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Occurrence of *Enterobacter sakazakii* (*Cronobacter*) and Other Enterobacteriaceae in Commercial Powdered Infant Formula in Abidjan, Ivory Coast

Kouadio Yao[1,2], Nanga Yessé Zinzendorf[2,3*], Guichard Bohoua[1], Kra Athanase Kouassi[1], Amian Koua[4], Luc Kouakou Kouadio[4,5], Patrice Kouamé[1], Guillaume Yao Loukou[2,3]

[1]Department of Sciences and Food Technology, University of Abobo-Adjamé, Abidjan, Côte d'Ivoire; [2]Department of Microbiology, National Laboratory of Public Health, Abidjan, Côte d'Ivoire; [3]Department of Bacteriology and Virology, University of Cocody, Abidjan, Côte d'Ivoire; [4]Deprtment of Hydrology and Public Health, University of Cocody, Abidjan, Côte d'Ivoire; [5]Laboratory of Biology, National Institut of Public Health, Abidjan, Côte d'Ivoire.
Email: [*]nangatchocht@yahoo.fr

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ABSTRACT

To determine the occurrence of *Enterobacter sakazakii* and other Enerobateriaceae in commercial powdered infant formula (PIF), 185 packages of PIF from different manufacturers, supermarkets and drug-stores in Abidjan were analyzed. Ten g of sample was homogenized in 90 ml of buffered peptone water (PBW, Biorad, Paris) for further studies. Enterobacteriaceae (coliforms) were enumerated according to French Association of Standardization methods. *E. sakazakii* was detected according to Kandhai's method. Bacteria were identified using API20 system. Thirty-eight samples (20.5%) were positive for Enterobacteriaceae. Twenty-four samples (13%) yielded *Enterobacter sakazakii*. Other Enterobacteriaceae isolated included *Pantoea spp*. 21 (11.5%), *Klebsiella pneumoniae subsp. pneumoniae* 8 (4.3%), *Citrobacter diversus* 1 (0.5%), *Citrobacter freundii* 1 (0.5%), *Enterobacter cloacae* 1 (0.5%), *Salmonella reading* 1 (0.5%), *Serratia ficara* 1 (0.5%) *Serratia odorifera* 1 (0.5%). This study is the first report to describe the contamination of PIF from Abidjan with *E. sakazakii* and several other Enterobacteriaceae that could be opportunistic pathogens. Therefore, well-controlled studies need to be conducted to assess the extent of risk associated with contaminated PIF for infants in Abidjan.

Keywords: Enterobacteriaceae; *Enterobacter sakazakii*; Powedered Infant Formulat

1. Background

Powdered Infant formula (PIF) is defined as powdered form specially manufactured and presented to be used by infants, either as beastmilk substitute after preparation with water or to modify prepared breastmilk substitutes or fortify human milk. PIF has been used to feed millions of infants for years, and it constitutes the majority of infant formula worldwidly used. This product is formulated to mimic the nutritional profile of human breast milk [1]. As PIF is not a sterile product, it is an excellent medium to support bacterial growth. Bovine milk is an essential ingredient of PIF and a potential source of bacteria that are pathogenic to humans. On occasion, bacterial pathogens have been cultured from PIF, including *Citrobacter*, *Enterobacter*, *Klebsiella*, *Staphylococcus*, *Streptococcus*, and *Yersinia* species. Should *E. sakazakii* multiply in PIF, it can result in infection [2]. A definitive link between the presence of *E. sakazakii* in an unopened can of PIF and an outbreak of infection has been reported [3]. Cases of invasive *E. sakazakii* infection have recently been added to the list of notifiable diseases in New Zealand, after the death of an infant due to *E. sakazakii* meningitis in July 2004 [4]. These actions highlight the importance of this opportunistic pathogen and the risk exposed to vulnerable infants.

The organism is ubiquitous in food products, being found in milk powder, rice, vegetables, cheese, sausage meat, teas, and various spices [5,6]. Moreover, *E. sakazakii* has been identified in the hospital environment in association with infant bottle brushes and food preparation equipment such as blenders [7]. However, most of the attention to *E. sakazakii*-related contamination of food products has focused on powdered infant formula.

According World Health Organization (OMS) and Food and Agricultural Organization (FAO), between 50 and 80% of PIF cases are the vehicle and the source of *E.*

[*]Corresponding author.

sakazakii-illness in the world [8]. In 2002, the US Food and Drug Administration (FDA) published a warning regarding the presence of *E. sakazakii* in baby formula [9]. In addition, there have been several recalls of formula in Asia, Europe, and North and South America as a result of *E. sakazakii* contamination [5,10,11]. This has resulted in increased efforts to implement appropriate strategies to reduce health risks associated with the use of PIF. In contrast, there is dearth information on contamination of PIF sold in Africa, particularly in Côte d'Ivoire. The aims of this study is to determine the occurrence of *E. sakazakii* and other Enterobacteriaceae in commercial PIF in Abidjan.

2. Material and Methods

2.1. Powered Infant formula (PIF) and Preparation of Inoculum

The PIF were purchased from supermarkets, drug-stores and food industries in Abidjan. Ten grammas of sample were homogenized in 90 ml of buffered peptone water (PBW, Biorad, Paris) for further studies. Each assay was repeated twice.

2.2. Enterobacteriaceae (Coliforms) Enumeration

Coliforms were enumerated according to AFNOR methods (NF V 08 015). Decimal dilutions (1 ml) of the food homogenate in BPW were used to inoculate Violet Red Bile Lactose agar (VRBLA, Biorad, Paris) using incurporation method. The plates were incubated at 30˚C for 24 h. On VRBLA coliforms form small red colonies (~0.5 mm diameter), with or without a red precipitate.

2.3. Identification of Enterobacteriaceae

Non-yellow colony types were identified using API20E (bioMérieux) to determine which of the Enterobacteriaceae were present in the sample after enrichment.

2.4. *E. sakazakii* Isolation and Identification

E. sakazakii isolation method was used according to Kandhai *et al*. [12]. Briefly, full homogenate in BPW was incubated for 20 h at 37˚C. One loofull was streaked on VRBGA (Biorad, Paris) from The overnight BPW broth and colony morphology observed after incubation at 37˚C for 24 h. Five red colonies were picked off and streaked on Trytone Soy Agar (TSA), (Biorad, Paris). Colonies that produced yellow pigment after incubation at 25˚C for 48 - 72 h were termed presumptive *E. sakazakii.* Non-yellow colony types were also picked. Presumptive colonies of *E. sakazakii* were tested for oxidase, using Oxidase DrySlide (Biorad, Paris). The oxidase-negative isolates were preliminary identified by α-glucosidase activity as described by Kandhai *et al.* [13]. α-glucosidase positive isolates were further identified using API 20 E test system (BioMérieux, SA, Marcy Etoile, France) and the corresponding identification software (ATB Expression version 6.0) using *E. sakazakii* ATCC 29544 as type strain.

2.5. Statistical Analyse

Descriptive statistical analyse was performed using Microsoft Office Excell program. The percentages were calculated on the basis of the total number of PIF analysed.

3. Results

A total of 185 PIF were analysed. Thirty-eight samples (20.5%) were positive for Enterobacteriaceae. The distribution of Enterobacteriaceae detected was seen in **Table 1**. Twenty-four samples (13%) yielded *E. sakazakii*. Other Enterobacteriaceae isolated included *Pantoea spp*. 21 (11.5%), *Klebsiella pneumoniae subsp. pneumoniae* 8 (4.3%), *Citrobacter diversus* 1 (0.5%), *Citrobacter freundii* 1 (0.5%), *Enterobacter cloacae* 1 (0.5%), *Salmonella reading* 1 (0.5%), *Serratia ficara* 1 (0.5%) and *Serratia odorifera* 1 (0.5%). Within Enterobacteriaceae, coliforms were used as indicator of hygienic control in factory production lines. Microbiological specifications for coliform counts in PIF is <3 Colony Forming Unit/ gramma (CFU/g) of the current Codex code [5]. A total

Table 1. Distribution of powdered infant formula according to Enterobacteriaceae detected.

	Powdered infant formula (N = 185)	
	Number (N)	Frequency (%)
Enterobacteriaceae detected		
Presence	38	20.5
Absence	147	79.5
Enterobacteriaceae species		
Enterobacter sakazakii	24	13
Pantoea spp.	21	11.5
Klebsiella pneumoniae	8	4.3
Citrobacter diversus	1	0.5
Citrobacter freundii	1	0.5
Enterobacter cloacae	1	0.5
Salmonella reading	1	0.5
Serratia ficaria	1	0.5
Serratia odorifera	1	0.5

of 180 PIF were standard and 5 were no standard. *E. sakazakii* was detected in standard and no standard PIF respectively in 11.7% (21/180 and 60% (4/5) of cases (**Table 2**).

4. Discussion

To our knowledge, this is the first report to describe the contamination of dehydrated powdered infant formula in Ivory Coast with *E. sakazakii* and several other Enterobacteriaceae that could be opportunistic pathogens. Mutyjens and al showed that entrobacteriaceae were present in 52% of 141 different formulas from 35 countries [5]. In dehydrated powdered infant formula manufactured in Indonesia and Malaysia, Enterobacteriaceae were detected in 47% of the cases [14].

Cronobacter spp. have been repeatedly reported as remarkably resistant to osmotic stress and dryness and moderately thermotolerant as some encapsulated *Cronobacter* spp. were still recoverable from desiccated infant formula after storage for up to 2.5 years [15,16]. The composition of dry foods and infant formula combined with their low *aw* (ca. 0.2) significantly affected the survival of *Cronobacter* spp. in these foods [16-18]. With 13% of cases, *E. sakazakii* is the Enterobacteriacea more detected in food collected in Abidjan. These results are in accordance with those described by Estuningsih *et al.* [14]. In Jordan, Jaradat and al isolated *E. sakazakii* in 1.4% of infant's foods [19].

According to microbial specifications for coliforms [5, 20], a total of 21 (11.7%) standard PIF were contaminated by *E. sakazakii*. The results obtained are agreed with the study of Muytens *et al.* [5]. who analysed 141 PIF from 13 countries and founded 20 positive culture, yet all met the microbiological specification counts in PIF. Such a formula has been linked to outbreaks [20].

Table 2. Distribution of Enterobacteriaceae detected in powdered infant formula according to microbial specifications for coliforms.

Microorganisms detected	Powdered infant formula (N = 185)	
	Number (N)	Frequency (%)
Enterobacteriaceae detected		
Standard	180	97.3
No Standard	5	2.7
***E. sakazakii* detected**		
Standard	21	11.7
No standard	4	60

Microbiological specifications for coliform counts in PIF is <3 Colony Forming Unit/gramma (CFU/g) [5]. Standard: <3 CFU/g; No standard: >3 CFU/g.

Iversen *et al.* 2004 [21] examined clinical and food strains and found that the generation times for *E. sakazakii* in reconstituted formula were 13.7 hours, 1.7 hours and 19 - 21 minutes at 6˚C, 21˚C and 37˚C respectively. Therefore, it is obvious that improper storage of contaminated reconstituted PIF can support rapid growth of *E. sakazakii*. Thus, low levels of contamination of *E. sakazakii* in PIF were considered to be a risk factor given potential for multiplication during the preparation and holding time prior to consumption of reconstituted formula. Now Ivory Coast is an underdeveloped country and characterized by a poor sanitary conditions. Risk reduction can be achieved by information and education about basic hygiene practices in connection with food handling, storage and preparation.

While *E. sakazakii* has caused disease in all the groups on the basis of the age distribution of reported cases, it was deduced that the group at particular risk is infants (*i.e.* children <1 year). Among infants, those who are immunocompromised and neonates (≤28 days) are considered to be a greatest risk, particularly neonates of low birth weight (<2500 g) [22]. Infants of HIV-positive mothers are also of concern, because they may specially require infant formula and they may be more susceptible to infection [23]. Ivory-Coast is characterized by a high proportion of special subpopulation consisting of low-birth weight infants and infants of HIV-infected mothers [24]. In these circumstances the use of PIF may be increasing in Ivory Coast. To minimize the coast, Ivory Coast imports PIF from a few countries: for example from Indonesia and Malaysia where reports have shown that some batches of PIF are contaminated by *E. sakazakii*. For all considerations, well-controlled studies needs to be conducted to assess the extent of risk associated with contaminated PIF for infants in Ivory Coast.

In Ivory Coast, PIF are from very different origins. There are an official circuit and unofficial ones. The unofficial circuits are not controlled by the State administration and there are no data about them. And yet, the results showed were obtained from samples collected in the official distribution facilities. A study that takes into account all the PIF suppliers and a wider sample size is therefore helpful to assess the level of bacterial contaminations in PIF on the national scale. In addition, there are no data on *E. sakazakii* infections among newborn babies in Ivory Coast while the results obtained side with a contamination by PIF consumed in Abidjan. A distinctive emphasis should be put on research about *E. sakazakii* during infections among newborn babies under bottle-feeding. At the same time, a study should be conducted to assess the contamination by *E. sakazakii* in food dressed from PIF.

Cronobacter is often isolated from the environment in

milk powder and PIF manufacturing facilities [25,26]. Pif contamination results from the introduction of the organism to the PIF at some stage during the manufacturing process. It is generally assumed that *Cronobacter* contamination of a product occurs in the processing environment at stages after pasteurization, including drying or packing. The prevalence of *Cronobacter* following the drying stage may be due (in part) to the ability of the organism to resist to drying or osmotic stress [27,28]. In Ivory Coast, commercial PIF were imported or produced locally. A total of 65 PIF were manufactured from Ivory Coast. All the isolates of *E. sakazakii* were detected in food produced locally, giving 36.9% of contaminated PIF. Studies conducted in Ivorian manufactures showed there is a default of hygiene in many food industries. Such reports may explain the prevalence of *Cronobacter* in PIF made in Ivory Coast [29]. The Ivorian infant food industry should be encouraged to reduce the concentration and prevalence of *E. sakazakii* in both the manufacturing environment and PIF. To this end, the infant food industry should consider implementing an effective environmental monitoring programme.

5. Conclusion

The results obtained showed an important level of PIF contaminated by *E. sakazakii*. Investigation and reporting of sources and vehicles, including PIF, of infection by *E. sakazakii* should be encouraged. This could include the establishment of laboratory-based network in Ivory Coast.

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