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MICROBIOLOGICAL EVALUATION OF OYSTERS (CRASSOSTREA SPP) MARKETED IN THE STATE OF PARÁ, BRAZIL

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ABSTRACT

Oysters, bivalve mollusks, live predominantly in coastal regions and are valued for their high nutritional and culinary value. However, the ability of these mollusks to filter large volumes of water makes them susceptible to the bioaccumulation of pathogenic microorganisms, such as Escherichia coli and Salmonella. These contaminants pose a significant risk to public health, particularly due to the frequent consumption of raw oysters. The objective of this study was to analyze the presence of Salmonella spp and Escherichia coli in oyster samples marketed in the state of Pará, Brazil. There were 23 oyster samples, collected in 6 municipalities in the State of Pará. Being 03 samples, from oyster growers' cooperatives and 20 samples collected from street vendors on beaches located on the coast of Pará. For the detection of Salmonella spp, the ISO 6579:2002 method was used, while the analysis of Escherichia coli followed the Embrapa protocol. The analyses revealed the presence of Salmonella spp. and Escherichia coli in 100% of the samples, regardless of the origin (cooperatives or street vendors). The contamination of oysters highlights failures in management and marketing. Our findings highlight the need for strict controls in the production chain to ensure the food safety of oyster consumers.

Keywords: Oyster farming. Pathogens in Molluscs. Oyster Commercialization. Crassostrea Gasar.

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INTRODUCTION

Oysters, bivalve mollusks, live predominantly in coastal regions and are valued for their high nutritional and culinary value, bivalves constitute natural stocks of renewable resources that depend on a balanced ecosystem to meet their physiological demands, ensuring their development (Nuernberg *et al*., 2022).

The extraction of these mollusks is of great importance in several communities in the north and northeast of Brazil, where about 50 thousand people live exclusively from the removal of crustaceans such as oysters (Pereira *et al*., 2017). Sustainable mariculture is part of aquaculture, and contributes to the reduction of hunger and poverty in coastal regions, standing out as a relevant source of income for communities (Silva *et al*., 2021).

Oyster farming in the state of Pará has sanitary requirements, however, the state, due to its large territorial area, presents logistical difficulties for execution. Since, there is a wide commercialization of this mollusk, and consequently an increase in risks associated with contamination by pathogens (Da Silva *et al.,* 2024), which can affect public health, since these microorganisms generate serious infections and poisoning (Ballesteros *et al.*,2016). The species that are currently being cultivated in the Northeast of Pará are the native oysters *Crassostrea gasar (Deshayes, 1830) and C. rhizophorae,* being marketed alive, with an average size of 60 to 120 mm (Moura *et al*, 2024).

Oysters basically feed on small microscopic algae (called phytoplankton), microorganisms in general (bacteria, fungi) and detritus (very small pieces of decomposing plants and animals). These organisms filter large volumes of water for food, which also makes them susceptible to the accumulation of pollutants that are present in the water. The cause of oyster contamination is due to the method of obtaining food, carried out exclusively by filtering the particles that are suspended in the water, accumulating toxins in their tissues (Pruzzo *et al.,* 2005). By filtering water for oxygen and nutrients, oysters can bioaccumulate microorganisms and chemicals in their tissues. (Mendes *et al.,* 2023).

The accumulation of these toxins in the mollusk does not affect its health, much less alter its sensory characteristics such as odor, color, taste, and texture (Buzin *et al.,* 2011). Thus, the more they filter, the more the concentration of toxic substances in their meat increases. However, when consuming the contaminated raw material, the consumer may experience serious symptoms, such as fever, gastrointestinal pain, and even brain problems and cancer. The habit of consuming raw oysters contributes to the emergence of these cases of foodborne diseases (DTAs), and highlights the need for studies related to

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the quality of this food, since there is a high consumption of the population (Forcelini *et al.,* 2009).

Bivalve mollusks are also susceptible to the action of parasites that can cause diseases and lead to significant mortalities, both in natural banks and in cultivation environments. Monitoring these parasites along the oyster production chain is essential to ensure food safety and public health. One of the most relevant parasites is the protozoan *Perkinsus marinus*, which causes the "oyster disease". According to Hégaret *et al.* (2021) and da Silva *et al.* (2014), P*. marinus* infection can lead to high mortality rates in oyster populations, as well as posing a potential risk of transmission to humans who consume them.

A relevant group of contaminating bacteria are those of the genus *Salmonella*., which cause Salmonellosis, can be found in oysters (Liu *et al.,* 2022; Iwamoto *et al.*, 2010). In addition, another bacteria that causes gastrointestinal disease is *Escherichia coli* can also be a contaminant present in oysters and (Iwamoto *et al.,* 2010; Lhafi & Kühne, 2007). It is also possible to highlight other pathogenic bacteria such as *Listeria monocytogenes* and *Staphylococcus aureus* as potential contaminants of this crustacean, and have a potential capacity to cause serious diseases (Aubourg *et al.,* 2021; Vongkamjan *et al.,* 2017). These bacteria can be naturally present in the marine environment and proliferate in warm waters, where oysters are farmed (Han *et al.,* 2021).

In this sense, the objective of this study is to perform a microbiological study on oysters commercialized in the state of Pará, Brazil and to evaluate the contamination by *Salmonella* spp. and *Escherichia coli.*

METHODOLOGY

SAMPLE COLLECTION

Twenty-three (23) oyster samples were collected from 6 municipalities in the State of Pará (Figure 01). 03 samples, collected directly from oyster farmers, located in the community of Alto Pereru (1) and Pereru de Fátima (1), both located in the municipality of São Caetano de Odivelas-PA, and in the community Lauro Sodré (1), in the municipality of Curuçá-PA. The other 20 (twenty) samples were acquired from street vendors (originating from cultivation and extractivism in natural areas), who sold on beaches located on the coast of Pará, in Salinópolis-Pa, on Atalaia beach (09), Barcarena-Pa, on Caripi beach

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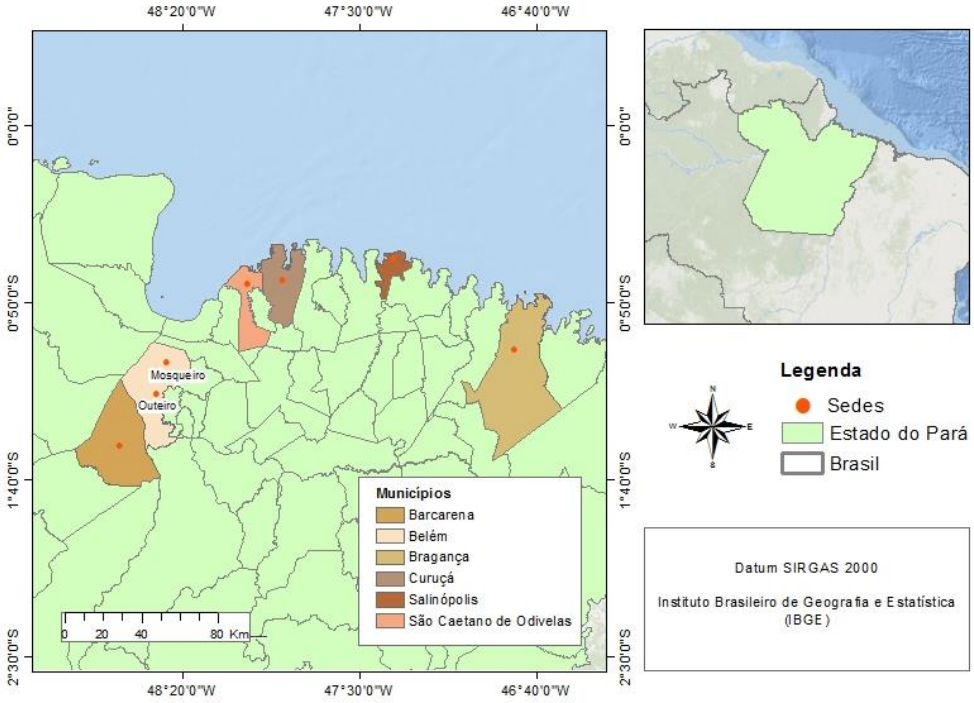
(05), in Belem-Pa, on the beach of the Outeiro District (02) and beach of the Mosqueiro District (02), finally in the city of Bragança-Pa, on Ajuruteua Beach (05).

Figure 1 - Location of oyster sample collection points in the state of Pará-Brazil



Source: Author, 2024.

The samples were collected on different days, from January to August 2024, and packed in isothermal boxes and transported under refrigeration, for a period of less than 6 hours, to the Food and Water Analysis and Research Laboratory (LAPAA), of the State University of Pará, Campus XX, to carry out microbiological analyses for the detection of *Salmonella* spp. and *Escherichia coli.*

DETECTION OF ABSENCE AND PRESENCE OF SALMONELLA SPP

For the analysis of *Salmonella* spp., the method proposed in ISO 6579:2002 was used. In the first stage of the analysis, pre-enrichment was performed with 25mL of the sample and 225mL of diluent solution, buffered peptone water, then homogenized and incubated at 35°C ±2°C for a period of 18±2h. After the period, the selective enrichment stage was carried out, transferring 1ml to a test tube containing 10mL of Tetrathionate Broth and incubated at 35°C ±2°C / 24±2h and transferred 0.1mL to the tube containing 10mL of *Rappaport-Vassiliadis* Broth (RV) Incubated in a water bath at 42°C ± 0.5°C for a period of 24 ± 2h. Subsequently, cultures were added to perform streaking on the Xylose Lysine Deoxycholate (XLD) Agar plates, and Salmonella *Shigella Agar* (SS). The plates

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were incubated at 36°C for 24 hours. In the final stage, characteristic colonies of each plate were collected for biochemical confirmation. The inoculation of the colonies was performed using tubes containing culture media, *Triple Sugar Iron* Agar (TSI), Iron Lysine Agar (LIA), Indole Sulfide Motility Agar (SIM) and Simmons Citrate Agar, which were incubated for 24 hours at a temperature of 36°C to confirm the presence/absence in the oyster samples. The results were expressed as absence or presence of *Salmonella* in 25g.

DETECTION OF ABSENCE AND PRESENCE OF ESCHERICHIA COLI

The identification of *Escherichia coli* followed the methodology for determining Absence and presence described by Embrapa (2011).

First, the presumptive test was performed, where three aliquots of three dilutions of the sample were inoculated in a series of three test tubes containing 10mL of Lauryl Sulfate Tryptose Broth (LST) and *Durham tube*. Incubated at 35°C/ 24-48h. And after that, microbial growth with gas production, characteristic of coliforms, was observed. Subsequently, the confirmation test was performed, which consisted of transferring a lift from each tube of positive LST broth to the selective medium E. coli broth (EC). Incubated in a bath at 45.5 ± 0.2°C/24 h. Then, microbial growth with gas production, characteristic of thermotolerant coliforms, was observed. Soon after, E. coli isolation and characterization was transferred from each positive EC broth tube to the surface of Petri dishes containing *Levine Eosin Blue Methylene* (EMB) agar, previously prepared and dried. Spread the inoculum with a Drigalski loop until all the excess liquid is absorbed. Incubate the plates at 35°C/24 h. Select typical colonies (black colonies with a metallic green luster) for confirmation by means of the following biochemical tests: Gram stain (negative), citrate (negative), *Voges Proskauer-VP* (negative), Methyl Red-VM (positive) and Indol (positive).

RESULTS

The results of microbiological analyses on oysters sold in the municipalities of São Caetano de Odivelas, Curuçá, Salinópolis, Barcarena, Belém and Bragança, in the State of Pará, can be seen in Table 1.

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Table 1. Results of microbiological analyses in oysters (*Crassostrea* spp.) marketed in the municipalities of São Caetano de Odivelas, Curuçá and Salinópolis, Barcarena, Belém and Bragança, in the State of Pará, Brazil.

Sample Code	Location	*Salmonella ssp.*	*Escherichia Coli*
A01	Ostreicultores de Alto Pereru, São Caetano de Odivelas-PA	Present	Present
A02	Ostreicultores de Pereru de Fátima, São Caetano de Odivelas-PA	Present	Present
A03	Ostreicultores de Lauro Sodré, Curuçá-PA	Present	Present
A04	Praia de Atalaia, Salinópolis- PA	Present	Present
A05		Present	Present
A06		Present	Present
A07		Present	Present
A08		Present	Present
A09		Present	Present
A10	Praia de Caripi, Bacarena-PA	Present	Present
A11		Present	Present
A12		Present	Present
A13		Present	Present
A14		Present	Present
A15	Praia do distrito de Outeiro, Belém- PA	Present	Present
A16		Present	Present
A17	Praia do distrito de Mosqueiro, Belém-PA	Present	Present
A18		Present	Present
A19	Praia de Ajuruteua, Bragança- PA	Present	Present
A20		Present	Present
A21		Present	Present
A22		Present	Present
A23		Present	Present

Source: Author, 2024.

The microbiological analyses of the oysters showed the presence of *Salmonella* spp. and *Escherichia coli in* 100% (23/23) of the samples studied. The oysters were collected from oyster farmers' cooperatives and street vendors on beaches. The purpose was to evaluate whether there would be a difference in the microbiological results, according to the origin of the sample. However, in the results, the oysters were also contaminated.

DISCUSSION

Studies carried out in Brazil have investigated the presence of *Salmonella* in oysters, one of the main foods of marine origin consumed by the population. In a study conducted by Silva *et al.* (2010), in the state of Rio de Janeiro, 120 oyster samples were

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analyzed, and *Salmonella* was detected in 5.8% of the samples. Salmonella serovars identified included *S. Enteritidis* and *S. Typhimurium.* Corroborating these findings, Figueiredo *et al.* (2013), analyzed 150 oyster samples collected in different regions of the state of Bahia and found *Salmonella* in 4.7% of the samples. The most prevalent serovars were S. *Typhimurium, S. Enteritidis and S. Abony.* More recently, in a study conducted by Oliveira *et al.* (2020), in the state of São Paulo, 200 oyster samples were subjected to microbiological analysis, and *Salmonella* was detected in 6.5% of the samples. The serovarieties identified included *S. Enteritidis, S. Typhimurium and S. infantis.*

The studies corroborate the findings of the present study, which is considered a concern for food safety and public health, as a result of the risk of contamination of consumers who eat this food, usually raw, especially in coastal regions, during the summer.

Salmonella contamination of oysters is also evidenced in other countries. Liu *et al.* (2022), in its research carried out in China between 2019 and 2020, found that out of 1,312 oyster samples, they revealed the presence of *Salmonella* in 3.7% of the samples. The *most commonly isolated* Salmonella serovarieties were *S. Typhimurium and S. Enteritidis.* In the results of Lhafi and Kühne (2010), of the 135 oyster samples collected in Germany between 2006 and 2007, 4.4% of the samples were contaminated by *Salmonella*. As well as in the study conducted by Iwamoto *et al.* (2010), in the United States, in which 5.9% of the oyster samples analyzed were contaminated by *Salmonella*. The most prevalent serovarieties were *S. Typhimurium, S. Newport and S. Enteritidis.* And finally, Vongkamjan *et al.* (2017), in a survey conducted in Thailand, found *Salmonella* in 4.8% of the 125 oyster samples analyzed. The identified serovarieties included *S. Weltevreden, S. Stanley and S. Rissen.*

In the research by Nuernberg et al. (2022), where oyster samples collected in natural environments and cultivation areas of Lagoa do Noca, in the municipality of Laguna, in Santa Catarina, showed contamination of thirteen species of Gram-negative bacteria, indicators of the presence of coliforms, were identified in 73.78% (76/103) of the samples. *Escherichia coli* had a prevalence of 21.05% of the mollusks analyzed. The authors point out that the absence of adequate sanitary infrastructure and the direct flow of urban waste are aggravating factors in the microbiological contamination of oysters. The results presented corroborate the findings in our research, where all samples showed contamination, emphasizing the need for constant monitoring and strict management practices of oysters.

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The oysters studied presented in their total, the pathogen *Salmonella.* Control can be controlled through monitoring

It is necessary to intensify monitoring and control along the production chain, considering that the presence of this enteric pathogen in bivalve mollusks represents a risk to public health, and may cause outbreaks of salmonellosis in consumers. In this context, Figueiredo and Goulard (2015) relate the consumption of oysters with Salmonella food outbreaks , caused by the conditions of cultivation, handling and storage, as well as the lack of adequate hygiene practices, and it is essential to apply decontamination processes such as hot water or high hydrostatic pressure.

The results confirmed a high prevalence of the bacterium *Escherichia coli* in all samples analyzed. This bacterium indicates fecal contamination, which increases the risk of disease transmission by enteric pathogens. Santos *et al.,* (2023), when evaluating oysters in São Luíz do Maranhão, detected *Escherichia coli* in only 26.66% of the oysters analyzed. In the studies by Ribeiro and Amaro (2006), in the state of São Paulo, 50 oyster samples were analyzed, and *E. coli* was detected in 26% of them. While in their research Soares *et al.* (2011), analyzed 120 oyster samples collected in the state of Santa Catarina and found *E. coli* in 30.8% of the samples. Oliveira *et al.* (2019), carried out its study in the state of Rio de Janeiro, where 80 oyster samples were subjected to microbiological analysis, and *E. coli was detected* in 22.5% of the samples.

Another problem in the contamination of this mollusk refers to post-harvest and/or capture management, because at this stage, the oysters are sold and consumed still alive, thus requiring care that can guarantee hygiene and quality during the handling of this mollusk, directly interfering with the health of the consumer. In this way, evaluating the microbiological characteristics of this food, and disseminating its results through scientific research, supports the inspection agencies in acting effectively to control and comply with sanitary requirements.

Therefore, monitoring and controlling the microbiological quality of oysters throughout the production chain, combined with appropriate hygienic practices, are essential to ensure the safety of consumption of this important food of marine origin.

CONCLUSION

The contamination of oysters highlights failures in management and marketing. Our findings highlight the need for strict control in the production chain to ensure the food safety

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of oyster consumers, especially because they are consumed raw. Regulatory agencies need to intervene on the need to apply conservation processes to control pathogens.

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