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Quantitative Assessment of the Risk Associated to Bacillus cereus Group for the Attieke Consumer in Daloa City (Côte d'Ivoire)

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Authors' contributions

This work was carried out in collaboration between all authors. Author KAC designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors KKB, DM and ADC performed the statistical analysis and managed the analyses of the study. Author NMF managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: *Attieke* is a fermented cassava product. It may be contaminated by species of *Bacillus cereus* group, responsible for food poisoning. The objective of this study was to assess the risk associated with *B. cereus* group for the *attieke* consumer in Daloa city (Côte d'Ivoire).

Place and Duration of Study: Department of Formation and Research in chemistry and food sciences, Laboratory of Industrial Process Synthesis and Environment, Felix Houphouet-Boigny National Polytechnic Institute, between April and November 2017.

Methodology: First, an investigation procedure was followed. In this method, 386 persons were interviewed in order to determine the general pattern of *attieke* consumption and potential

symptoms related to its suse. Then, physicochemical and microbiological analyses were carried out on thirty *attieke* samples collected in eight suburbs of Daloa city following standard methods. A probabilistic approach was followed to assess the risk related to *attieke* consumption quantitatively. Finally, the risk of ingesting the infectious dose of 10¹⁰ *B. cereus* cells was estimated by Monte Carlo simulation using both load distribution and consumption one. Data collected were subjected to statistical analyses.

Results: Physicochemical characterisation showed that the pH value, acidity and moisture content of *attieke* were 4.74 \pm 0.76, 49.08 \pm 28.93 meq.g/100 g and 51.46 \pm 1.72%, respectively. These characteristics are within the recommended standards for *attieke*. The enumeration of *B. cereus* group gave loads ranging from 10³ to 10⁷ CFU/g. Besides, all the *attieke* samples were contaminated by these germs. A proportion of 50% of samples had poor quality. It appears that the risk exists and is 0.13% i.e., 130 cases per 100,000 inhabitants.

Conclusion: *Attieke* was contaminated by bacteria belonging to *B. cereus* group. Its consumption represents a risk of food poisoning. This risk is estimated to be 130 cases per 100,000 inhabitants.

Keywords: Bacillus cereus; risk assessment; attieke; food poisoning; Daloa; Côte d'Ivoire.

1. INTRODUCTION

Attieke is a steam cooked fermented product made from cassava (*Manihot esculenta* Crantz) roots. Due in part to its cost considered affordable, it has become one of the most consumed foods in many African countries where rapid urbanisation and economic difficulties have led to an increase in the number of consumers [1,2].

In Côte d'Ivoire, annual consumption of *attieke* is estimated at over 450,000 tons [3,4]. It is a typical Ivorian food. Its production and marketing constitute an essential source of income for the actors of the cassava sector. Also, *attieke* contributes to the energy supply of the populations. In fact, it accounts for about 20.5% of the calories in the diet of many Ivorian people, including those in the Abidjan coastal region, who are recognised as primary producers and consumers [5].

Despite its importance as a staple food, attieke is produced mainly in a traditional way and in often deplorable conditions. Thus, taking into account the nonconformity of some unit operations and the characteristics of the production environment, it can be contaminated by microorganisms with pathogenic potentialities for humans, particularly by the Bacillus cereus group [6]. Also, attieke is sold fresh, ready to eat, in packaging that does not always guarantee microbiological safety. Besides, poor storage conditions and frequent post-cooking operations during marketing can increase the level of contamination to a level of loads exceeding acceptable health limits [7]. The species of *B. cereus* group belong to the genus Bacillus and the family of Bacillaceae. They are slightly large bacilli, Gram⁺ and optional aeroanaerobic, measuring from 1 to 1.8 µm in diameter and 4 to 8 µm long [8].

The development of *B. cereus* in attieke contributes to the rapid deterioration of the product and limits its shelf life [7]. In addition, the consumption of attieke contaminated by the species of this group could represent a risk of poisoning for the consumer. Indeed, many cases of food poisoning in the world have been attributed to *B. cereus stricto sensu* [9,10]. However, in Côte d'Ivoire, no cases of *B. cereus* intoxication have been reported so far, although, *B. cereus* spores have been isolated in the commercial attieke produced in the south of the country [6]. This risk could be assessed by a scientific and modern approach called the risk assessment [11].

The work on the quantitative assessment of the risk associated to *Clostridium perfringens* in the consumption of *attieke* has shown clearly the level of risk [12]. Similarly, that on the microbial risks related to the consumption of braised beef "Choukouya" in Côte d'Ivoire permit characterizing the risk [13]. Therefore, the objective of the present study is to quantitatively assess the risk associated with *B. cereus* group for the consumer of *attieke* produced in the city of Daloa following probabilistic approach.

2. MATERIALS AND METHODS

2.1 Raw Materials

In this study, ready-to-eat *attieke* samples produced in the city of Daloa were used (Fig. 1).

2.2 Study Area

The city of Daloa, in the centre-west of Côte d'Ivoire, was selected for this study (Fig. 2). The choice of this city is justified by the fact that it is an area of high production and consumption of *attieke*. In addition, this city is relatively populated with 212,670 inhabitants [14]. It is the third city of the country in terms of population, surface and density.



Fig. 1. Photograph of ready-to-eat attieke samples conditioned [15]

2.3 Investigation Procedure

To determine the consumption pattern of *attieke* (quantity consumed, mode of consumption and consumer characteristics) an investigation procedure was followed. It allows determining potential symptoms associated with foodborne illness related to consumption of *attieke*. A number of 386 persons were interviewed in Daloa city following questionnaire.

2.4 Sample Collection for Analyses

A number of thirty (30) *attieke* samples were collected in the city. These *attieke* samples were randomly purchased from markets, and other sales zones near the streets in eight suburbs of Daloa city. These suburbs were *Abattoir*, *Garage*, *Huberson*, *Kennedy*, *Labia*, *Lobia*, *Orly* and *Tazibouo* (Table 1). Samples were transported in cool conditions to Laboratory of Industrial Process, Synthesis and Environment (Felix Houphouet-Boigny National Polytechnic Institute) for physicochemical and microbiological analyses.

2.5 Physicochemical Analyses

The following analyses were conducted to characterize the *attieke* samples. Moisture content was determined following standard method [17]. Titrable acidity and pH were evaluated using method described by [18].

2.6 Microbiological Analysis

2.6.1 Preparation of the initial suspension and decimal dilutions

A suspension was prepared by taking 10 g of *attieke* at different points in the sample and then homogenizing for 1 min with 90 mL of peptone water in a Stomacher [19]. A volume of 10 mL of supernatant of this initial suspension was collected in a test tube and used to prepare the decimal dilutions. Successive decimal dilutions up to 10^{-5} were obtained by adding each time 1 mL of the lower dilution in 9 mL of peptone water.

2.6.2 Isolation and enumeration of Bacillus cereus group bacteria

The enumeration of the *B. cereus* group was carried out on a selective culture medium (MOSSEL, AES laboratories) [20]. An amount of 0.1 mL of the initial suspension, as well as each of the successive decimal dilutions up to 10^{-5} was spread on the surface of a Petri dish containing previously Mossel agar. The whole was incubated at 30° C for 24 - 48 hours. The initial suspensions and successive decimal dilutions were seeded in duplicate.

2.6.3 Determination of the microbiological guality of attieke samples

Taking into account the load of bacteria belonging to *B. cereus* group, the microbiological quality of *attieke* was determined through a three-class plan [21]. This plan is so designated because the results of the examinations are interpreted on the basis of three classes of contamination:

- That lower than or equal to criterion m;
- The one between the criterion m and the acceptability threshold M;
- The one above threshold M.

The criterion m is stated to 10^5 CFU/g of *B. cereus* group [8].

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Fig. 2. Location of study area (Daloa city) [16]

 Table 1. Number of samples collected by suburb in Daloa city

Suburbs	Abattoir	Garage	Huberson	Kennedy	Labia	Lobia	Orly	Tazibouo
Number of samples	4	4	4	3	4	3	4	4

2.7 Risk Assessment

The risk assessment of the *Bacillus cereus* group in *attieke* was carried out following the method of Codex Alimentarius Commission [22]. It consisted of 4 steps: hazard identification, hazard characterization, exposure assessment and risk characterization.

2.7.1 Hazard identification

Hazard identification has consisted of a literature review of the risk that may cause adverse health effects and may be present in a particular food group or food item [11]. The pathogen selected for this study was the *Bacillus cereus* group as a producer of thermotolerant spores, toxins and for its ability to grow at low pH. It induced toxiinfections with two main manifestations: diarrheal and emetic one.

2.7.2 Characterization of the hazard

A description of the severity of effects resulting from ingestion of the hazard in food was made at the hazard characterization stage. Then, a load of 10^{10} cells of *B. cereus* is chosen to be the infectious dose [8].

2.7.3 Exposure assessment

2.7.3.1 General outline of the evaluation model

The exposure assessment is based on contaminated food consumption scenarios [23]. Thus, the concentration (C) of *B. cereus* in the *attieke* portion is assumed to be equal to its initial concentration (C0) in the *attieke* samples on the market.

2.7.3.2 Modeling the exposure assessment

The number of ingested germs was obtained by multiplying the distribution of the concentration of *B. cereus* (C) by the portion size distribution (Q) of *attieke* consumed per meal per person. The method used to perform this operation was Monte Carlo simulation. The following relationship gave the number of ingested germs I (expressed in CFU), per person:

 $I = Q \times C$

The use of this relationship involved experimental data for *Bacillus cereus* group concentrations (expressed in CFU/g) in *attieke* samples and portion sizes consumed at each meal per person. The data collected on these two parameters gave their distributions. The uncertainty in the parameters of the evaluation model has been quantified by the bootstrap method [11]. In this study, a number of 5,000 iterations have been realized.

2.7.4 Characterization of the risk

The characterization integrates the results of hazard identification, hazard characterization and exposure assessment to obtain a risk estimate by making a quantitative estimation of the probability and severity of adverse effects likely to occur or produce in the population following consumption of attieke. In the probabilistic risk estimation, method used in this study, the distribution of portion consumed and the distribution of contaminant are involved. Then, by Monte Carlo simulations, the distribution of the ingestion of the contaminant by the population is determined. By comparing the value of the infectious dose of 10^{10} microbial cells [8] with the distribution of the ingestion of the contaminant, the interval of the value of the risk per 100,000 inhabitants is thus determined.

2.8 Statistical Analysis

2.8.1 Descriptive statistics

Descriptive statistical methods (frequency, mean, standard deviation, relative standard deviation) were used for the quantitative variables.

2.8.2 Hierarchical cluster analysis

In this study, the hierarchical cluster analysis used Ward's method as an aggregation one. To estimate the metric distance, the square of Euclidean distance that represents the shortest geographical distance between two points in a multidimensional space was chosen. According to [24], Ward's method gives a good performance when combined with the square of Euclidean distance. For these treatments, STATISTICA 7.1 software was used.

2.8.3 Monte Carlo simulation

In mathematics, a Monte Carlo method is any method of calculating a numerical value using random methods, that is, probabilistic techniques [25]. The MATLAB R 2015b software was used to determine the risk of toxi-infection related to *attieke* consumption. From the distribution of microbial loads and consumption portion sizes one, in application of the mathematical model established in the exposure assessment step, a Monte Carlo simulation results in a cumulative probability curve.

3. RESULTS

3.1 Identification of the Consumption Pattern of *Attieke*

3.1.1 Characteristics of attieke consumers

Table 2 shows the consumer characteristics of Daloa city. *Attieke* is consumed mainly by young people (58.28%) whose age is between 18 and 30 years old, adolescents (20.47%) and adults (15.54%) whose age is located between 30 and 40 years old. Adults over the age of 40 years old were only 4.67%. In this city, *attieke* consumers consisted mainly of men (53.37%) against 46.63% of women. All social groups were also represented with a predominance of informal sector actors (46.63%), pupils and students (42.49%) as well as a minority of civil servants (04.15%), housewives (04.15%) and unemployed (02.58%).

Table 2. Characteristics of attieke consumers in Daloa city

Character	Percentage (%)	
Gender	Mens	53.37
	Women	46.63
	<18 years old	20.47
Age	18-30 years old	58.28
groups	31-40 years old	15.54
	>40 years old	4.67
	Pupils and students	42.49
Social	Actors of informal sector	46.63
classes	Housewives	4.15
	Civil servants	4.15
	Unemployed	2.58

3.1.2 Mode of *attieke* consumption

In the city of Daloa, *attieke* was consumed immediately after purchase (87.82%) (Table 3). However, a proportion of 12.18% of the

consumers kept the *attieke* after purchase. Among these consumers, 78% stored at room temperature, 18% in the refrigerator and 4% in the freezer. However, only half (50%) of them warmed the *attieke* before consumption. They did so either by steam heating (39.29%) or by heating in a kettle (60.71%) (Table 3).

3.1.3 Quantity of attieke consumed per person and per meal

Fig. 3 shows the distribution of the consumed portions of *attieke* in Daloa city. The portions of *attieke* consumed ranged from 95.37 to 798.89 g. The most consumed portion corresponded to 226.16 g with a proportion of 53.37% of consumers. The least consumed portion is 572.26 g.

3.2 Physicochemical and Microbiological Characteristics of *Attieke*

<u>3.2.1 Average of physicochemical</u> <u>characteristics</u>

The average values of the physicochemical characteristics of the *attieke* samples analyzed are presented in Table 4. The analysis revealed that, *attieke* samples had an average pH of 4.74 \pm 0.76, an acidity of 49.08 \pm 28.93 meq.g/100 g and a moisture content of 51.46 \pm 1.72%. In addition, certain parameters such as pH and

titrable acidity have relative standard deviation (RSD) superior to 15%.

3.2.2 Microbiological characteristics of attieke

3.2.2.1 Distribution of B. cereus charges

The charges found in *attieke* samples produced in Daloa city varied from 10^3 to 10^7 CFU/g (Fig. 4). Among the samples, 10% had a load ranging from 1 to 9.99 x 10^3 CFU/g, 6.67% were between 10^4 and 9.99 x 10^4 CFU/g and 33.33% are located between 10^5 and 9.99 x 10^5 CFU/g. Samples with a load ranging from 10^6 to 9.99 x 10^6 CFU/g represented 46.67%. Finally, a small proportion of 3.33% had a load of 1.42 x $10^7 \pm$ 0.16 x 10^7 CFU/g.

Table 3. Mode of attieke consumption

Consumption modes	Percentage (%)
Immediate consumption	87.82
Deferred consumption	12.18
Storage at room temperature	78
Storage in the refrigerator	18
Freezer storage	4
Pre-consumption heat treatments	50
Absence of heat treatments	50
Steam heating	39.29
Reheating in a cooking pot	60.71



Fig. 3. Distribution of *attieke* portions consumed per person and per meal in Daloa city

 pH
 Titrable acidity (meq.g/100 g)
 Moisture (%)

 Means
 4.74 ± 0.76
 49.08 ± 28.93
 51.46 ± 1.72

 Relative standard deviation (%)
 15.20
 58.94
 3.33





Fig. 4. Distribution of B. cereus loads in attieke samples from Daloa city

3.2.2.2 Microbiological quality

The results of microbiological quality according to a three-class plan are shown in Table 5. The number of samples with unsatisfactory quality reached fifteen (15), i.e. 50% of the samples analyzed. Only a proportion of 30% had satisfactory quality and 20% with acceptable one.

3.3 Differentiation between the Attieke Samples from Eight Suburbs of Daloa City

3.3.1 Grouping attieke samples by cluster analysis

The *attieke* samples were subjected to a hierarchical cluster analysis taking into account all the physicochemical and microbiological characteristics studied. The dendrogram obtained is shown in Fig. 5. When the tree is cut at a certain distance, three classes of samples can be distinguished:

- A class containing samples from TAZIBOUO, HUBERSON and ABATTOIR suburbs;
- A second class composed by samples from LOBIA and GARAGE suburbs;

- A third class formed by samples from the suburbs of KENNEDY, LABIA and ORLY.

This typology, which takes into account 93.29% of the differentiations, reveals that differences seem existed between the samples collected.

Table 5. Microbiological quality of *attieke* samples collected in Daloa city

Microbiological quality*	Number of samples (n=30)	Percentage (%)
Satisfactory	9	30
Acceptable	6	20
Unsatisfactory	15	50
Corrupt	0	0

*Microbiological quality on the basis of the load of B. cereus group in attieke analyzed

3.3.2 Correlation between variables studied in the attieke samples

Table 6 presents the correlations between the physicochemical and microbiological parameters of the *attieke* samples analyzed. The correlation matrix of the variables showed significant relationships between some characteristics. Thus, there was a negative and significant

correlation between acidity and pH (Pearson correlation coefficient of -0.87); which means that samples with high acidity are those in which the pH is the lowest. The same is true for humidity and pH (Pearson correlation coefficient equal to 0.37). The most significant correlation exists between pH and acidity.

3.4 Characterization of the Risk Associated to *Bacillus cereus* Group

3.4.1 Potential symptoms associated with foodborne illness related to consumption of attieke

No consumer was ill at the time of the investigation procedure. Nevertheless, previous potential symptoms associated with foodborne

illness related to *attieke* consumption were reported by 26.68% of consumers in Daloa (Fig. 6). The most commonly reported symptoms are nausea and vomiting (58%), abdominal pain (24%) and diarrhea 9%.

3.4.2 Exposure assessment and risk characterization

Fig. 7 presents the results of the Monte Carlo simulation of the microbial load distribution (*B. cereus*) and the consumption one. It represent the distribution of the ingestion of the contaminant (*B. cereus*) by consumers. The risk of diarrheal and / or emetic syndrome related to *attieke* consumption in the city of Daloa is in the order of 0.13% i.e., 130 cases per 100,000 inhabitants (Fig. 7).





Table 6. Correlation between the variables characterizing attieke samples

Variables	рН	Acidity	Moisture	Loads	
рН	1				
Acidity	-0.87	1			
Moisture	-0.37	0.31	1		
Loads	-0.06	-0.09	-0.13	1	

In bold, significant values at 5% probability



Fig. 6. Symptoms encountered by attieke consumers in Daloa city



Fig. 7. Risk of toxi-infection related to *attieke* consumption in Daloa city according to the Monte Carlo simulation *x:* Log₁₀ CFU

F(x): Probability of ingesting infectious dose in the population

4. DISCUSSION

Before characterizing the risk associated to the B. cereus group for the attieke consumer, an investigation procedure was carried out. The aim of this procedure was to identify the general consumption pattern of attieke. Thus, the results revealed that attieke is consumed by all social groups and by almost all age groups in Daloa Similar results have been reported [7]. city. In fact, attieke is an available food and accessible one due certainly to its low cost (compared to other derivatives of cassava) and to its character of ready-to-eat food. High consumption is recorded at the level of pupils and students, and actors in the informal sector. These results can be explained by the fact that attieke is an energy food, ready to consume [1], [18]. Also, it should be noted that informal sector actors have very difficult jobs that require physical strength. The quantity consumed being linked to the intensity of the activity carried out, it is therefore right that these actors are among the people with the highest consumption [26]. Although the most consumed portion is 226 g, they can consume up to 790 g of attieke per meal. In addition, the majority of the people surveyed immediately consume attieke after purchase. A method of preservation by cold (refrigeration or freezing) is adopted a few times.

The physicochemical characteristics of the attieke samples were determined. This characterization revealed that the average pH is acid. Indeed, taking into account the pH, the products are classified as weakly acid (pH> 4.5), acid (4 - 4.5) and very acid (pH <4) foods [27]. In addition, the average pH recorded in this study is close to that of 4.4 ± 0.21 recorded in fresh attieke [28]. The average pH is also within the recommended standard ranged between 4 and 5 [29]. The moisture content was also determined. The results revealed that attieke has a high moisture content. The moisture content recommended in the edible attieke is between 45 and 55% [29]. In the present study, value of moisture content of attieke was within the recommended standard. In addition. the relatively high moisture content does not promote storage at room temperature over a long period. The average humidity recorded in the attieke samples is close to 48.25 ± 0.05% reported in dishes made from cassava [30]. The high moisture would be related to the drying mode of the fermented cassava paste. Indeed, in the manufacturing process of attieke, the drying step reduces the moisture content of the finished product [18].

The study of the correlations between variables that characterized the attieke samples revealed that the most significant correlation exists between the pH and the acidity. The negative correlation clearly indicates that attieke samples, are characterized by their acidity and confirm that attieke is a fermented product. The negative correlation between these parameters has already been reported [31]. In addition, some physicochemical parameters such as titrable acidity and pH have RSD superior to 15%. It suggests that differences seem existed between the attieke samples analyzed. The cluster analysis done on the physicochemical and microbiological parameters of samples collected in eight suburbs confirms this fact. Indeed, three classes can be distinguished between samples from Daloa city. This grouping takes more than 93% of the differentiation.

To characterize the risk associated to the B. cereus group for the attieke consumer, a microbiological characterization was performed. It consisted of a count of germs belonging to the *B. cereus* group in each sample. The distribution of microbial loads showed that all attieke samples are contaminated by this group. The presence of *B. cereus* in attieke samples for sale has been reported [6,7]. The loads recorded in this study are superior to those of 2.4 x 10^2 CFU/g reported in attieke produced in the informal sector in southern of Côte d'Ivoire [7]. Loads of *B. cereus* ranging between 10^2 to 3.2 x 10⁷ CFU/g were reported in some foods in France [32]. Differences observed might due to several factors including the safety of the production environment, the cooking time of the attieke, the various manipulations during the sale. In addition, attieke can undergo initial contamination during conditioning after cooking if the air is polluted [12]. The results of the study of the microbiological quality with regard to these germs, according to a three-class plan indicated that 50% of the samples coming from the city of Daloa, had unsatisfactory quality. The consumption of these samples presents a risk of toxi-infection due to B. cereus load that is greater than 10° CFU/g [8]. Although no consumers were ill during the surveys, potential symptoms associated with foodborne illness related to attieke consumption were reported by 26.68% of the consumers in Daloa. The most frequently mentioned symptoms are nausea and vomiting, abdominal pain and diarrhea. Similar symptoms to those mentioned in this study were reported in a survey of *attieke* consumption [7]. It is therefore important to characterize the risk associated to *B. cereus* group.

From the distribution of microbial loads and that of consumption and, in application of the mathematical model established in the exposure assessment step, a Monte Carlo simulation results in a cumulative probability curve. The distribution of the ingestion of the contaminant by the population is thus determined. The results of the B. cereus risk assessment for the attieke consumer revealed that the risk is 0.13% in the city of Daloa. This leads to 130 cases per 100,000 inhabitants. The risk associated to B. cereus group for the consumer is real. The risk of disease would be accentuated by the phenomenon of cross-contamination. Indeed, many manipulations take place after purchase without cleaning the hands. In France, B. cereus has been implicated in meaningful cases of poisoning from 2006 to 2010 [32]. Several methods can help reducing the risk associated to the B. cereus group. Indeed, members of this group are ubiquitous of soil and environment; so the implementation of good hygienic and manufacturing practices by producers can reduce primary contaminations. These include the sanitation of the production environment, the conditioning of attieke just after cooking and the respect of the cooking time. For consumers, it is necessary to warm the attieke stored before consumption. Indeed, a hydrothermal reheating at 90°C for at least 15 minutes of an amount of attieke ranging from 150 to 450 g could reduce a proportion of 56.1% of the initial B. cereus load [7].

5. CONCLUSION

The present study on the risk assessment associated to B. cereus group for attieke consumer was conducted in Daloa city. It showed that attieke is consumed by all social classes and all age groups, especially pupils, students and actors in the informal sector. Physicochemical analyses of attieke samples gave an average pH of 4.6 ± 0.68; a titratable acidity of 58.21 ± 28.79 meq.g/100 g and an average humidity of 51.83 ± 1.79%. Attieke produced in Daloa had physicochemical characteristics in line with Ivorian standards. Microbiological analyses also demonstrated the presence of B. cereus in all samples at loads ranging from 10³ to 10⁷ CFU/g. Besides, the risk assessment revealed that the risk associated

with the *B. cereus* group for the *attieke* consumer in Daloa exists. Taking into account the infectious dose of 10^{10} cells, the probability of developing a food poisoning due to *B cereus* by consumption of *attieke* portions is 0.13% that is 130 cases per 100,000 inhabitants. These results should attract the attention of the authorities and serve as a guide for public awareness campaigns as well as producers on the reality of foodborne microbiological hazards. Because of the fact that the *B. cereus group* includes several species, further studies are needed to determine the potential contribution of each member. In addition, the study must be extended to other cities of high production and consumption.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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