33]. These samples, even if the levels of contamination were low, were nevertheless considered at risk. This is due to the microorganism ability to grow in the reconstituted product after preparation, during handling before use, and during preservation if at room temperature. Such data are supported by the opinion recently expressed by EFSA (2004 meeting) on microbiological risks regarding foods for newborns and infants. Experts reported that *E. sakazakii*, in the same way as *Salmonella*, is the microorganism raising the highest concern in respect to the above mentioned foods. It has been pointed out that the presence of this pathogen represents a serious risk if conditions after reconstitution allow its replication [4].

It should be noted that the reduction in the frequency of *E. sakazakii* contamination of infant formula powder might reduce four to five times the risk of infection, while minimising the lapse of time between preparation and consumption might reduce thirty times the risk [4].

Since *E. sakazakii* does not withstand the temperatures at which milk is pasteurized, but is easily found in the environment, post-pasteurization, as well as preparation and handling before consumption, represent critical points. That is why these are the steps during which a preventive action is needed to eliminate or avoid risks of contamination and replication of the microorganism.

It is worth pointing out that breastfeeding should always be supported and encouraged since a mother's milk constitutes the preferred food for newborn infants especially in their early months. When this is not possible, a mother should be well informed and trained on the importance of hygiene while handling, preparing and storing powdered milk.

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