

**Microbiological standards for food: what has changed in 18 years?**

**Padrões microbiológicos para alimentos: o que mudou em 18 anos?**

**Normas microbiológicas para los alimentos: ¿qué ha cambiado en 18 años?**

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**Allana Dayana de Souza Salvador da Silva**

ORCID: <https://orcid.org/0000-0002-1588-7316>

Universidade Federal da Paraíba, Brazil

E-mail: [allana.souza@gmail.com](mailto:allana.souza@gmail.com)

**Juliana Marinho de Oliveira**

ORCID: <https://orcid.org/0000-0002-3646-1373>

Universidade Federal da Paraíba, Brazil

E-mail: [marinhoju43@gmail.com](mailto:marinhoju43@gmail.com)

**Steffany Marielly de Macêdo Gomes**

ORCID: <https://orcid.org/0000-0001-6800-4604>

Universidade Federal da Paraíba, Brazil

E-mail: [mariellymacedo954@gmail.com](mailto:mariellymacedo954@gmail.com)

**Fernando Luiz Nunes de Oliveira**

ORCID: <https://orcid.org/0000-0002-4188-7467>

Universidade Federal da Paraíba, Brazil

E-mail: [fernando.oliveira.ufpb@gmail.com](mailto:fernando.oliveira.ufpb@gmail.com)

**Geíza Alves Azerêdo**

ORCID: <https://orcid.org/0000-0003-3725-690X>

Universidade Federal da Paraíba, Brazil

E-mail: [geiza.azeredo.ufpb@gmail.com](mailto:geiza.azeredo.ufpb@gmail.com)

**Abstract**

Objective: This study aimed to show the main changes in the microbiological standards for food, occurred after the repeal of Resolution - RDC No. 12, of January 2, 2001, by Resolution - RDC No. 331, of December 23, 2019. Methods: A comparative study of RDC was performed to identify the modifications regarding food groups, the number of sample units to be collected, indication of the number of acceptable samples, types of microorganisms, and their tolerance limits. Results: The following main changes were observed: 1) inclusion of

aerobic mesophiles and enterobacteria (hygienic indicators), *Escherichia coli* (fecal microorganism), *Cronobacter* spp. (for infant foods), and microbial toxins and metabolites; 2) increased demand for tolerance limits for some food groups; and 3) the creation of specific categories for certain food groups, such as poultry meat. Conclusion: RDC No. 331, of December 23, 2019, provided more security to consumers, as it is applied to foods ready for consumption, implying greater rigor in their production, especially those easily accessible to the population, with high nutritional content and intended for groups at risks such as infants and newborns.

**Keywords:** Resolution; Quality control; Microbiological standards.

### Resumo

Objetivo: apontar as principais mudanças que ocorreram nos padrões microbiológicos para alimentos, após revogação da Resolução - RDC Nº 12, de 02 de janeiro de 2001 pela Resolução - RDC Nº 331, de 23 de dezembro de 2019. Métodos: Foi realizado um estudo comparativo entre as resoluções para identificar as modificações que ocorreram quanto aos grupos alimentares contemplados, número de unidades amostrais a serem coletadas, indicação do número de amostras aceitáveis, tipos de microrganismos e os seus limites de tolerância permitidos. Resultados: Verificou-se como principais mudanças: 1) a inserção de mesófilos aeróbios e enterobactérias (indicadores higiênicos), de *Escherichia coli* (microrganismo de origem fecal), de *Cronobacter* spp. (para alimentos infantis), e de toxinas e metabólitos microbianos; 2) o aumento da exigência nos limites de tolerância para alguns grupos alimentícios; e 3) a criação de categorias específicas para determinados grupos alimentícios, como a de carne de aves. Conclusão: A Resolução - RDC Nº 331 de 23 de dezembro de 2019 trouxe mais segurança aos consumidores, pois se aplica aos alimentos que já estão prontos ao consumo, implicando em um maior rigor na produção, especialmente daqueles de fácil acesso à população, com alto teor nutritivo e destinados a grupos de risco, como lactentes e recém-nascidos.

**Palavras-chave:** Resolução; Controle de qualidade; Padrões microbiológicos.

### Resumen

Objetivo: señalar los principales cambios ocurridos en los estándares microbiológicos para alimentos, luego de la derogación de la Resolución - RDC No. 12, de 2 de enero de 2001 por Resolución - RDC No. 331, de 23 de diciembre de 2019. Métodos: Se realizó un estudio comparativo entre las resoluciones para identificar las modificaciones ocurridas respecto a los

grupos de alimentos contemplados, número de unidades de muestra a recolectar, indicación del número de muestras aceptables, tipos de microorganismos y sus límites de tolerancia permitidos. Resultados: Los principales cambios fueron: 1) la inserción de mesófilos aeróbicos y enterobacterias (indicadores higiénicos), *Escherichia coli* (microorganismo fecal), *Cronobacter* spp. (para alimentos para lactantes) y toxinas y metabolitos microbianos; 2) la mayor demanda de límites de tolerancia para algunos grupos de alimentos; y 3) la creación de categorías específicas para ciertos grupos de alimentos, como la carne de ave. Conclusión: La Resolución - RDC No. 331 del 23 de diciembre de 2019 trajo más seguridad a los consumidores, ya que se aplica a los alimentos que están listos para el consumo, lo que implica mayor rigor en la producción, especialmente de aquellos que son de fácil acceso para la población, con alto contenido nutricional y destinado a grupos de riesgo, como lactantes y recién nacidos.

**Palabras clave:** Resolución; Control de calidad; Estándares microbiológicos.

## 1. Introduction

Foods are naturally subject to chemical changes, by enzymatic activity, and microbiological changes, by deteriorating and/or pathogenic microorganisms. This is due to certain intrinsic characteristics of most foods, such as high-water activity, adequate pH, and balanced nutritional and chemical composition (Fellows, 2019). Under ideal conditions, enzymatic and microbial activities cause changes in color, taste, odor, texture, and nutritional value.

Nevertheless, microbial growth can also represent a series of risks to consumers, although it is influenced by and related to the infectivity and virulence potential of microorganisms, as well as to the host's pre-existing immune status and diseases (Dubugras; Pérez-Gutiérrez, 2008). These risks involve bacteria, viruses, parasites, spores, toxins, or even chemicals, which can be responsible for causing foodborne diseases.

According to estimates by the World Health Organization (WHO, 2015), foodborne diseases affect 1 in 10 people worldwide, causing about 420 thousand deaths per year, of which 1/3 in children under 5 years old. Also, norovirus, *E. coli*, *Campylobacter*, and non-typhoid *Salmonella* are the main etiologic agents of foodborne diseases.

Because this is a serious public health problem and seeking a way to offer the consumer a safe food, from a microbiological point of view, the legislation of a country should establish microbiological criteria from a sampling plan to indicate the acceptability of

food or batch of foods. In Brazil, these criteria were established by Resolution - RDC No. 12, of January 2, 2001 (Brasil, 2001), which was revoked only in 2019 by Resolution - RDC No. 331, of December 23, 2019 - ANVISA (Brasil, 2019).

Due to this update in the current microbiological standards, we sought to address the main changes, occurred after 18 years, and assess their impacts in the search to ensure the quality of food products.

## 2. Methodological Procedures

This exploratory qualitative study consisted of analyzing RDC No. 12/2001 and Normative Instruction No. 60, of December 23, 2019, which is applied in a complementary way to the Collegiate Board Resolution - RDC No. 331, of December 23, 2019. This RDC provides for microbiological standards for food and their application and will come into force on December 23, 2020. The components of a microbiological standard include the food, the specific point of the chain at which this standard is applicable, the microorganism, the microbiological limits, and the sampling plan.

Therefore, the main changes in Resolution were analyzed regarding the contemplated food groups, the number of sample units to be collected, indication of the number of acceptable samples, types of microorganisms, and their tolerance limits. Then, based on data from the literature, we sought to discuss, critically, the implications of these changes, regarding the acceptability of a food batch or process, protection of consumers, or even as an indication of a risk assessment.

## 3. Results and Discussion

The National Health Surveillance Agency (ANVISA) regulates standards for food microbiological analysis, i.e., it establishes rules for companies, industries, and food businesses, in general, to ensure the safest possible food for consumers. The establishment of RDC No. 331, of December 23, 2019, led to significant changes in these regulations, especially regarding intolerance, even higher to pathogenic microorganisms and substances harmful to consumers.

RDC No. 331 included the following microorganisms, toxins, and metabolites: *Escherichia coli*/g, Enterobacteriaceae/g, molds and yeasts, *Salmonella* Enteritidis /25g, *Salmonella* Typhimurium/25g, aerobic mesophiles/g, *Clostridium perfringens*/g, histamines

(mg/kg), staphylococcal enterotoxins, presumptive *Bacillus cereus*, *Cronobacter* ssp./10g, coagulase-positive staphylococci, *Enterococcus*, *Pseudomonas aeruginosa*, *Clostridium perfringens* spores/50mL, meeting their specificities according to the needs of a food category or group.

RDC No. 12/2001 included most of these bacteria but in a more generalized way. This RDC required the analysis of coliforms at 45°C, whereas according to RDC No. 331/2019, coliforms are also analyzed but through the identification of *E. coli*, one of the four etiological agents responsible for causing foodborne diseases worldwide (WHO, 2015). Therefore, the current RDC No. 331/2019, requires an analysis more focused on and specific to some potential microorganisms.

Coliforms at 45°C indicate the presence of pathogens in the gastrointestinal system of humans and animals, whereas *E. coli*/g, Enterobacteriaceae/g, and *Enterococcus*, although belonging to the coliform group, when analyzed individually, as required in RDC No. 331/2019, serve as a parameter to indicate sanitary control at a more specific stage of the process. The presence of Enterobacteriaceae and *Enterococcus*, e.g., indicates contamination after pasteurization and *E. coli* is an indicator of fecal contamination in processed foods. The most significant changes between these resolutions are shown in Tables and discussed throughout the text.

In the case of food groups, more specifically fruits and derivatives, the number of samples to be analyzed increased, and the number of acceptable samples and the maximum allowed value decreased (Table 1). This greater rigidity may be due to the various sources of contamination during agricultural practices, derived from fertilizer, soil, and/or irrigation water, which can carry microorganisms such as *E. coli*/g and *Salmonella*, as well as molds and yeasts, which are indicators of food deterioration.

**Table 1.** Comparison between RDC No. 12/2001 and RDC No. 331/2019, regarding the microbiological standards for fruits and derivatives.

Food group	Microorganisms		Number of samples to be analyzed and contaminated samples				Maximum allowed value	
			RDC No. 12/2001		NI No. 60/2019		RDC No. 12/2001	NI No. 60/2019
	n*	c**	n	c				
1. FRUITS AND DERIVATIVES	RDC No. 12/2001	NI No. 60/2019						
a) "In natura",	Coliforms	<i>Escherichia coli</i> /g	5	2	5	2	2x10 <sup>3</sup>	10 <sup>3</sup>

whole, selected or not	at 45°C							
	<i>Salmonella</i> sp./25g	<i>Salmonella</i> sp./25g	5	0	5	0	Absent	Absent
b) Prepared (whole, peeled or fractionated), sanitized, chilled or frozen	Coliforms at 45°C	<i>Escherichia coli</i> /g	5	2	5	2	5x10 <sup>2</sup>	10 <sup>2</sup>
	<i>Salmonella</i> sp./25g	<i>Salmonella</i> sp./25g	5	0	10	0	Absent	Absent
c) Bleached or cooked	<i>Salmonella</i> sp./25g	<i>Salmonella</i> sp./25g	5	0	10	0	Absent	Absent
	Coliforms at 45°C	<b>Enterobacteriaceae</b> /g	5	2	5	1		10 <sup>3</sup>
d) Dried, dehydrated or lyophilized	<i>Salmonella</i> sp./25g	<i>Salmonella</i> sp./25g	5	0	10	0	Absent	Absent
	Coliforms at 45°C	<i>Escherichia coli</i> /g	5	2	5	2		10 <sup>2</sup>
		<b>Molds and yeasts</b> /g			5	1		10
e) Pulp and purees	<i>Salmonella</i> sp./25g	<i>Salmonella</i> sp./25g	5	0	10	0	Absent	Absent
	Coliforms at 45°C	<i>Escherichia coli</i> /g	5	2	5	2	10 <sup>2</sup>	10 <sup>2</sup>
	Molds and yeasts/g	Molds and yeasts/g	5	2	5	1	10 <sup>4</sup>	10 <sup>4</sup>
f) Sweets in pastes or pasta and similar products, including jams and sweets in syrup	Molds and yeasts/g	<i>Salmonella</i> sp./25g			10	0		Absent
		<b>Enterobacteriaceae</b> /g			5	1		10 <sup>2</sup>
		Molds and yeasts/g	5	2	5	1	10 <sup>4</sup>	10 <sup>4</sup>
g) Fried or baked, with or without the addition of other ingredients		<i>Salmonella</i> sp./25g			10	0		Absent
		<i>Escherichia coli</i> /g			5	2		20

Source: RDC No. 12, of January 2, 2001, and Normative Instruction No. 60, of December 23, 2019. Bold font indicates changes in the resolutions. \*n = number of sample units to be randomly collected

from the same batch and individually analyzed. \*\*c = the size of the analytical unit and the indication of the number of tolerated sample units with intermediate quality.

RDC No. 12/2001 required the analysis of only four pathogenic microorganisms: *Salmonella* sp./25g, coagulase-positive staphylococci/g, *Clostridium* sulfite reducer at 46°C, and coliforms at 45°C. RDC No. 331/2019 determined the regulation of two more microorganisms or groups: *E. coli*/g and aerobic mesophiles. The latter ones are indicators of food contamination at room temperature, in addition to indicating problems in disinfection stages, temperature control during industrial processes, transport, and storage (Silva *et al.*, 2010).

There was also the creation of a category for poultry meat, which in RDC No. 2001 was classified in the same category of beef, pork, among other meats, for which only the analysis of coliforms at 45°C was required, whereas based on RDC No. 331/2019, poultry meat must be analyzed as for *Salmonella* Enteritidis, *Salmonella* Typhimurium, *E. coli*, and aerobic mesophiles.

The current RDC No. 331/2019 started to demand the analysis of *Salmonella* Typhimurium/25g and *Salmonella* Enteritidis/25g. This is because ANVISA considered *Salmonella* serotypes prevalent in outbreaks, following the safety criteria adopted by the European Community (Regulation EC 2073/2005). Brazilian data reaffirm the worldwide importance of enteric *Salmonella* serotype Enteritidis and enteric *Salmonella* serotype Typhimurium as the two most relevant *Salmonella* serotypes for public health. *Salmonella* Enteritidis affects both humans and animals differently from *Salmonella* Typhimurium; however, both are prevalent in human salmonellosis (Brasil, nd).

According to results presented by EMBRAPA (Brazilian Agricultural Research Corporation) in 2017 (Brasil, 2017), regarding a study of the projection of Brazilian agricultural production for the next decade, the marketing of poultry in Brazil will have the highest growth over 10 years, until 2027.

Poultry is one of the most consumed meats in Brazil and worldwide, due to its high production potential and demand of the population for a healthier and more nutritious diet since it has low-fat content and a good source of proteins, in addition to its low cost. Thus, a greater rigor for this group of food is justified, which also has intrinsic characteristics conducive to the growth of pathogens, such as high-water activity.

Montezani *et al.* (2018), after analyzing the presence of *Salmonella* spp. in 70 samples of frozen and chilled chicken carcasses and cuts, in the municipality of Tupã (São Paulo,

Brazil), found a positive result for the presence of this pathogen. Sousa *et al.* (2020), analyzing the microbiological quality of “*in natura*” and seasoned chicken meat, sold in Santa Helena de Goiás (Brazil), recorded the presence of *Salmonella* spp. in 75% of the samples. Freitas *et al.* (2019) analyzed chicken thighs and drumsticks, sold in bulk, and found contaminated samples considered unfit for consumption because of the presence of *E. coli* (10%) and *Salmonella* spp. (20%). These authors also reported that, because of collective health risks related to the consumption of food contaminated with human disease-causing agents, it is evident the need for good manufacturing, hygiene, and handling practices throughout the entire production chain to reduce final product contamination.

Meat food category or products containing meat in their composition should be highlighted, as RDC No. 331/2019 included another change regarding them – the requirement for the analysis of *Clostridium perfringens*. Analyzing the presence of this bacterium is extremely important for human health since the consumption of food contaminated by *C. perfringens* can cause consequences, ranging from diarrhea to death of the consumer. Some isolates of *C. perfringens* produce an enterotoxin (CPE) responsible for clinical symptoms in cases of food toxoinfection (Otuki, 2010).

Bottled waters, fish, vegetables, roots, tubers, edible fungi and derivatives, and infant food had significant changes regarding their analysis, tolerance limit, and analyzed samples. Among these categories, ‘7. Fish’ (Table 2) and ‘13. Infant Food’ (Table 3) will be addressed below.

In the category ‘7. Fish’, according to RDC No. 12/2001, three microorganisms were analyzed: *Salmonella* spp./25g, coagulase-positive staphylococci/g, and coliforms at 45°C. In RDC No. 331/2019, the coliform group was replaced by *E. coli*, and histamine analysis (mg/Kg) was included only for fish with high histidine content such tuna, mackerel, chub mackerel, “guaraiúba”, crevalle jack, yellowfin tuna, shrimp, crustaceans, among others.



**Table 2.** Comparison between RDC No. 12/2001 and RDC No. 331/2019, regarding microbiological standards for fish.

Food group	Microorganisms		Number of samples to be analyzed and contaminated samples				Maximum allowed value	
	RDC No. 12/2001	NI No. 60/2019	RDC No. 12/2001		NI No. 60/2019		RDC No. 12/2001	NI No. 60/2019
			n*	c**	n*	c**		
a) Fished (fish, crustaceans, mollusks) and “miúdo” (roe, gizzards, swimming bladder) raw, seasoned or not, fresh, chilled or frozen		<b>Histamine (mg/Kg), only for fish with high histidine content (Carangidae, Gempylidae, Istiophoridae, Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae, Scombrosidae)</b>			<b>9</b>	<b>0</b>		<b>The maximum limit of histamines must be 100 mg/kg of muscle tissue, based on a sample composed of nine sample units, and no sample unit can present a result higher than 200 mg/kg.</b>
	<i>Salmonella</i> sp./25g	<i>Salmonella</i> /25g	5	0	5	0	Absent	Absent
	Coagulase positive staphylococci/g	Coagulase positive staphylococci/g	5	2	5	2	10 <sup>3</sup>	10 <sup>3</sup>
		<i>Escherichia coli</i> /g, for non-consumed raw products			5	2		5x10 <sup>2</sup>
		<i>Escherichia coli</i> /g, for products consumed raw			5	2		10 <sup>2</sup>
b) Alive bivalve mollusks and alive echinoderms, tunicates and gastropods, eaten raw	<i>Salmonella</i> sp./25g	<i>Salmonella</i> /25g	5	0	10	0	Absent	Absent
	Coagulase positive staphylococci/g	<i>Escherichia coli</i> /g	5	2	5	1	10 <sup>3</sup>	7
	Coliforms at 45°C/g		5	2			5x10	

Products based on fish ground or chopped meat, seasoned or not, chilled or frozen (hamburgers meatballs, raw breaded, raw sausages)		<b>Histamine (mg/Kg), only for fish with high histidine content (Carangidae, Gempylidae, Istiophoridae, Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae, Scombresosidae)</b>				<b>9</b>	<b>0</b>		<b>The maximum limit of histamines must be 100 mg/kg of muscle tissue, based on a sample composed of nine sample units, and no sample unit can present a result higher than 200 mg/kg.</b>
	<i>Salmonella</i> sp./25g	<i>Salmonella</i> sp./25g	5	0	5	0	Absent	Absent	
	Coagulase positive staphylococci/g	Coagulase positive staphylococci/g	5	2	5	2	10 <sup>3</sup>	10 <sup>3</sup>	
	Coliforms at 45°C/g	<i>Escherichia coli</i> /g	5	2	<b>5</b>	<b>2</b>	10 <sup>3</sup>	<b>5x10<sup>2</sup></b>	
d) Fished (fish, crustaceans, mollusks) and “miúdos” (roe, bladder swimming) salty or dried salty, anchored or in brine		<b>Histamine (mg/Kg), only for fish with high histidine content (Carangidae, Gempylidae, Istiophoridae, Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae, Scombresosidae)</b>				<b>9</b>	<b>0</b>		<b>The maximum limit of histamines must be 100 mg/kg of muscle tissue, based on a sample composed of nine sample units, and no sample unit can present a result higher than 200 mg/kg.</b>
	<i>Salmonella</i> sp./25g	<i>Salmonella</i> /25g	5	0	5	0	Absent	Absent	
	Coliforms at 45°C/g	<i>Escherichia coli</i> /g	5	3	<b>5</b>	<b>2</b>	10 <sup>2</sup>	<b>10<sup>2</sup></b>	
	Coagulase positive staphylococci/g		5	2			10 <sup>2</sup>		

e) Fished semi-elaborated dehydrated, smoked or not, breaded or not, chilled or frozen (nuggets, steaks, fingers)		<b>Histamine (mg/Kg), only for fish with high histidine content (Carangidae, Gempylidae, Istiophoridae, Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae, Scombrosidae)</b>			<b>9</b>	<b>0</b>		<b>The maximum limit of histamines must be 100 mg/kg of muscle tissue, based on a sample composed of nine sample units, and no sample unit can present a result higher than 200 mg/kg.</b>
	<i>Salmonella</i> sp./25g	<i>Salmonella</i> /25g	5	0	5	0	Absent	Absent
	Coagulase positive staphylococci/g	Coagulase positive staphylococci/g	5	2	5	2	5x10 <sup>2</sup>	10 <sup>4</sup>
	Coliforms at 45°C/g	<b><i>Escherichia coli</i>/g</b>	5	2	<b>5</b>	<b>3</b>	10 <sup>2</sup>	<b>5x10<sup>2</sup></b>

Source: RDC No. 12, of January 2, 2001, and Normative Instruction No. 60, of December 23, 2019. Bold font indicates changes in the resolutions. \*n = number of sample units to be randomly collected from the same batch and individually analyzed. \*\*c = the size of the analytical unit and the indication of the number of tolerated sample units with intermediate quality.

Histidine is an amino acid that gives rise to histamine when fish after death is subjected to inappropriate conservation and storage. Under conditions favorable to microbial growth, histidine is converted to histamine, which is responsible for undesirable effects. The symptoms are similar to those of allergic reactions, such as hypotension, flushing, headache, urticaria/pruritus, palpitations/tachycardias, and other gastrointestinal problems. These effects were reported by people who consumed *bonito* fish (*Sarda sarda*) involved in outbreaks of food intoxication. *S. sarda* samples had a concentration of 6,407.9 mg/kg of histamine, whereas current microbiological standards indicate a maximum limit of 200 mg/kg (Takemoto *et al.*, 2019).

Category ‘13. Infant Food’ also deserves attention regarding the changes in resolution (Table 3). This category now includes other groups of microorganisms to be analyzed, especially formulations for infants and newborns, for which only the analysis of coliforms at

45°C was required; after such change, *Salmonella*, *Cronobacter* spp., presumptive *Bacillus cereus*, Enterobacteriaceae, and aerobic mesophiles were included.

**Table 3.** Comparison between RDC No. 12/2001 and RDC No. 331/2019, regarding microbiological standards for infant food.

Food group	Microorganisms		Number of samples to be analyzed and contaminated samples				Maximum allowed value	
	RDC No. 12/2001	NI No. 60/2019	RDC No. 12/2001		NI No. 60/2019		RDC No. 12/2001	NI No. 60/2019
			n*	c**	n*	c**		
a) Powdered infant formulations for infants (up to six months old), infant formulations intended to specific therapeutic diet needs, nutrient formulations presented or indicated for newborns at high-risk, and other foods, especially those formulated for infants		<i>Salmonella</i> /25g	5	0	60	0	20	Absent
		<i>Cronobacter</i> spp./10g			30	0		Absent
		Presumptive <i>Bacillus cereus</i> /g			5	1		5x10 <sup>2</sup>
	Coliforms at 45°C mL	Enterobacteriaceae/g			10	0		Absent
		Aerobic mesophiles/g			5	2		5x10 <sup>3</sup>
b) Powdered infant formulations for infants and early childhood children and other foods, especially those formulated for	<i>Salmonella</i> /25g (mL)	<i>Salmonella</i> /25g	5	0	60	0	Absent	Absent
	<i>B. cereus</i> /g (mL)	Presumptive <i>Bacillus cereus</i> /g	5	1	5	1	10 <sup>2</sup>	5x10 <sup>2</sup>
	Coliforms at 35°C/g (mL)	Enterobacteriaceae /10g	5	2	5	0	10	Absent
	Coliforms at 45°C/g (mL)	Aerobic mesophiles/g	5	0	5	2	-	5x10 <sup>3</sup>

infants and early childhood children	Coagulase positive staphylococci/g (mL)		5	0					
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Source: RDC No. 12, of January 2, 2001, and Normative Instruction No. 60, of December 23, 2019. Bold font indicates changes in the resolutions. \*n = number of sample units to be randomly collected from the same batch and individually analyzed. \*\*c = the size of the analytical unit and the indication of the number of tolerated sample units with intermediate quality.

There was a significant increase in the number of samples used for the analysis of *Salmonella* spp. (Table 3) in powdered formulations for infants and early childhood children. Before such change, only 5 samples were required for analysis; now 60 samples are required. *Salmonella* is a pathogen that, when affecting adults, can cause nausea, stomachache, and other disturbances, but in young children, the presence of this bacterium acts more aggressively, as it can cause diarrhea, meningitis, or even death (Cahill *et al.* , 2008).

These formulations are characterized as a medium of high nutritional value; therefore, they have a high potential for the growth of pathogenic microorganisms. Infant formulations have compounds similar to those of mother’s milk, which assist in feeding the child, acting in the replacement of food, in whole or in part. The target audience of this food category are children who are still developing their immune and metabolic system, and precisely because of this immunological immaturity, the newborn becomes more vulnerable to infections, being even more exposed to possible contamination (De Mello; De Oliveira Rosa, 2017). Strapasson (2019) stated that powdered infant formulations have been related to bacteria that cause necrotizing enteritis, meningitis, meningoencephalitis, septicemia, and even death.

Given the above, the analyses of pathogens provided for in RDC No. 331/2019 are based on the concern to ensure higher food quality, aiming at protecting consumer health. To better group the categories and specify in more detail the microorganisms to be analyzed, some categories were created or included in RDC.

In addition to the changes above-mentioned, some categories were also changed, such as (A) ‘3. Nuts and Seeds’, which in RDC No. 12/2001 was classified in category ‘14. Ready-to-eat solid products’, but in RDC No. 331/2019 became part of a single category, meeting the specifications for nuts, almonds, and edible seeds; (B) ‘4. Other plant products’, for which *Salmonella* sp./25g, coliforms at 45°C, and coagulase-positive staphylococci/g were analyzed, but RDC No. 331/2019 included other two microorganisms (Enterobacteriaceae/g and molds and yeasts); and (C) ‘14. Formulations for enteral nutrition’, which was included in RDC No. 331/2019.

The microbiological standards for the food group “supplements” are shown in Table 4. There were a relevant replacement and inclusion of new microorganisms, such as *E. coli*/g (only for bar supplements), Enterobacteriaceae/g (only for powdered supplements), coagulase-positive staphylococci/g, staphylococcal enterotoxins (ng/g) (only for protein-based products), molds and yeasts/g, and Enterobacteriaceae/g, in addition to applying parameters of non-sterile products, as established in the Brazilian Pharmacopoeia (n = 5; c = 0).

**Table 4.** Comparison between RDC No. 12/2001 and RDC No. 331/2019, regarding the microbiological standards for supplements.

Food group	Microorganisms		Number of samples to be analyzed and contaminated samples				Maximum allowed value	
			RDC No. 12/2001		NI No. 60/2019		RDC No. 12/2001	NI No. 60/2019
	n*	c**	n*	c**				
15. SUPPLEMENTS  a) Powdered and bar supplements	Coliforms at 45°C/g	<b><i>Escherichia coli</i>/g, only for bar supplements</b>	5	2	5	2	10	<b>10<sup>2</sup></b>
	<i>Salmonella</i> /25g	<i>Salmonella</i> /25g	5	0	10	0	-	-
	<i>B. cereus</i> /g	<b>Enterobacteriaceae/g, only for powdered supplements</b>	5	2	5	0	5x10 <sup>2</sup>	-
	Coagulase positive staphylococci/g	Coagulase positive staphylococci/g	5	2	5	1	5x10 <sup>2</sup>	<b>10<sup>2</sup></b>
		<b>Staphylococcal enterotoxins (ng/g), only for protein-based products</b>			5	0		-
		<b>Molds and yeasts/g</b>			5	1		<b>10<sup>4</sup></b>
b) Liquid and gel supplements, not	Coliforms at 45°C/g	<i>Salmonella</i> /25g	5	2	5	0	5	-
		<b>Enterobacteriaceae</b>			5	2		<b>10<sup>2</sup></b>

commercially sterile		/g						
		<b>Molds and yeasts/g</b>			<b>5</b>	<b>2</b>		<b>10<sup>3</sup></b>
c) Supplements in capsules, pills and tablets	Coliforms at 45°C/g	<b>Apply the parameters of non-sterile products, as established in the</b>	5	2			10	
	Coagulase positive staphylococci/g	<b>Brazilian Pharmacopoeia</b>	5	2			5x10 <sup>2</sup>	
	<i>Salmonella</i> /25g	<b>(n=5; c = 0).</b>	5	0			-	

Source: RDC No. 12, of January 2, 2001, and Normative Instruction No. 60, of December 23, 2019. Bold font indicates changes in the resolutions. \*n = number of sample units to be randomly collected from the same batch and individually analyzed. \*\*c = the size of the analytical unit and the indication of the number of tolerated sample units with intermediate quality.

Food supplements are preparations and/or substances intended to complement the human diet, providing micro and macronutrients. Although healthy people use supplements, such as athletes, they are commonly used by sick patients due to their functional characteristic, as mentioned by Silva *et al.* (2020), who assessed the influence of flaxseed oil supplementation in patients on chronic hemodialysis, and by Da Silva *et al.* (2020), when conducting an integrative literature review on the analysis of the supplementation effect of certain antioxidants on adjuvant cancer treatment.

From the concept of “sick”, considering the above-mentioned studies, it is understood that patients are in a weakened state, with low immunity, which justifies the greater rigor in the microbiological standards established in RDC No. 331, of December 23, 2019.

#### 4. Final Considerations

Based on what was presented here, it was clear that RDC No. 331/2019 provides more security to consumers, as it was intended for food ready for consumers, demanding greater rigor in the production of food, especially those easily accessed by the population, with high nutritional content and intended for risk groups, such as infants and newborns.

*Salmonella* Typhimurium/25g, *Salmonella* Enteritidis/25g, aerobic mesophiles, histamine (mg/kg), *Clostridium perfringens*/g, staphylococcal enterotoxins, presumptive *B. Cereus*, *Cronobacter* ssp./10g, coagulase-positive staphylococcus, enterococcus, *Pseudomonas aeruginosa*, and spores of *Clostridium perfringens*/50mL are examples of

pathogenic microorganisms and hygienic indicators, metabolites, and/or toxins, whose analyses were included in RDC No. 331, of December 23, 2019.

The demand for these analyses for fish, poultry, vegetables, supplements, infant food, among other categories, modified/included in the aforementioned Resolution is directly related to people who have increasingly searched for a healthier diet.

Thus, from the analysis performed here, we can conclude that ANVISA has new regulatory microbiological standards for food, with the expansion of regulatory convergence based on the main international guidelines dealing with such issue, correction of previous inconsistencies, and expansion and inclusion of new food categories, in order ensure the population's safe access to food products, until their expiration date.

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**Percentage of contribution of each author in the manuscript**

Allana Dayana de Souza Salvador da Silva - 30%

Juliana Marinho de Oliveira – 25%

Steffany Marielly de Macêdo Gomes – 10%

Fernando Luiz Nunes de Oliveira – 5%

Geíza Alves Azerêdo – 30%