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Research article

SUBSTANTIATION OF WAYS TO REDUCE CONTAMINATION BY BACTERIA OF THE GENUS CRONOBACTER OF DRY SPECIALIZED PRODUCTS FOR BABY FOOD DURING THEIR PRODUCTION

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Prevention of morbidity in the child population from septic foodborne infections caused by the new bacterial pathogen Enterobacter sakazakii (according to the new classification - Cronobacter spp.) is becoming increasingly relevant due to an expanding contingent of susceptible individuals and the proven ability of low doses of the pathogen to quickly increase a population in dry specialized products for formula feeding after rehydration.

In this regard, it is important to assess the risk of accumulation of thermoresistant coliform enterobacteria, including Cronobacter spp., in residual microflora of such products during their production in order to determine ways to minimize it.

To identify a hazardous factor in specialized infant formula of domestic production, we summarized and analyzed expert data on contamination of 245 samples of infant formula and 182 cereals with the entire spectrum of coliform enterobacteria, which were previously identified as Enterobacter sakazakii (Cronobacter spp.). Cronobacter spp. was detected in 4 samples of instant formula (1.6 %) in amounts ranging from 0.04 to 0.5 CFU/g, which is above the hazardous level (≥ 0.003 CFU/g) for susceptible children. No pathogen was isolated from dry mixtures for cooking and instant porridges produced by dry mixing but the content of heat-resistant Enterobacter spp. was 10 times higher than those produced during the full cycle.

Using a risk process model and assuming the content of coliforms in raw milk at the level of the regulated microbial number, probability of pathogen survival in dry mixtures was assessed under standard parameters of spray drying technology. The calculation results showed that under this scenario of raw material contamination, 0.3–0.5 CFU of heat-resistant E.sakazakii (Cronobacter spp.) can be retained in 1 g of a finished product. This substantiates the necessity to introduce the strongest possible requirements for the microbiological quality of raw milk.

Keywords: *Enterobacter sakazakii (Cronobacter spp.), enterobacteria, food safety, microbial contamination, instant milk powder formulas, infant food products, microbiological risk assessment, risk process model.*

Provision of microbiological safety of specific food products used in infant formula feeding and prevention of their contamination with an emergent bacterial pathogen *Enterobacter sakazakii* (*Cronobacter* spp. according to the latest classification) are top priorities along the whole chain of supplying such products to infants.

Cronobacter spp. is a gen. nov created within *Enterobacteriaceae* family by reclassification of a genetic variant of *Enterobacter sakazakii* into separate *species C. sakazakii*

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ssps. *sakazakii & malonaticus*, *C. Turicensis*, *C. muytjensii*, *C. dublinensis* [1]. They are officially labeled by the WHO as obligate pathogens that can cause infections in neonates.

Cronobacter spp. and, primarily, *C. sakazakii* are able to cause necrotizing enterocolitis (NEC), meningitis, bacteremia and sepsis in infants; the diseases can be registered both as sporadic cases and outbreaks as well. The total number of such diseases is unknown since *Cronobacter*-associated infections are not still subject to registration as independent nosologies in most countries worldwide, Russia included [2]. G.L. Linchevskiy with colleagues point out that over the last 20 years the agent has been identified in 70 % of the patients with NEC [3]. Neonates are at the highest risk, especially low birth weight and premature ones. Incidence associated with *C. sakazakii* accounts for 13 and 25 % of the total infection cases respectively according to K. Abdesselam и F. Pagotto [4]. The case fatality rate reaches 60 % among infected neonates with low birth weight and outcomes of the disease (intellectual disability and hydrocephaly) result in permanent disability.

Epidemiological evidence has been provided for an association between *C. sakazakii*-related incidents and powdered breast milk substitutes but a dose of the infectious agent has not been definitely established yet. Experts believe it to be extremely low (below 1 CFU/g) and the infectious agent is considered to be able to form colonies in the intestines due to their immaturity in neonates, absence of protection provided by breast milk and low diversity of gut microbiota [5].

Cronobacter spp. do not differ from allied coliforms as per their cultural characteristics but they are closer to invasive enterobacter species as regards their certain metabolic properties. *Cronobacter* spp. are more resistant to heat and drying than the latter, can survive low pasteurization, able to increase a population in reconstituted formula very rapidly (over 4 hours) even from single cells and within a wide temperature range between 5.5 and 45 °C. They effectively form biofilms on surfaces of bottles, nipples, syringes, and polymer tubes for enteral feeding [6].

A hygienic regulation has been fixed as a measure to manage health risks in the CU TR 021/2011 On Food Safety[1]. It stipulates absence of *E. sakazakii* in 300 grams of powdered infant formulas. Control over occurrence of this pathogen is to be performed in case of detection of *Enterobacteriaceae*, not belonging to *E. coli* and salmonellas, in rated masses (the two-stage approach).

Group and individual prevention of *Cronobacter*-associated infections puts main emphasis on product consumption as the final stage, namely, on preventing the pathogen from growing in reconstituted powdered formulas or on their re-contamination from the environment. Recommendations have been developed for healthcare workers and parents on how to store and prepare formulas, including duration of safe storage of ready-to-feed formulas for healthy children or prohibition of any delayed use for sick ones [7–9].

These measures can need certain revisal and actualization given the contemporary demographic and economic processes. Thus, a population group susceptible to *Cronobacter* spp. is growing in Russia: since 2000, the number of premature newborns has increased by 1.5 times against 1990 and starting from 2012, in accordance with WHO recommendations, neonates born after 22 weeks of gestation have also been considered premature [10]. Moreover, imported infant formulas used to dominate the market in the past but recently the situation has changed and now domestic production of such products has grown considerably. This requires the strictest

[1] TR TS 021/2011. O bezopasnosti pishchevoi produktsii: Tekhnicheskii reglament Tamozhennogo soyuza (s izmeneniyami na 25 noyabrya 2022 goda), utv. Resheniem Komissii Tamozhennogo soyuza ot 9 dekabrya 2011 goda № 880 [CU TR 021/2011. On Food Safety: Technical Regulations of the Customs Union (with latest alterations made on November 25, 2022), approved by the Decision of the Customs Union Commission on December 9, 2011 No. 880]. *KODEKS: electronic fund for legal and reference documentation.* Available at: https://docs.cntd.ru/document/902320560 (November 03, 2023) (in Russian).

assessment of safety of manufactured powdered infant formulas and their components as regards the analyzed infectious agent. It is also necessary to improve control of products imported from South-East Asia and China where applied production technologies might not be reliable enough as regards contamination with *Cronobacter*.

Given all the aforementioned and to substantiate ways to minimize contamination of powdered infant formulas with the analyzed infectious agent, it is important to investigate risk factors associated with *Cronobacter* spp., which can have certain impacts on effectiveness of managing than at the very first stage, namely, during production.

Materials and methods. We analyzed and generalized data reported in foreign and Russian publications and results of our own sanitary-epidemiological inspections about quantitative characteristics of contamination by thermal resistant enterobacter species, including coliforms and those from *Cronobacter* genus, identified in powdered breast milk substitutes, cereals for infants and components for their production.

We analyzed typical domestic technological instructions on how to produce instant powdered infant formulas to assess impacts of technological factors on levels of microbial contamination in products. On this basis, a schematic risk process model (RPM) was created in conformity with the Methodical Guidelines MR 2.1.10.0067-2012[2] at the production stage.

Results. ***Analysis of prevalence and quantitative characteristics of contamination by thermal resistant enterobacter species, including those from Cronobacter genus, in powdered infant formulas.*** Cronobacter spp. are identified (as a rule, when testing coliform occurrence) in a in a wide variety of cereal-based foods, vegetables, herbs, spices, ready-to-eat foods, and foods from other categories. This pathogen was also found in cultivation environments, such as soils, compost, animal feces, rice and vegetable crops, as well as food processing industries, and domestic environments, thus demonstrating possible contamination routes for them to occur in ready-to-feed formulas [11]. Biological properties and behavior of Cronobacter spp. in different objects have been studied well enough; however, despite this fact, there are only scarce data in literature about their prevalence, quantities and concrete species occurring in baby formulas sold on the consumer market. Table 1 summarizes data on Cronobacter spp. identification in powdered infant formulas and their components in different countries. This includes studies where such data were collected within control of products incriminated during outbreaks of Cronobacter-associated infections.

Data analysis indicates that *Cronobacter* spp. can be found everywhere in instant powdered infant formulas and supplementary feeds for infants, including those used as part of dietetic therapy. In America, frequency of *Cronobacter* spp. identification in powdered formulas (when a sum of positive samples was recalculated per their sum for a specific product) equaled 22.5 % in the USA, 8.6 % in Brazil, and 6.25 % in Chile. In Eurasia (Russia, Jordan, and Egypt), it varied between 2.5 and 17.5 %. In China, powdered formulas were contaminated only slightly (less than 1 %) whereas cereals were contaminated in 13 % of cases. It is worth noting that contamination identified in rice flour and flour made of other cereals was 26.6 and 14 % accordingly and infant formulas and cereals in China are reported to be made of basic components used in manufacturing mass food products instead of some specialized ones. *Cronobacter* spp. contamination was identified in 120 samples of

[2] MR 2.1.10.0067-2012. Otsenka riska zdorov'yu naseleniya pri vozdeistvii faktorov mikrobnoi prirody, soderzhashchikhsya v pishchevykh produktakh. Metodicheskie osnovy, printsipy i kriterii otsenki [Assessment of health risks caused by exposure to microbial factors in food products. Methodical essentials, assessment principles and criteria]: methodical guidelines, approved by the Head of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, the RF Chief Sanitary Inspector on August 10, 2012. Moscow, the Federal Center for Hygiene and Epidemiology of Rospotrebnadzor, 2012, 53 p. (in Russian).

Table 1

Cronobacter spp. identification in powdered infant formulas and their components

Product	Number of samples	Share of contaminated		Association with disease (cases)	Year	Country	Reference
		abs.	%				
1	2	3	4	5	6	7	8
PIFs (younger than 6 months)	14	0	0	Not proven	2022	USA	[2]
PIFs (6–12 months)	8	0	0				
PIFs	80	5	6.25	n.d.	2018–2020	Chile	[12]
Powdered milk	20	7	35	n.d.	2020	Serbia	[13]
PIFs (younger than 12 months)	4050	7	0.17	n.d.	2014–2019	China	[14, 15]
Supplementary cereal-based feeds	8055	1048	13				
Rice flour	410	109	26.6				
Cereal flour	85	12	14				
PIFs	400	70	17.5	n.d.	2017–2018	Egypt	[16]
Manufactured herbal infusions	500	45	9				
PIFs (younger than 6 months)	47	0	0	n.d.	2016–2018	Brazil	[17]
PIFs (6–12 months)	30	0	0				
Dry cereals	75	13	17.3				
PIFs	71	21	29.5	71	1961–2018	USA	[18]
PIFs (for premature/low birth weight neonates)	14	3	21.4	2	2012	Brazil	[19]
PIFs (0–6 months)	15	3	20				
PIFs (6–12 months)	7	6	85.7				
PIFs (0–12 months)	6	0	0				
Fortified powdered milk	5	0	0				
PIFs	40	1	2.5	n.d.	2008	Jordan	[20]
PIFs (for children with malabsorption)	1 lot	n/a	-	3	2001	USA, Tennesy	[21]
Instant PIFs	2	2	100	Not analyzed	2005	RF	[22]
Instant PIFs (0–12 months)	157	2	1.3	Not analyzed	2007	RF	[3]

Note: PIFs are powdered infant formulas; n.d. means no data available.

powdered infant formulas out of total 891 (that is, 13.5 %). The pathogen was detected in powdered milk, a basic component of such formulas, much more frequently, namely in 7 out of 25 cases (28 %).

Obviously, all aforementioned comparisons are formal in their essence, both due to analyzed samples being rather small and use of different analysis techniques in different studies. Also, data on frequency of the pathogen identification were obtained by alternative investigation in all foreign studies and no data are provided on quantity of the pathogen per 1 gram of a product, including cases of

[3] Sheveleva S.A. Analiz mikrobiologicheskogo riska kak osnova dlya sovershenstvovaniya sistemy otsenki bezopasnosti i kontrolya pishchevykh produktov [Analysis of microbiological risk as grounds for improving the system for safety assessment and control of food products]: dissertation … for the Doctor of Medical Sciences degree. Moscow, 2007, 329 p. (in Russian).

outbreaks. When the same approach was used (calculating a sum of 33 positive samples out of 137 samples analyzed during outbreaks (Table 1)), frequency of *Cronobacter* spp. identification on average did not exceed 24 %. Similarly, any association with the pathogen was confirmed only in 24 cases out of 84 incidences with analyzed products (28.6 %). Considering a usually big size of a sample analyzed to identify *Cronobacter* spp. (300 grams of a product), we can state that negative results of the test aimed at the pathogen detection in epidemiologically proven incidents provide another evidence that extremely low content of the pathogen in powdered infant formulas (≤ 0.003 CFU/g) can still pose a serious threat for infants who consume them.

Given that, it seemed important to get a clear idea about frequency of potentially hazardous doses of *Enterobacter sakazakii* (*Cronobacter* spp.) for infants in domestic powdered infant formulas and to substantiate ways to minimize this contamination. To achieve that, we searched for data that provided quantitative characteristics of contamination. However, practically no research works on the subject have been published in the RF since 2008. Given that, we relied on generalizing and analyzing our own retrospective data obtained during sanitary-epidemiological inspections of such products in the process of their registration to be permitted for sale on the consumer market in the RF.

The sample includes results of tests performed on 247 samples of instant powdered infant formulas and 182 instant cereals, both milk and cereal-based ones, that had to be boiled prior to consumption. The tests were aimed at identifying the whole range of coliforms with *Enterobacter sakazakii* (*Cronobacter* spp.) being identified in their structure. For comparison, we also analyzed data on contamianiton by such pathogens in another group of specialized products for infants, namely, fermented milk products for babies.

Data on coliforms in a mass (volume) of these products obtained by an alternative way were transformed into CFU/g. While doing it, we assumed that a sample with no growth identified in 1 gram of it was considered as a clean one, not containing one single intact microbial cell. If coliforms were not identified in 0.1 and 0.01 gram of a product, their number was considered to be above 0 but below 10 and 100 CFU/g accordingly; in 10, 100 grams, above 0 but below 0.1 and 0.01 accordingly. Results were given as a mean value of a sum of the upper and lower boundaries of established ranges[4].

Table 2 provides quantitative characteristics of coliform enterobacter pathogens detected in residual microflora of infant formulas and foods for babies.

Obviously, *Enterobacter* species were the major contaminant identified in all ready-to-feed instant powdered products out of all analyzed coliform enterobacter pathogens. They prevailed over *E. coli, Citrobacter, Klebsiella* spp. and other gram negative bacteria (non-fermenting, Acinetobacter) as regards both frequency and contents. Starting from the 90-th percentile, levels of bacteria belonging to this species, which had previously included *Cronobacter* spp., varied between 0.04 CFU/g in formulas and 5 CFU/g in cereals.

Identification of isolated *Enterobacter spp.* strains revealed that *E. Aerogenes* was the prevailing species among those contaminating instant powdered breast milk substitutes. Its average contents in products equaled 0.11 CFU/g (0.5 CFU/g as per the 95-the percentile). *E. sakazakii* (*C. sakazakii*) was isolated from 4 samples of instant infant formulas in quantities ranging between 0.04 and 0.5 CFU/g (in the 95-th percentile of the sample), that is, above the level established by the WHO as hazardous for susceptible groups among infants.

[4] Sheveleva S.A. Analiz mikrobiologicheskogo riska kak osnova dlya sovershenstvovaniya sistemy otsenki bezopasnosti i kontrolya pishchevykh produktov [Analysis of microbiological risk as grounds for improving the system for safety assessment and control of food products]: dissertation … for the Doctor of Medical Sciences degree. Moscow, 2007, 329 p. (in Russian).

Table 2

Levels of contamination by coliform enterobacter pathogens identified in infant formulas and food products for babies

Species	Number of contaminated samples		CFU/g						
			Range		*M*	*Me*	75%-perc.	90%-perc.	95%-perc.
	abs.	%							
Powdered breast milk substitutes									
Tasteless instant powdered formulas reconstituted under 50 °C and below, *n* = 126									
E.coli	11	8.9	0	5	0.08	0	0	0	0.5
Enterobacter spp.	19	**16.6**	0	5	0.2	0	0	0.05	0.5
Citrobacter spp.	9	7.25	0	0.5	0.02	0	0	0	0.05
Klebsiella spp.	1	0.8	0	0.5	0.004	0	0	0	0
Other gram(-) bacteria	9	7.25	0	5	0.18	0	0	0	0
Instant powdered formulas reconstituted under 70 °C, *n* = 33									
E.coli	2	6.1	0	5	0.3	0	0	0	5
Enterobacter spp.	4	**12.1**	0	0.5	0.06	0	0	0.5	0.5
Citrobacter spp.	3	9.1	0	0.5	0.045	0	0	0	0.5
Klebsiella spp.	2	6	0	0.5	0.03	0	0	0	0.5
Other gram(-) bacteria	2	6	0	0.5	0.045	0	0	0	0.5
Formulas to be boiled, *n* = 88									
E.coli	6	6.8	0	49.5	0.84	0	0	0	5
Enterobacter spp.	50	**56.8**	0	49.5	4.3	5	5	5	5
Citrobacter spp.	7	7.95	0	5	0.4	0	0	0	5
Klebsiella spp.	5	5.7	0	5	0.3	0	0	0	5
Other gram(-) bacteria	27	30.7	0	5	0.57	0	0	0.04	0.04
Dry supplementary feeds									
Instant milk cereals, *n* = 142									
E.coli	2	1.4	0	0.5	0.007	0	0	0	0
Enterobacter spp.	16	**11.3**	0	5	0.49	0	0	0.46	5
Other CA(+) coliforms, total	2	1.4	0	0.13	<0.001	0	0	0	0
Other gram(-) bacteria	7	4.9	0	0.04	0.007	0	0	0	0
Instant cereals, *n* = 40									
E.coli	4	10	0	5	0.387	0	0	0.25	5
Enterobacter spp.	6	**15**	0	5	0.75	0	0	5	5
Other CA(+) coliforms, total	0	0	0	0	0	0	0	0	0
Other gram(-) bacteria	4	10	0	0.08	0.12	0	0	0	0
Liquid fermented milk products, *n* = 234									
E.coli	3	1.3	0	100	0.47	0	0	0	0
CA(+) coliforms, total	0	0	0	0	0	0	0	0	0
Gram(+) microbes (*Enterococcus spp.*, yeast and mold)	22	9.4	0	1610	51.1	0	0	0	0

Note: 0 means not identified in bacterial inoculation.

E. cloacea prevailed in formulas to be boiled prior to consumption. They were isolated in 22 samples and occurred in the 76-th percentile of the series in a quantity equal to 5 CFU/g.

It should be emphasized that tests aimed at detecting all species of enterobacter pathogens in instant infant formulas involved a stage of preliminary non-selective fortification.

Analyzed fermented milk products were only slightly contaminated with coliforms in general; *Enterobacter* spp. were not identified in them at all. This most probably indicates that high active acidity typical for such products due to active fermenting microflora and occurrence of its metabolites create rather unfavorable conditions for the development of these enterobacter pathogens.

Contents of enterobacter pathogens contaminating powdered instant formulas and cereals depended on a technology. For example, contents of *Enterobacter* spp. in formulas reconstituted under 70 °C and cereals made by dry mixing of prepared components were 10 times higher than in formulas reconstituted under 50 °C and below and in milk cereals produced in a full cycle process accordingly.

Given solid evidence of cause-effect relations between low doses of certain *Enterobacter* spp. and *Cronobacter* spp. species and infections in infants and weakened people [23, 24], the obtained results indicate there is a necessity to perform profound examination of technologies facilitating their concentration in instant products and possible ways to reduce it.

Assessing likelihood of E. sakazakii (Cronobacter spp.) survival in a risk process model (RPM). The task was to assess likelihood of *Enterobacter sakazakii* (*Cronobacter* spp.) survival during production of powdered breast milk substitutes manufactured using spray drying, the most common technology in the sphere, as well as a risk of contamination by them in ready products. To do that, we created an element of the stage I in microbiological risk assessment, a risk process model (RPM), in accordance with the MR 2.1.10.0067-2012[5]. Parameters of the spray drying technology (modules) were introduced into the RPM for powdered instant formulas reconstituted in water under temperature below 50 °C. The model also covered data available in literature about basic microbiological processes occurring in different stages in production (including data on how enterobacter pathogens from raw milk behave under heating) since at present no data are available in literature as regards such characteristics of *Cronobacter* spp. [25–27]. The Figure below presents the scheme.

Next, we assumed that raw milk was contaminated only or predominantly with coliforms and predicted contents of their thermal resistant specimen in a ready formula made of milk, which conformed to the safety requirements established by the CU TR 033/2013 On Safety of Milk and Milk Products[6] as per microbiological indicators.

The RPM scheme obviously shows that only those enterobacter pathogens that can survive temperatures ranging between 72 and 80 °C (in milk) and between 78 and 82 °C (in cream) at the stage I (pasteurization) can mostly persist in residual microflora in a ready product given the outlined interchanging thermal processes. Literature data outlined above indicate that *Enterobacter* spp. are the most likely to persist among such microorganisms. Since the entire milk microflora was assumed to be represented by coliforms, then we can believe their initial contents to equal 300,000 CFU/ml (*lg* 5.47) for raw milk used in infant formula production category and 500,000 CFU/ml (*lg* 5.69) for milk or cream sold on the consumer market.

These figures were considered as well when we included critical control points (CCP) and CP (control points) into the RPM. Obviously, pasteurization reduces *Enterobacter* spp. contents by 5 *lg*-orders at the first CCP (pasteurization) [28, 29]. Accordingly, by the moment CP2 is reached (evaporation), 0.47–0.69 *lg* CFU/ml are left of the initial population. In accordance with the RPM, a mean

[5] MR 2.1.10.0067-2012. Otsenka riska zdorov'yu naseleniya pri vozdeistvii faktorov mikrobnoi prirody, soderzhashchikhsya v pishchevykh produktakh. Metodicheskie osnovy, printsipy i kriterii otsenki [Assessment of health risks caused by exposure to microbial factors in food products. Methodical essentials, assessment principles and criteria]: methodical guidelines, approved by the Head of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, the RF Chief Sanitary Inspector on August 10, 2012. Moscow, the Federal Center for Hygiene and Epidemiology of Rospotrebnadzor, 2012, 53 p. (in Russian).

[6] TR TS 033/2013. O bezopasnosti moloka i molochnykh produktov [CU TR 033/2013. On Safety of Milk and Milk Products]: Technical Regulations of the Customs Union (with latest alterations made on September 23, 2022), approved by the Decision of the Customs Union Commission on October 9, 2013 No. 67. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/499050562 (November 05, 2023) (in Russian).

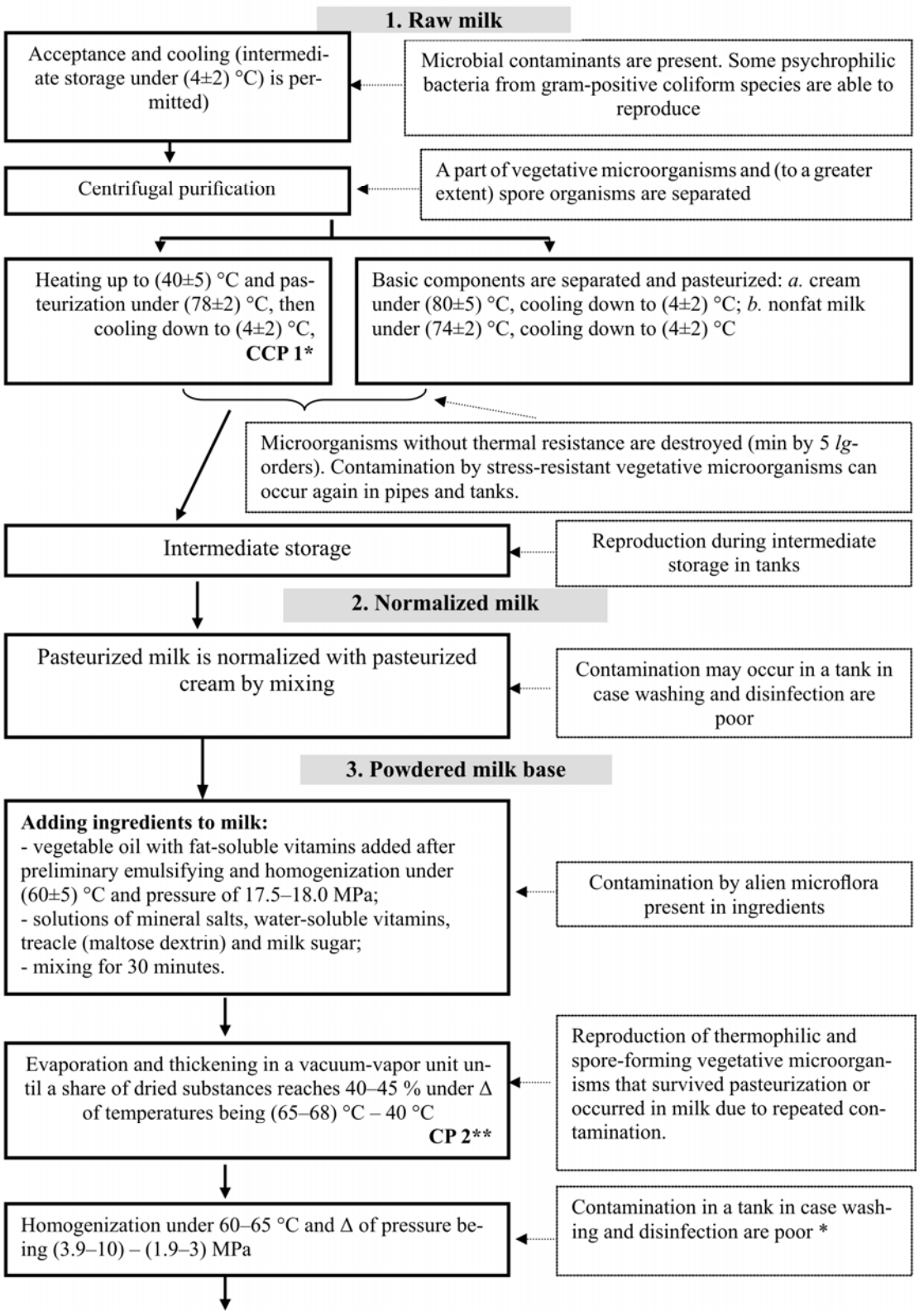
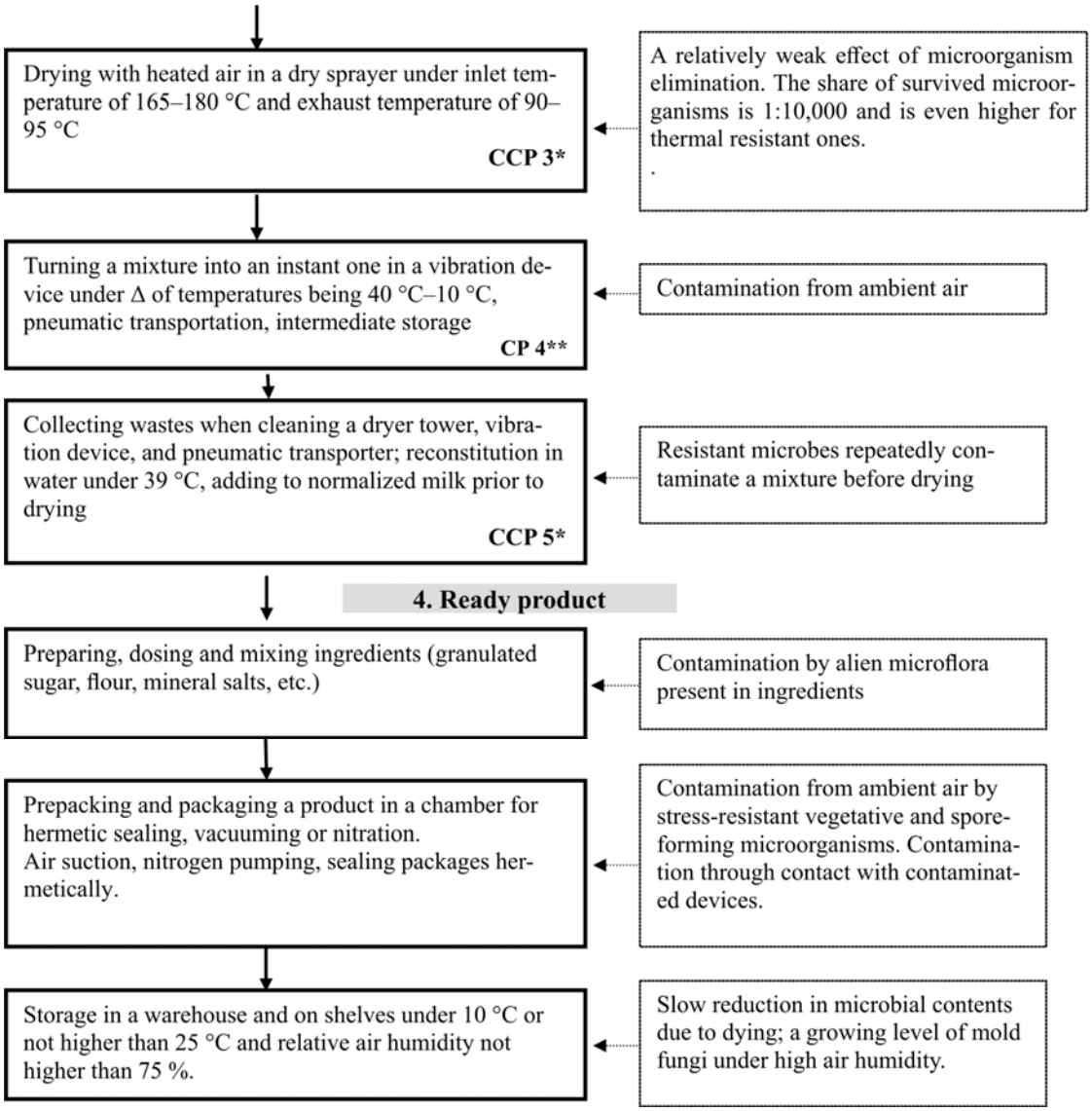


Figure. The risk process model for survival of *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered breast milk substitutes produced by using spray drying technology: *CCP are critical control points at different stages in the technological process where contents of coliforms, enterobacter pathogens included, should be checked; **CP are critical points at different stages in the technological process where contents of coliforms, enterobacter pathogens included, should be checked; CCP 1 is a tank for storing milk after heating; CP 2 is at the exhaust from an evaporator to a tank; CCP 3 is a powder collector; CP 4 is at the exhaust from a vibration device; CCP 5 is at a centrifugal collector



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evaporation temperature is within 52.5–54 °C (53.25 °C). If we again assume that a death rate fixed for *Enterobacter* species in instant formula for 56 °C [30], which is close to 53.25 °C, is correct, then their population reduces by one *lg*-order, that is, down to 0.047–0.069 *lg* CFU/ml at this production stage over 19.1 minutes. If other variables are neglected, then these contents correspond to 0.3–0.5 CFU of *Enterobacter* species, *E. sakazakii* included, in 1 gram of a ready product manufactured under conventional production conditions. Obviously, the calculation results are fully consistent with the actual data on contamination of powders by *Enterobacter* spp. ($M_{cp.}$ = 0.2 CFU/g) since the figures are within one *lg*-order given authentically higher frequency of their occurrence among all other coliform enterobacter pathogens.

Undoubtedly, results of such calculations depend on initial contents of *Enterobacter* species in raw materials. If this estimation and assumptions are correct, then absence of all *Enterobacter*, including *Enterobacter sakazakii*, in 1 gram of a ready product can be ensured under the outlined production conditions only by using milk with coliform contents not exceeding 100,000 CFU/ml (*lg* 5.0). Use of milk with coliform contents ranging between 300,000 and 500,000 CFU/ml does not ensure their absence in powdered instant formulas.

Therefore, use of a simple qualitative risk process model as an element of microbial risk assessment made it possible to objectively identify behaviors of the most thermal resistant enterobacter pathogens *Enterobacter* spp., including pathogenic *Enterobacter sakazakii* (*Cronobacter* spp.) that contaminate milk during production of instant powdered instant formulas and to substantiate a relationship between contamination by them in a ready product and initial levels of microbial contamination in raw milk. Accordingly, manufactures of powdered instant formulas should aim at using raw milk with the lowest possible contents of coliform enterobacter pathogens in it.

Conclusion. Biological properties of a new emergent bacterial pathogen *Enterobacter sakazakii* (*Cronobacter* spp.) include thermal resistance, resistance to drying, and fast growth activation due to rehydration. They have actually become selection factors in technological parameters of production and use of powdered breast milk substitutes and supplementary feeds due to their ability to create risks of severe infections in susceptible infants. Therefore, this study is an attempt to substantiate that, apart from microbiological regulation of the pathogen in such products and development of relevant prevention activities provided for their practical use in healthcare organization and at home, it is also vital to perform investigations aimed at assessing microbiological risks when developing, implementing or improving technologies applied in their production.

In this study, we used some elements of simple qualitative microbiological risk assessment, such as identification of a hazard factor represented by thermal resistant enterobacter pathogens from *Enterobacter* species including *E. sakazakii* (*Cronobacter* spp.); we also analyzed their taxonomic identity, quantitative indicators of their frequency and contents in CFU/g in detail and estimated impacts exerted on these contents by technological parameters by using a risk process model. As a result, we confirmed a priority association between these bacteria and powdered instant formulas and cereals. In addition to that, this methodology made it possible to predict likely accumulation of thermal resistant enterobacter pathogens in residual microflora of ready-to-feed instant powdered infant formulas. This substantiates a recommendation to introduce the strongest possible requirements for the microbiological quality of raw milk into HACCP plans as a prevention measure aimed at reducing microbial contamination.

The results obtained by analyzing available data on microbial contamination of ready products and examination data that are evidence of high frequency of thermal resistant *Enterobacter* spp., *E. sakazakii* (*Cronobacter* spp.) included, in instant serials produced by spray drying indicate the necessity to perform a profound investigation of production technologies and their impacts on the analyzed hazard factor. This is necessary for developing targeted measures aimed at reducing contamination and for achieving more effective control of cereal-based components by introducing relevant laboratory tests in addition to assessment of covering documents.

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