



Microbiological quality evaluation of ready-to-eat foods at the mass event Rock in Rio 2022, Rio de Janeiro, Brazil

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Abstract

Food-borne diseases are a global concern, exacerbated by unchecked urbanization and population growth. They have a significant impact on public health and economy. Brazil, known for its vibrant mass events, such as the Rock in Rio festival, presents a unique challenge concerning food safety. This study focused on the microbiological assessment of ready-to-eat foods at the 2022 9th Rock in Rio mass event. Over the seven event days, 102 ready-to-eat food samples from the 30 vendors at the festival were collected. The foods were assessed according to the Brazilian Resolution No. 724/2022 and Normative Instruction No. 161/2022. Microbiological analysis revealed ten (9.80%) samples were unsatisfactory and three (2.94%) had intermediate safety. *Escherichia coli*, *Bacillus cereus*, and coagulase-positive *Staphylococcus* were among the predominant microorganisms identified. Although no foodborne disease outbreaks were associated with the Rock in Rio event, the attributed establishments incurred appropriate penalties in line with health regulations. The findings emphasize the risk associated with mass event food service practices. Overall, this study highlights the critical role of Health Surveillance in proactively ensuring public health during mass events and underscores the significance of disseminating these findings for improving future control measures in similar settings.

Keywords: food quality; health surveillance; food-borne diseases; food security; *Escherichia coli*; *Bacillus cereus*.

Practical Application: Microbiological assessment at mass event Rock in Rio 2022 flags food safety risks.

1 INTRODUCTION

The number of nationally reported food-borne diseases (FBD) continues to increase. This is due to population size increase, along with inadequate housing and sanitation (Chen et al., 2022; Chhetri et al., 2021). Furthermore, FBD is a recognized economic problem as its worldwide morbidity and mortality affects the quality of public health and productivity (Bartsch et al., 2018; Chen et al., 2022; Dhaliwal et al., 2021). Consequently, effective control actions by public health agencies need to have a significant impact by reducing the frequency of outbreaks and their related risks (Kirk et al., 2015).

Brazil has a United Health System which covers hygiene measures for restaurants, street cafés, snack parlors, kiosks and street food operators. It also covers the accuracy of labels for health-related products and veterinary residues in foods with animal origin (Silva et al., 2018). Brazilian Health Surveillance Secretariat of the Department of Health developed the National Epidemiological Surveillance System of Foodborne Diseases that aims to provide information to promote health actions and reduce the incidence of FBD in Brazil (Brazil, 2022d).

Mass events are complex activities, for a set period of time, which involve an exceptional concentration or flow of people

(Nunes et al., 2016; Sousa & Aguiar, 2019). Brazil has a long tradition in holding large mass events, such as Carnival, religious events, and folkloric festivities (Vilani & Machado, 2015). These events have direct impacts on lodging, food and beverages, local transportation, tours and attractions and shopping. There are also indirect impacts, such as salaries and suppliers, such as the industry that supplies training, real estate, hospitals, entertainment, and logistics (FGV & Ministério da Cultura, 2018). The Carnival in Rio in 2022 had an economic value of R\$ 4.0 billion (~ \$ 821 millions) (Rio de Janeiro, 2022). In 2014 and 2016, Rio de Janeiro was the host city of the Soccer World Cup and World Olympic Games, respectively. These events are of concern with respect to the role of health surveillance in order to prevent the spread of FBD (Ritter & Tondo, 2014; Teixeira et al., 2018).

Rock in Rio is recognized as one of the biggest music festivals on the planet, having its origin in Rio de Janeiro in 1985. There has been a total of 22 Rock in Rio events, with more than 11.2 million people in attendance; 22 thousand jobs generated in the 2022 festival, and 73 million trees in the Amazon funded by the event. Currently, the event is held biannually in the city of Rio de Janeiro, in early Spring. It is considered the eighth best music festival in the world, attracting many tourists from other states and countries to the city (Rock In Rio, 2024).

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During mass events, a significant commercialization of ready-to-eat (RTE) foods occurs, typically provided through food trucks or temporary sites specifically established for the event. This involves adherence to specific arrangements in compliance with Good Practices for Food Services to ensure the food quality offered at these events (Sousa & Aguiar, 2019). Brazil has implemented Resolution No. 656/2022, which governs the provision of food services during mass events (Brazil, 2022c).

The Municipal Institute of Sanitary Surveillance, Zoonosis Surveillance and Agricultural Inspection of Rio de Janeiro (IVISA-RIO) is part of the Municipal Public Health Laboratory of Rio de Janeiro. It is responsible for monitoring the quality of health-related products sold in the municipality of Rio de Janeiro, through some of its programs, complaints, or concerns after inspections (Rio de Janeiro, 2020). The Institute has been working in all Rock in Rio events, performing inspection and ensuring the monitoring of the quality of food offered to the population participating in the event. In September 2022, the 21st Rock in Rio took place. During the event, the IVISA-RIO inspected all the foodservices and collected food samples for microbiological analysis. The analyses included looking for specific microbial pathogens, as well as hygiene indicators (Brazil, 2022a). The aim of this study was to consider the microbiological safety of RTE food collected by IVISA-RIO which was on sale at the 2022 mass event 9th Rock in Rio.

2 MATERIALS AND METHODS

2.1 Description of 9th Rock in Rio mass event

The 9th Rock in Rio mass event was held in Cidade do Rock, in the municipality of Rio de Janeiro, on the 2nd to 4th, and 8th to 11th of September, 2022. It is estimated that 700 thousand people participated in the event over seven days; with 420 thousand coming from outside the state of Rio de Janeiro. Additionally, there were 10 thousand visitors from 31 different countries, and the event received support from 30 food service retailers.

2.2 Food samples

IVISA-RIO collected 102 samples of RTE foods from all 30 food establishments. These samples were transported in thermal boxes, containing recyclable ice to maintain the refrigeration temperature of $5 \pm 3^\circ\text{C}$. The temperature of the samples was measured at the time of collection and at the time of entry into the laboratory for analysis. On receipt they were categorized according to Brazilian Normative Instruction No. 161/2022 (Brazil, 2022b), that divided the ready-to-eat foods in subcategories, defined based on the composition of each sample to be analyzed according to Table 1.

2.3 Bacterial strains and culture conditions

The reference strains used as controls during the microbiological analysis are listed in the Supplementary Table A1. Stock cultures were prepared and maintained at less than -70°C in Brain Heart Infusion broth (BHI; Merck, Darmstadt, Germany) containing 20% glycerol (Merck, Darmstadt, Germany).

Inocula were prepared by transferring one loopful from the stock tube into 3 mL fresh BHI broth and incubating at $37 \pm 1^\circ\text{C}$ for 24 hours. For daily use, cultures were maintained at $5 \pm 3^\circ\text{C}$ on nutrient agar (Merck, Darmstadt, Germany).

2.4 Microbiological analysis of food samples

Salmonella spp. analyses were performed on 25 g of each sample using the selective enrichment technique according to the standardized methodology described in Food and Drug Administration's Bacteriological Analytical Manual online (Andrews et al., 2023).

Escherichia coli, *Bacillus cereus* and coagulase-positive *Staphylococcus* enumeration were performed using the semi-automated system TEMPO[®] equipment Version B Ref. 80.033 (BIOMERIEUX, Marcy-l'Étoile, France) according to the manufacturer's instructions. Results were expressed as Colony Forming Unit (CFU)/g.

Samples were classified as 'satisfactory', 'satisfactory with intermediate quality' or 'unsatisfactory' according to Brazilian Resolution No. 724/2022 (Brazil, 2022a), showed in Table 1.

3 RESULTS AND DISCUSSION

The categories of the samples collected during the event are shown in Table 2. Due to the dynamic characteristics of the event and the large number of people in attendance, it was impractical to collect multiple ($n = 5$) representative samples as required by the Normative Instruction No. 161/2022 (Brazil, 2022b). Consequently, only single samples were collected from each vendor. This restricted number of samples obtained from each food outlet limited determination of the microbiological range of organisms from each food outlet. However, the results shown in Table 2 still reflect the microbiological quality of RTE foods sold at the event, which was the primary aim of the study.

Of the 102 samples analyzed, ten (9.80 %) were considered unsatisfactory and three (2.94 %) were deemed satisfactory even though they were at borderline intermediate quality. The highest percentage of unsatisfactory samples was in the category 'sweets and desserts' (33.33 %), followed by 'RTE foods prepared using heat' (12.90 %). Unfortunately, no statistical analysis could be carried out on the results because the number of samples was not homogeneous between the categories of samples collected. Therefore, prior planning with a homogeneous collection strategy between the categories is important to allow for a more robust analysis of the results obtained after the mass event.

In total, 327 assays were carried out, of which 93.27% gave satisfactory results, 11 (3.36%) presented satisfactory results with intermediate quality and 11 (3.36%) unsatisfactory results (Table 3). The most frequent microorganism found was *E. coli*, with five (4.8 %) samples being classified as unsatisfactory due to counts above the specification; 2×10 to $5 \times 10^2/\text{g}$ (Table 1). Counts of *B. cereus* and coagulase-positive *Staphylococcus* were in the unsatisfactory range for greater than the specification 3.7 and 3.3% of the samples, respectively (Table 3).

Table 1. Parameters for microbiological control of ready-to-eat foods in accordance with Brazilian Normative Instruction No. 161/2022.

Group 21. Ready-to-eat prepared foods					
Specific categories	Microorganism	n	c	m	M
a) Ready-to-eat foods prepared with the use of heat	<i>Salmonella</i> /25 g	5	0	Abs	-
	<i>Bacillus cereus</i> presumptive/g, only for preparations containing cereals or sauces	5	1	10 ²	5x10 ²
	<i>Clostridium perfringens</i> /g, for meat preparations only	5	1	10 ²	5x10 ²
	Coagulase-positive <i>Staphylococcus</i> /g	5	2	10 ²	10 ³
	<i>Escherichia coli</i> /g	5	2	10	20
b) Ready-to-eat prepared foods containing products of animal origin, prepared without the use of heat, consumed raw.	<i>Salmonella</i> /25 g	5	0	Abs	-
	Coagulase-positive <i>Staphylococcus</i> /g	5	1	10 ²	10 ³
	<i>Escherichia coli</i> /g	5	2	10	10 ²
c) Ready-to-eat prepared foods containing exclusively products of plant origin, prepared without the use of heat	<i>Salmonella</i> /25 g	5	0	Abs	-
	<i>Escherichia coli</i> /g	5	2	10	10 ²
d) Sandwiches	<i>Salmonella</i> /25 g	5	0	Abs	-
	<i>Bacillus cereus</i> presumptive/g	5	1	10 ²	5x10 ²
	<i>Clostridium perfringens</i> /g	5	1	10 ²	5x10 ²
	Coagulase-positive <i>Staphylococcus</i> /g	5	2	10 ²	10 ³
	<i>Escherichia coli</i> /g	5	2	5x10	5x10 ²
e) Sweets and desserts	<i>Salmonella</i> /25 g	5	0	Abs	-
	Coagulase-positive <i>Staphylococcus</i> /g	5	2	10 ²	5x10 ²
	<i>Bacillus cereus</i> presumptive/g	5	2	10 ²	5x10 ²
	<i>Escherichia coli</i> /g	5	2	10	10 ²

n: number of sample units that are to be drawn independently and randomly from the lot; c: maximum allowable number of sample units yielding unacceptable (or defective) test results; m: a microbiological limit which separates good quality from unacceptable (or defective) quality; M: results above are unacceptable (or defective); Abs: absence.

Table 2. Categorization and microbiological quality evaluation of ready-to-eat prepared food samples ($n = 102$) collected during the mass event and analyzed in this study.

Category¹ (No. of samples)	Classification¹ (%)		
	Satisfactory	Satisfactory with intermediate quality	Unsatisfactory
21 a) Ready-to-eat foods prepared with the use of heat (62)	53 (85.48)	1 (0.62)	8 (12.90)
21 b) Ready-to-eat foods containing products of animal origin, prepared without the use of heat, consumed raw (3)	3 (100)	0 (0)	0 (0)
21 c) Ready-to-eat foods containing exclusively products of plant origin, prepared without the use of heat (12)	12 (100)	0 (0)	0 (0)
21 d) Sandwiches (22)	19 (86.36)	2 (9.09)	1 (4.55)
21 e) Sweets and desserts (3)	2 (66.66)	0 (0)	1 (33.33)
Total ($n = 102$)	89 (87.25)	3 (2.94)	10 (9.80)

¹Criteria are shown in Table 1 as per Brazilian Normative Instruction No. 161/2022 (Brazil, 2022b).

Table 3. Results of the microbiological assays performed on the ready-to-eat prepared food samples ($n = 102$) collected during the mass event and analyzed in this study.

Microorganisms (No. of samples)	Classification¹ (%)		
	Satisfactory	Satisfactory with intermediate quality	Unsatisfactory
<i>Salmonella</i> spp. (52)	52 (100)	0 (0)	0 (0)
<i>E. coli</i> (102)	93 (91.2)	4 (3.9)	5 (4.8)
Coagulase-positive <i>Staphylococcus</i> (91)	84 (92.3)	4 (4.4)	3 (3.3)
<i>B. cereus</i> (82)	76 (92.6)	3 (3.7)	3 (3.7)
Total (327)	305 (93.3)	11 (3.4)	11 (3.4)

¹See Table 1 for criteria as according to Brazilian Normative Instruction No. 161/2022 (Brazil, 2022b).

These results are in agreement with Batista et al. (2022), who evaluated the epidemiological profile of FBD notifications in Brazil from 2016 to 2021 and also reported that pathogenic *E. coli* was the most frequently reported organism (7.9%). However, in other countries, the main FBD pathogens identified in outbreaks are different. Chhetri et al. (2021) reported that *Salmonella* species were the most common pathogen causing FBD in Bhutan, accounting for 42.9% of cases. The World Health Organization estimated the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases in 2010. Norovirus was the leading cause of FBD causing 125 million cases, while *Campylobacter* spp. caused 96 million (Kirk et al., 2015). Outbreak surveillance data from the US Centers for Disease Control and Prevention (CDC), from the period of 2009–2018, revealed that norovirus (2,798; 47%), and *Salmonella* (1,191; 20%) were the most confirmed or suspected etiology agents (White et al., 2022).

Among the samples considered unsatisfactory due to the identification of *E. coli*, four had a count between 1.1×10 and 10^2 CFU/g, and one had a count between 1.1×10^2 and 10^3 CFU/g. Regarding coagulase-positive *Staphylococcus*, one sample had a count between 1.1×10^3 and 10^4 CFU/g, and two showed $> 10^4$ CFU/g. All samples classified as unsatisfactory due to the identification of *B. cereus* showed levels between 1.1×10^3 and 10^4 CFU/g (Table 4).

Most unsatisfactory samples were due to the presence of *E. coli* which was above the recommended limit (Tables 1 and 3). Considering the structure of a mass event, possibly the identification of *E. coli* can be due to the lack of hygiene of the food handlers. This may have been due to not washing their hands when necessary on the production line, as well as not using protective equipment (mask, gloves and caps) correctly. The non-recovery of *Salmonella* spp. from the samples (Table 3) may be due to their sensitivity to heat treatments by cooking (Dawoud et al., 2017).

The identification of coagulase-positive *Staphylococcus* is closely related to improper handling of food, as this microorganism is often found on the skin, mucous membranes and upper respiratory tract of humans (Ercoli et al., 2017). In the present study, three samples were considered unsatisfactory (Table 3) and two showed concentrations $> 10^4$ CFU/g (Table 4). Coagulase-positive *Staphylococcus* contamination can also be related to other causal factors such as prolonged exposure to ambient temperature as well as inadequate refrigeration (Bianchi et al., 2022; Ercoli et al., 2017).

B. cereus food poisoning can occur when foods are prepared and kept without refrigeration for several hours, with rice, meat, vegetables, vanilla sauce, creams, soups, and raw sprouts being the most incriminated foods (Tallent et al., 2020). In the present

study, counts of *B. cereus* above the recommended limits were found in three samples (Table 4). In mass events, foods will be exposed to high temperatures if they are not packaged correctly, as they commonly remain at room temperature for long periods, rather than refrigeration temperature. *B. cereus* is known as a mesophilic microorganism, with an optimal temperature for growth between 35–40°C. However, strains have been described as psychotropic, being able to grow at refrigerated temperatures, and cause deterioration of refrigerated foods (Can et al., 2022).

Mixed food, water and eggs and meat products are the foods most frequently involved in FBD outbreaks in Brazil (Draeger et al., 2018). This fact was similar to that observed in the present study, where the highest number of unsatisfactory samples was identified in 'RTE prepared foods prepared using heat' (Table 2). This may be related to causal factors, such as improper handling of foods, prolonged exposure to room temperature and insufficient refrigeration. These factors have a high likelihood of occurring during mass events since they involved temporary structures for food preparation and distribution (Nunes et al., 2016; Ritter & Tondo, 2014).

According to Vidal et al. (2022), among the factors that may lead to deficient manufacturing practices are lack of knowledge about methods for hygienic food production, the cost of their implementation, and inefficient quality control by food producers. These conditions can be evident at a mass event, since temporary structures for food preparation and distribution will be used for just a few days. Improper food handling seems to be the main source of food contamination, and therefore food handlers have a key role in ensuring food safety. Therefore, food handlers should be trained continuously and as commensurate with their duties.

The Brazilian Integrated Manual for Surveillance, Prevention and Control of Foodborne Diseases (Brazil, 2022d) reports that foods prepared several hours before consumption result in an increased risk of infection. The increased risk is related to the use of bacterially-contaminated raw materials, the presence of foodborne pathogens into the preparation environment, allowing the occurrence of cross-contamination such as common worksurfaces for raw materials and RTE foods. These circumstances can easily happen during mass events, especially as the temporary production site differs from that of conventional kitchens. Another pertinent reason mentioned in the Brazilian Integrated Manual is inadequate cleaning utensils and equipment used in food preparation.

4 CONCLUSIONS

In conclusion, considering the risk of FBD spreading during mass events, it is essential to develop specific strategies to prevent the occurrence of outbreaks, as this would avoid serious consequences for public health, especially in events involving

Table 4. Ranging counts (CFU/g) of the samples classified as unsatisfactory, with respect to the identified microorganisms.

Microorganism	Count (Colony-forming units/g)			
	$1.1 \times 10 - 10^2$	$1.1 \times 10^2 - 10^3$	$1.1 \times 10^3 - 10^4$	$> 10^4$
<i>E. coli</i>	4	1	0	0
Coagulase positive <i>Staphylococcus</i>	0	0	1	2
<i>B. cereus</i>	0	0	3	0

international attendees (Ritter & Tonto, 2014). Health surveillance plays a fundamental role, acting preventively to protect health and promote quality of life by inspecting and guiding establishments that sell or produce food, among other items, being able to eliminate risks and intervene in health problems (Ritter & Tonto, 2014). There were no complaints or notifications of FBD outbreaks related to the Rock in Rio 2022, which indicates that the control measures implemented by the Rio de Janeiro Sanitary Surveillance contributed to preventing attributable FBD. The establishments that sold the foods identified as unsatisfactory were duly charged and fined, in accordance with the Rio de Janeiro Municipal Health Code and Ordinance S-IVISA-RIO No. 222/2021 (Rio de Janeiro, 2021). The results obtained from these analyses and their dissemination to the scientific community are of direct relevance for the improvement of future control actions in mass events that may occur in the municipality, or in other locations.

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