

***Bacillus cereus* as the main casual agent of foodborne outbreaks in Southern Brazil: data from 11 years**

Bacillus cereus como principal agente etiológico em surtos de intoxicação alimentar no Sul do Brasil: dados de 11 anos

Bacillus cereus como el principal agente causante de brotes de enfermedades alimentarias en el Sur de Brasil: datos de 11 años

Silvia Adriana Mayer Lentz ¹
Paula Marques Rivas ²
Marisa Ribeiro de Itapema Cardoso ³
Daiana de Lima Morales ⁴
Fabiana Cassel Centenaro ²
Andreza Francisco Martins ^{1,5}

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Abstract

Foodborne diseases are a global concern. In Brazil, the most prevalent pathogen found in foodborne outbreaks is *Salmonella* sp. (14.4%), followed by *Staphylococcus aureus* (7.7%), *Escherichia coli* (6.5%), and *Bacillus cereus* (3.1%). With the aim to perform a regional detailed analysis of foodborne intoxication, we analyzed 253 outbreaks' profile reports to Food Surveillance team of the General Secretariat of Health Surveillance of Porto Alegre, Rio Grande do Sul State, between 2003 and 2013. In contrast to what was most notified in Brazil, in Porto Alegre the main outbreak agent identified was *Bacillus cereus* (32.2%) and, based on the patient symptoms, most cases were linked to enterotoxin production. The majority of the outbreaks were linked to the ingestion of food containing cereals or sauces poorly kept at environment temperature during the stock or preparation. We believe that, due to the compulsory use of pasteurized eggs in our city, *Salmonella* sp. outbreaks are less important here.

Bacillus cereus; Foodborne Diseases; Enterotoxins; Disease Outbreaks

Correspondence

A. F. Martins
Programa de Pós-graduação em Microbiologia Agrícola e do Ambiente, Universidade Federal do Rio Grande do Sul.
Rua Sarmento Leite 500, sala 210, Porto Alegre, RS
90050-170, Brasil.
andrezafm20@gmail.com

¹ Programa de Pós-graduação em Microbiologia Agrícola e do Ambiente, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil.

² Coordenadoria Geral de Vigilância em Saúde de Porto Alegre, Porto Alegre, Brasil.

³ Faculdade de Medicina Veterinária, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil.

⁴ Laboratório de Pesquisa em Resistência Bacteriana, Hospital das Clínicas de Porto Alegre, Porto Alegre, Brasil.

⁵ Departamento de Microbiologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil.



The occurrence of foodborne diseases has increased significantly worldwide. They are caused by ingesting food or water contaminated with chemical or biological agents, mainly viruses and/or bacteria ¹. According to the Department of Health Surveillance, Brazilian Ministry of Health, between 2000 and 2015, there were 10,666 foodborne diseases outbreaks in Brazil, affecting 209,240 people and with a mortality rate of 0.05%. *Salmonella* sp. was the most prevalent pathogen (14.4%), followed by *Staphylococcus aureus* (7.7%) and *Escherichia coli* (6.5%). *Bacillus cereus* was the fourth causative agent (3.1%). It is noteworthy that in most cases (58.5%), it was not possible to identify the microorganism associated with the outbreak ².

In the United States, most bacterial foodborne illnesses were caused by non-typhoid *Salmonella* spp. (11%) ³. It has caused one million of foodborne illnesses, with 19,000 hospitalizations and 380 deaths ⁴. On the other hand, in Republic of Korea, where rice and grains are widely consumed, the potential risk of emetic syndrome associated to toxin-producing *B. cereus* was reported to be high ⁵. Furthermore, the *B. cereus* was the second most frequent causative agent associated to foodborne outbreaks in France between 2006 and 2014, followed by *S. aureus* ⁶. Its importance in this country is partly due to the participation of national health and food safety authorities in the epidemiological and microbiological investigations of suspected foodborne outbreaks ⁷. *B. cereus* is recognized as an opportunistic pathogen, causing gastrointestinal symptoms associated with the production of cereulide (emetic toxin) or enterotoxin (diarrheal syndrome associate). Therefore, the selective enrichment of emetic strains and the ability to sporulate made these organisms able to enter easily in the food chain; consequently, they are of great concern in recent years ⁸.

Thus, the aim of this study was to evaluate the data from foodborne outbreaks notified in Porto Alegre, Rio Grande do Sul State, Brazil, that were associated to *B. cereus*.

Methodology

Data source

This was a retrospective study, using data obtained in the surveillance system from 2003-2013. A foodborne disease outbreak is defined as an occurrence of two or more cases with similar illness, resulting from ingestion of a common food or beverage ⁹. The notification was done by hospitals, boarding schools, schools, prisons, or the community ¹⁰. Each outbreak have been reported to the Department of Food Surveillance from Porto Alegre, recorded on the Figure 1a (Form 1), and inserted on the Brazilian National Information System for Notifiable Diseases (SINAN). Local health authorities interviewed patients and/or parents to obtain information about the exposure (Figure 1a; Form 2). After analyzing the data, the hypotheses about the etiologic agent and suspected food were elaborated, and the inspection was done with focus on processing controls ¹⁰.

Patients' and suspected foods' samples were collected for analysis by the Regional Reference Laboratory (LACEN/RS), according to the compendium of methods for microbiological examination of foods of the American Public Health Association ¹¹, as part of the outbreak surveillance program investigated in our city. The definition of incriminated food and causative agent is based on the epidemiological-clinical data collected during the investigation. The final report (Figure 1b; Form 5) of the outbreak is inserted in the SINAN ¹⁰.

Epidemiologic investigation

Epidemiological research was conducted in all outbreaks caused by *B. cereus*. Data were collected from research reports (Figure 1a; Forms 1 and 2) and cases were grouped in: exposed (who have eaten the incriminated food) and affected (who had eaten the incriminated food and presented symptoms).

Figure 1 (continued)

1b) Form 5

FORM 5 EPIDEMIOLOGICAL SURVEILLANCE PROGRAM FOR FOODBORNE DISEASES FINAL REPORT ON EPIDEMIOLOGICAL INVESTIGATION OF FOODBORNE DISEASE OUTBREAK										NOTIFICATION NUMBER		
REGISTRATION DATA										REG. NUMBER (CITY)	REG. NUMBER (STATE)	
NAME:										DATE OF START:		
ADDRESS:										DATE OF THE NOTIF.:		
CITY:										DATE OF INVESTIG.:		
SYMPTOMS	Nº	%	EXPOSED	Nº	%	AGE	M	F	Total	%		
NAUSEA			EXPOSED			< 1						
VOMITING			INTERVIEWED			1 to 4						
ABDOM. CRAMP			REAL PATIENTS			5 to 9						
ABDOM. STUFFING			ESTIMATED PATIENTS			10 to 19						
DIARRHEA			HOSPITALIZED			20 to 49						
FEVER			DEATHS			50 and more						
HEADACHE						IGNORED						
						TOTAL						
INCUBATION PERIOD												
MEDIAN:										MINIMUM:		
										MAXIMUM:		
PREPARATION PLACE					CONSUMPTION PLACE							
<input type="checkbox"/> Residence	<input type="checkbox"/> Industry				<input type="checkbox"/> Residence	<input type="checkbox"/> Industry						
<input type="checkbox"/> Trade	<input type="checkbox"/> Hospital				<input type="checkbox"/> Trade	<input type="checkbox"/> Hospital						
<input type="checkbox"/> School, nursery, asylum	<input type="checkbox"/> Industrial kitchen				<input type="checkbox"/> School, nursery, asylum	<input type="checkbox"/> Industrial kitchen						
<input type="checkbox"/> Clubs and associations					<input type="checkbox"/> Clubs and associations							
<input type="checkbox"/> Community hall, church, sport					<input type="checkbox"/> Community hall, church, sport							
<input type="checkbox"/> Others					<input type="checkbox"/> Others							
CAUSE / LOCAL FACTORS:					(P) PREPARATION (C) CONSUMPTION () IGNORED							
<input type="checkbox"/> P	<input type="checkbox"/> C	<input type="checkbox"/> Maintenance in improper cooling			<input type="checkbox"/> P	<input type="checkbox"/> C	<input type="checkbox"/> Contaminated water					
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> Maintenance in inadequate heat			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> Infected handler					
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> Improper cooking			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> Poor cleaning of equipment and utensils					
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> Improper reheating			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> Improper handling					
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> Maintained at ambient temperature for more than 2 hours			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> Raw material without inspection					
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> Cross contamination			<input type="checkbox"/>	<input type="checkbox"/>	Others:					
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> Contamination with toxic chemicals			<input type="checkbox"/>	<input type="checkbox"/>						
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> Use of toxic utensils			<input type="checkbox"/>	<input type="checkbox"/>	Unidentified					
RESULTS OF LABORATORY ANALYSIS												
REAL PATIENTS			TYPE OF EXAM						RESULT			
HANDLERS												
FOODS												
INCRIMINATED FOOD			FOR THE ATTACK RATE(= %)			CONFIRMED BY:			SUSPECTED BY			
						<input type="checkbox"/> Statistical Analysis			<input type="checkbox"/> Anamnesis			
						<input type="checkbox"/> Laboratory Analysis			<input type="checkbox"/> Unidentified			
ETIOLOGICAL AGENT						CONFIRMED BY:			SUSPECTED BY			
						<input type="checkbox"/> Bromatological Analysis			<input type="checkbox"/> Epidemiological Analysis			
						<input type="checkbox"/> Coproculture			<input type="checkbox"/> Unidentified			
						<input type="checkbox"/> Nail swab						
						<input type="checkbox"/> Skin lesion						
ADMINISTRATIVE MEASURES ADOPTED:												
DATE: _____ CITY _____ STATE _____ Epidemiologic Surveillance Department												
SIGNATURE/STAMP: _____												
RECOMMENDATIONS												
DATE: _____ SIGNATURE/STAMP _____ CITY _____ STATE _____ R.H.C. _____												

- The suspected foods were grouped as follow:
- Snack bar: the food was prepared and/or ate at this type of premise, such as pastel, sandwich, cake, fried eggs, and hot dog;
 - Food with sauce: all foods prepared with sauce, such as meat, vegetables, pasta, shrimp, and cream soup;
 - Cereals: all foods prepared with cereals, such as bean, lentil, and rice;
 - Others: any other food associated to an outbreak.

Results

During 11 years, 253 outbreaks were notified, involving 9,592 (68/100,000 inhabitants/year) subjects. Thirty-nine outbreaks were excluded from the study due to the lack of information. Of the 214 outbreaks included, in 118 (55.14%) it was possible to identify the pathogen, as follows: 38 (32.2%) *B. cereus*, 33 (27.97%) *C. perfringens*, 28 (22.89%) *Salmonella* sp., 27 (22.88%) *S. aureus* and 9 (7.62%) *E. coli*. It is noteworthy that in 22 (18.64%) outbreaks, more than one pathogen was identified.

Considering only the outbreaks associated with *B. cereus*, 2,435 (17/100,000 inhabitants/year) people were exposed and 346 (2.4/100,000 inhabitants/year) became ill. The central area of the city recorded the highest number of cases (50%), followed by the northwestern area (21%). The mean time between the onset of symptoms and the notification was 2 days, and between notification and investigation was less than 1 day.

Among the affected, 182 (52.6%) were men, and most were aged between 20-49 years (64.7%). The main symptoms reported by the affected were: diarrhea, cramps, and sickness. The mean incubation time was 12 hours (IQR = 5.75 hours). Three patients (0.87%) were hospitalized, and no deaths were reported. Table 1 summarizes the characteristics of the patient cases. We found that, in 73% of the cases, the suspected food was prepared and eaten in restaurants; moreover, snacks and foods with sauce were the main incriminated foods. The causative factors were: to be exposed to ambient temperature for over two hours (17.6%) or stored in inadequate temperature (16.2%) (Table 2).

Table 1

Characteristics of cases' patients, *Bacillus cereus*. Porto Alegre, Rio Grande do Sul State, Brazil, 2003-2013.

Characteristics	Case patient (N = 346) n (%)
Symptoms	
Diarrhea	225 (65.0)
Cramp	212 (61.0)
Sickness	155 (45.0)
Vomit	82 (24.0)
Headache	29 (8.0)
Bloating	29 (8.0)
Fever	2 (1.0)
Others	2 (1.0)
Incubation in hours (median ± IQR)	12 ± 5.75
Men	182 (52.60)
Age in years (range)	
< 1	0 (0.0)
1-4	25 (7.23)
5-9	22 (6.36)
10-19	36 (10.4)
20-49	224 (64.74)
≥ 50	33 (9.54)
Ignored	6 (1.73)
Total exposed	2,435
Affected	346 (100.0)
Hospitalized	3 (0.86)
Deaths	0 (0.0)

IQR: inter-quartile range.

Table 2Characteristics reported during the outbreaks, *Bacillus cereus*. Porto Alegre, Rio Grande do Sul State, Brazil, 2003-2013.

Factors	Outbreak (N = 38) n (%)
Food preparation site	
Home	3 (7.90)
Restaurant *	28 (73.70)
Hospital	0 (0.00)
Institution **	4 (10.50)
Others	3 (7.90)
Food consumption site	
Home	8 (21.00)
Restaurant *	20 (52.60)
Hospital	0 (0.00)
Institution **	8 (21.00)
Others	2 (5.40)
Suspected foods	
Snack	9 (23.70)
Food with sauce	9 (23.70)
Cereals	5 (13.20)
Others	6 (15.70)
No identified	9 (23.70)
Suspected causality	
Inadequate cooling	7 (10.29)
Inadequate heat	11 (16.18)
Inadequate cooking	2 (2.94)
Room temperature over 2 hours	12 (17.65)
Cross contamination	5 (7.35)
Contaminated water	0 (0.00)
Improper manipulation	8 (11.76)
Unsatisfactory hygiene	3 (4.41)
Others	6 (8.82)
Unidentified	14 (20.59)

* Including snack bar, fast food, self-service;

** Including school, church, club, home-care, headquarters, day care.

Discussion

Porto Alegre is a city with 496,682km² area and 1,409,351 inhabitants. In our study, most of the cases (50%) were reported in the central region of the city. This data is reasoned by the pronounced number of people who work in this region, plus the considerable number of inhabitants (276,799) and, in addition, approximately 20% (7,771) of the total food service establishments are located in the city center. Another argument is the high Human Development Index – HDI (0.805 of 1.0), that indicates a high socio-economic and cultural level of the inhabitants, who can have more meals in restaurants and have a better understanding of the importance of food intoxication reporting ¹².

Several toxins have been described in *B. cereus* outbreaks, and they cause two types of foodborne diseases. The enterotoxins have been linked to the diarrheal form of the disease, while the cereulide has been linked to emetic form ¹³. In the diarrheal syndrome, toxin production occurs after the bacterial growth in the patient guts (8 to 24 hours of incubation), while in the emetic syndrome the cereulide, the toxin is formed and consumed within the food ¹⁴.

In our study, the mean incubation period reported by the affected patients was 12 hours and the most common symptom was diarrhea, followed by abdominal pain. These results assume that most of the outbreaks were associated with diarrheal syndrome. This situation is similar to that found in Hungary, Finland, Bulgaria, and Norway, where the *B. cereus* diarrheal-type intoxication has been reported more frequently than the emetic syndrome ¹¹.

The main foods incriminated in our study were snacks and food with sauce, and the main cause of contamination was its exposition at ambient temperature for more than two hours and storage in an inadequate temperature. Blackburn & McClure ¹⁵ reported that the diversity of countries involved in *B. cereus* outbreaks reflects the different eating habits and diverse niches where it can proliferate. Meals based on cold or cooked rice, pasteurized cream, spaghetti, mashed potatoes, and vegetable sprouts are common in Japan, Great Britain, Finland, and the USA, where emetic intoxication is more prevalent. Soups, milk and dairy products, meat dishes, and spices are usually consumed in Europe and North America, where diarrheal intoxication predominates ¹⁵. In our study, the incriminated foods suggest the influence of eating habits from the central region, where there is a large circulation of people who choose faster meals, such as snacks or self-service restaurants.

As reported in other studies, *B. cereus* could enter the food production chain at different points due to its ability to sporulate ¹⁶. Furthermore, the months of May, November, and December were those with the highest incidence of cases. These months are characterized by heat and humidity, favoring the microbial proliferation.

According to the Food Surveillance team, *Salmonella* sp. is not the most prevalent pathogen in Porto Alegre. A hypothesis for explaining this finding is that in only 55.14% of the cases it was possible to identify the pathogen. The substantial number of cases with unidentified agent can be explained by the difficulty in recovering the food due to a late notification. Another situation that contributed to these results was the implementation of a program to prevent and control the *Salmonella*'s outbreaks by restricting the use of raw eggs in commercial food services. In addition to that, this result can be justified by actions of the Department of Food Surveillance from Porto Alegre, Ministry of Agriculture, South Association of Poultry, and the State Department of Health on the production and trading of poultry and eggs in the Southern state, with focus on inspections, sanitary education, and more control over the raw materials that can carry the *Salmonella* sp. to food.

Finally, our findings show the importance of *B. cereus* in foodborne diseases, and the need for constant monitoring these cases to detect failures in the food preparation process.

Contributors

S. A. M. Lentz, P. M. Rivas and F. C. Centenaro participated in the data collection and article writing. D. L. Morales contributed in the article writing. M. R. I. Cardoso and A. F. Martins participated in the data analysis and article writing.

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Resumo

As doenças de veiculação alimentar representam um problema de ordem global. No Brasil, o patógeno mais prevalente em surtos de intoxicação alimentar é a *Salmonella sp.* (14,4%), seguido pelo *Staphylococcus aureus* (7,7%), *Escherichia coli* (6,5%) e *Bacillus cereus* (3,1%). Com o objetivo de realizar uma análise regional detalhada da intoxicação alimentar, examinamos os relatórios de 253 surtos, notificados à equipe de Vigilância de Alimentos da Coordenadoria Geral de Vigilância em Saúde de Porto Alegre, Rio Grande do Sul, entre 2003 e 2013. Ao contrário do que é notificado no Brasil, em Porto Alegre, identificamos como principal agente etiológico, o *Bacillus cereus* (32,2%) e de acordo com os sintomas dos pacientes, a maioria dos casos esteve associada à produção de enterotoxina. A maioria dos surtos foram associados à ingestão de alimentos que continham cereais ou molhos mantidos à temperatura ambiente durante o armazenamento ou preparação. O uso compulsório de ovos pasteurizados em Porto Alegre pode explicar a relativa escassez de surtos de *Salmonella sp.* no município.

Bacillus cereus; Doenças Transmitidas por Alimentos; Enterotoxinas; Surtos de Doenças

Resumen

Las enfermedades transmitidas a través de la comida son una preocupación de carácter global. En Brasil, el patógeno encontrado de forma más prevalente en los brotes de epidemias alimentarias es la *Salmonella sp.* (14,4%), seguida por el *Staphylococcus aureus* (7,7%), *Escherichia coli* (6,5%), y el *Bacillus cereus* (3,1%). Con el fin de realizar un análisis regional detallado de los brotes de infecciones alimentarias, analizamos el perfil de 253 brotes, registrados por el servicio de Vigilancia Alimentaria de la Secretaría General de Vigilancia en Salud de Porto Alegre, Rio Grande do Sul, entre 2003 y 2013. En contraste con lo que fue más notificado en Brasil, en Porto Alegre el principal agente causante de brotes fue identificado como el *Bacillus cereus* (32,2%) y, basado en los síntomas del paciente, la mayoría de los casos estaban relacionados con la producción de la enterotoxina. Muchos de los brotes estaban relacionados con la ingesta de comida que contenía cereales o salsas mal conservadas a temperatura ambiente durante el almacenaje o la preparación. Creemos que, debido a la utilización obligatoria de huevos pasteurizados en nuestra ciudad, los brotes de *Salmonella sp.* son menos relevantes aquí.

Bacillus cereus; Enfermedades Transmitidas por los Alimentos; Enterotoxinas; Brotes de Enfermedades

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