O MUNDO DA SAÚDE

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Microbiological study of the invasive oyster *Saccostrea cucullata*: seasonal variation of total and thermotolerant coliforms in the Cananéia Estuarine Complex during the 2018-2019 season

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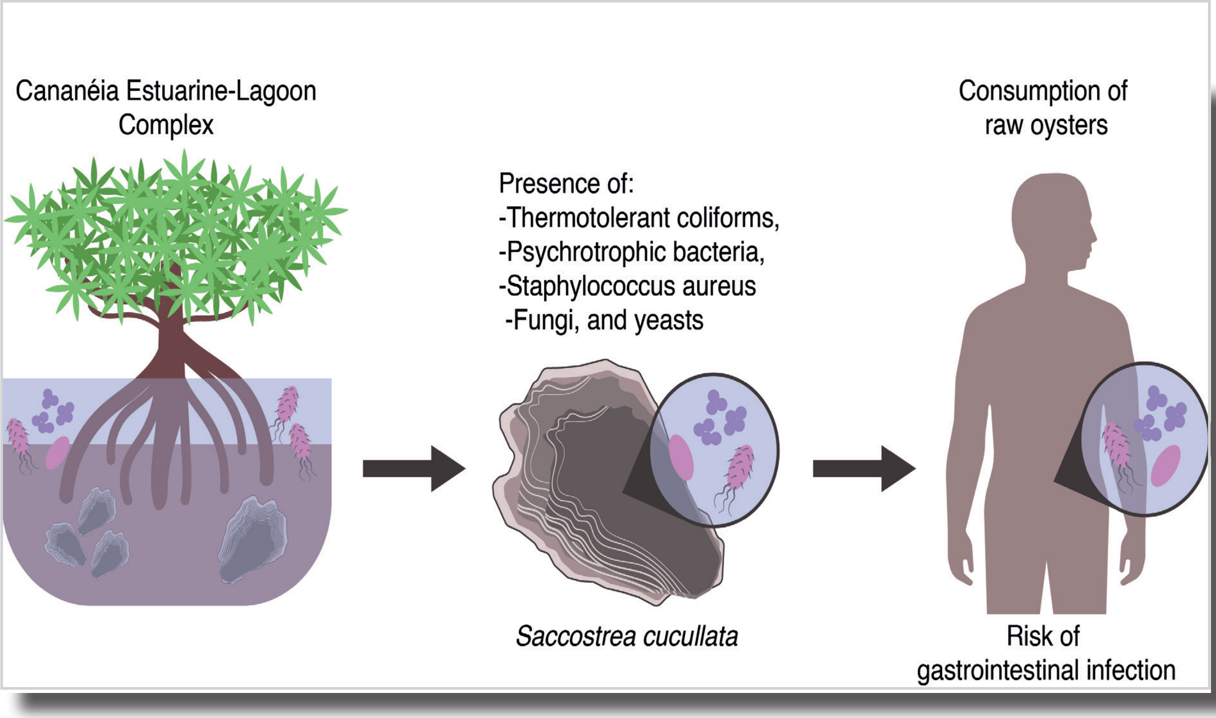
Graphical Abstract



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Abstract

In the estuarine lagoon complex of Cananéia, state of São Paulo, southeast region, there is fresh consumption of the invasive oyster *Saccostrea cucullata*, mainly in summer, high season. It is a filter-feeding mollusk that presents a risk of serious pathologies of the gastrointestinal system due to the risk of storing microbiological agents that affect humans. The lack of monitoring, good manufacturing practices, water quality and control measures to ensure the safety and quality of oyster consumption is necessary for the prevention of pathogens in public health. This study carried out microbiological analyzes of oyster samples, focusing on mesophilic bacteria, psychrotrophic bacteria and fungi/yeasts. Furthermore, seasonal concentrations of total and thermotolerant coliforms were investigated in oyster samples collected over a year. Counts of mesophilic bacteria in oysters ranged from 1.45±0.22 to 3.32±0.28 log CFU g^{-1}, with average values of 2.24±0.86 log CFU g^{-1}. For psychrotrophic bacteria in oyster samples it ranged between 1.34±0.29 and 3.12±0.45 log CFU g^{-1}. The data revealed that fungal and yeast counts ranged from 2.65±0.23 to 3.57±0.22 log CFU g^{-1}. The maximum *S. aureus* count was 1.24 log CFU g^{-1}, and 83.5% of the samples tested negative for this microorganism. No presence of *Salmonella* spp. was detected in the analyzed data. These results provide important insights into seasonal variation and microbiological counts in oyster samples, highlighting the relevance of microbiological monitoring and control in marine food products.

Keywords: Cananéia. Oyster. Food security. Microbiology. Enterobacteriaceae.

INTRODUCTION

The detection of coliforms in oysters is a highly relevant topic in the context of food safety and public health[1,2,3]. Oysters, bivalve molluscs widely appreciated in different parts of the world for their distinctive flavor and nutritional benefits, present the additional challenge of accumulating pollutants and microorganisms present in the water they inhabit, including thermotolerant coliforms[4,5,6].

Thermotolerant coliforms make up a bacterial group found in the feces of endothermic animals, including humans[7,8,9]. Their presence in oysters may indicate contamination of cultivation water, often associated with human or farm animal waste[10,11]. Contamination by enterobacteria is a concern, as these bacteria can harbor disease-causing pathogens such as *Escherichia coli* and *Salmonella* spp.[12].

Consumption of oysters contaminated with thermotolerant coliforms can result in foodborne illnesses, including serious gastrointestinal infections[7], with symptoms such as diarrhea, nausea, vomiting, fever and abdominal pain. Individuals with compromised immune systems, children, the elderly and pregnant women are particularly vulnerable to these diseases[13], requiring medical treatment and hospitalization in more severe cases[14].

Therefore, it is imperative to conduct studies and monitoring of contamination by pathogenic microorganisms in oysters to identify areas and sources of contamination, implement preventive and control measures, and ensure consumer safety and prevent foodborne illnesses and protect consumer health. Continued research in this field contributes to the development of more effective guidelines and regulations on the cultivation, processing and consumption of oysters, aiming to protect public health and preserve the reputation of the seafood industry[13], in addition to promoting sustainability and environmental responsibility in the seafood industry.

With the confirmation of the presence of the invasive oyster *Saccostrea cucullata* on the coast of Santa Catarina, Paraná, São Paulo and Rio de Janeiro, and its incorporation into the diet of the local population, as documented by Galvão *et al.*[15] and Amaral *et al.*[16], this study proposed a general assessment of the microbiological quality of these oysters, abundantly found in the Cananéia Estuarine-Lagunar Complex, located on the south coast of São Paulo. These oysters are consumed fresh and shelled by local inhabitants. This study carried out microbiological analyzes of oyster samples, focusing on mesophilic bacteria, psychrotrophic bacteria and fungi/yeasts. Furthermore, seasonal concentrations of total and thermotolerant coliforms were investigated in oyster samples collected over a year.

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METHODOLOGY

Samples collected

For the analysis of total and thermotolerant coliforms, a total of ten samples per month of oysters of the species *Saccostrea cucullata* were collected in front of the pier of the *Instituto de Pesca* (Fisheries Institute) (S 25°01'10.344", W 47°55'30.6804") located in the municipality of Cananéia (SP), from January to December 2019 (Figure 1). On the other hand, samples isolated in the months of August, October and December 2018 and February 2019 were used for more specific analyzes such as the presence of *Staphylococcus aureus*, *Salmonella* spp. mesophilic, psychotrophic bacteria, fungi and yeasts.

Quantification of total and thermotolerant coliforms

For the analysis of total and thermotolerant coliforms, tissue samples were collected from *S. cucullata oysters*, and the soft tissue of the oysters were analyzed in the laboratory of the Fisheries Institute, Cananéia. Samples from 10 oysters were subjected to monthly analysis to determine the MPN (Most Probable Number) of total and thermotolerant coliforms, following the methodology described by the Standard Methods For The Examination Of Water And Wastewater[17, 18].

The samples were diluted from a 1/10 dilution of the sample (10 g of sample in 90 mL of sterile 0.1% peptone water), prepared in dilutions of 1/100 to 1/1000, totaling ten dilutions. After this procedure, they were cultivated in Sodium Lauryl Sulfate Broth (Himedia®, Mumbai, India). The tubes with oyster samples were incubated at 35 ºC for 48 hours. Those that showed gas production in the Durham pipes and/or acidified the medium (yellowish color) were considered positive. To confirm coliforms, cultures with a positive result from the previous test were inoculated in Brilliant Green Bile Broth (2%) (Himedia®, Mumbai, India), incubated at 35±1 °C for 48 hours. To confirm the presence of *E. coli*, the samples were transferred and incubated in EC Broth (Himedia®, Mumbai, India) for an interval of 18 to 24 hours at 44.5±0.2 °C.

Detection and quantification of mesophylls, psychotrophs, fungi and yeasts

With the aim of obtaining specific information regarding the detection and quantification of mesophilic, psychrotrophic bacteria, fungi and yeasts, *Staphylococcus aureus* and *Salmonella* spp., the stomach and digestive diverticula were isolated by dissection and grouped to obtain 25 g of tissue. Aliquots of 25 g of each sample were aseptically weighed into sterile plastic bags and homogenized with 225 mL of 0.1% (w/v) peptone water (Himedia®, Mumbai India), using a Colworth Stomacher® circulator for optimal homogenization. of the material, and incubated in a BOD chamber model 347 CD (Fanem® - São Paulo, Brazil). Decimal dilutions of 10:1 were prepared in tubes containing 9.0 mL of 0.1% peptone water.

To determine mesophilic bacteria, samples were incubated at 35±2 °C for 48 h and for psychrotrophic bacteria at 7±2 °C for 10 days, for molds and yeasts at 25±2 °C for 5 days, following the plate technique. propagation method was used to analyze mesophilic and psychrotrophic bacteria, molds and yeasts[18].

The determination of the *Staphylococcus aureus* count was carried out using the Compact Dry® XSA kit (Nissui Pharmaceutical Company LTD - Ibaraki, Japan), and the samples were incubated at 35±1°C for 24 hours[19]. For detection of *Salmonella* spp. detection, the BAX® kit (DuPont Qualicon - Wilmington, USA) was used and the samples were incubated at 35±2 °C for 24 hours[20].

Statistical analyzes

The Shapiro-Wilk statistical test was carried out, which showed that the distribution of the data did not adjust to normality. As the distribution was not normal, the non-parametric Kruskal-Wallis test (KW-H) $p<0.05$ was used to compare the level of contamination between months and seasons.

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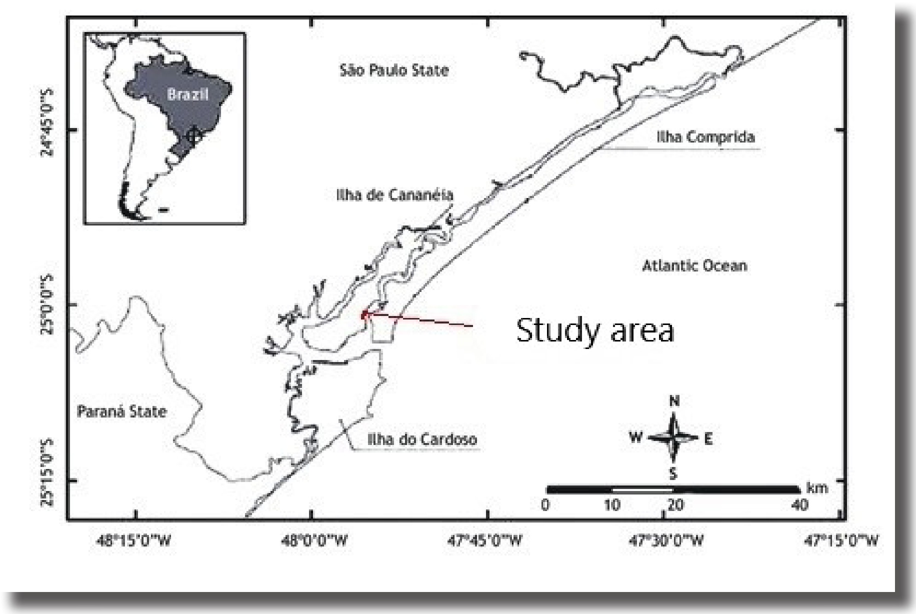

Figure 1 - Map of the Cananéia-Iguape Estuarine-Lagunar Complex region. The coordinates of the collection area were: S 25°01'10.344", W 47°55'30.6804".

RESULTS

Concentrations of total coliforms decreased from June to September, as illustrated in Figure 2. On the other hand, concentrations of thermotolerant coliforms decreased from April to September (Figure 3). Regarding seasonality, an increase in the concentration of thermotolerant coliforms was observed during the summer and spring months. On the other hand, total coliform concentrations increased during the summer and decreased significantly in the winter, as indicated in Figure 4 and 5.

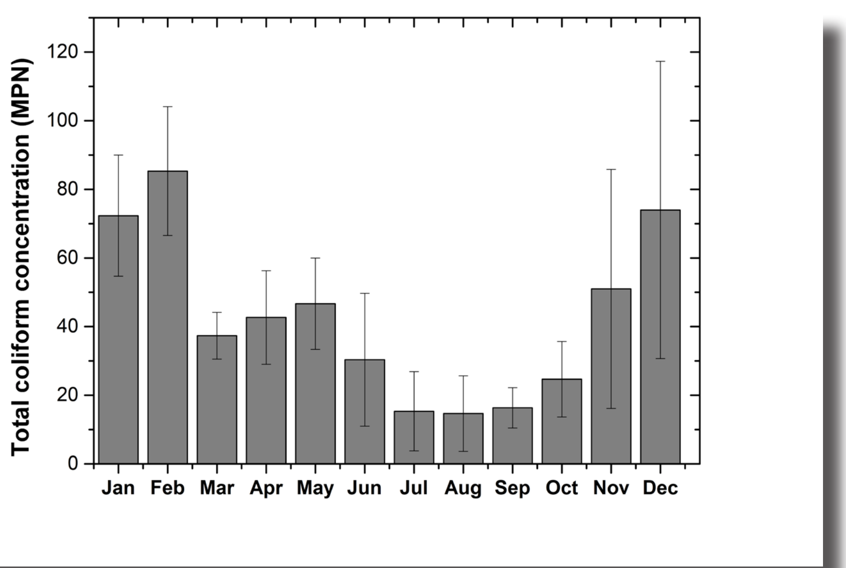

Figure 2 - Means of total coliforms (N=10) in oyster tissues analyzed monthly. The bars show their respective standard deviations. The averages for the months of July, August and September were statistically different from those of January, February, March, April, May and December (p<0.05).

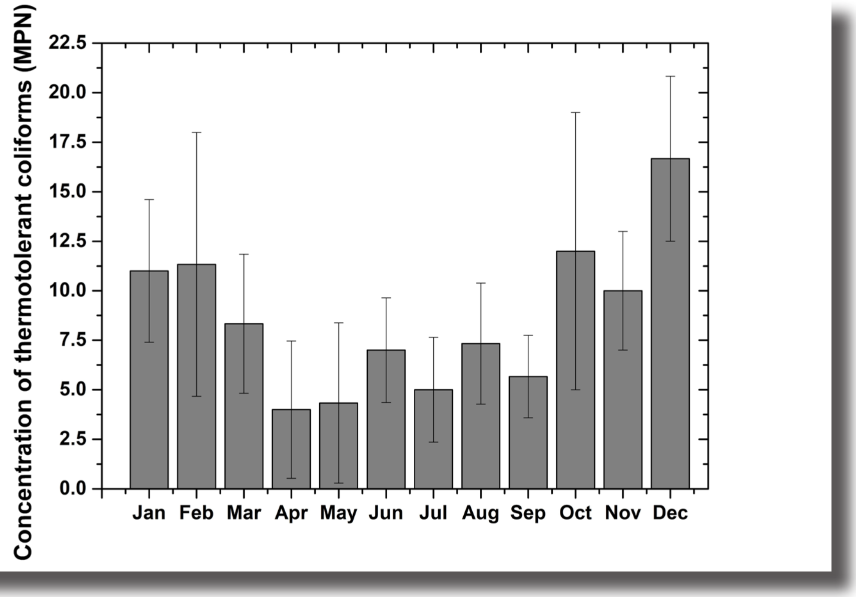

Figure 3 - Means of thermotolerant coliforms (N=10) in oyster tissues analyzed monthly. The bars show their respective standard deviations. The averages for the months of January, November and December were statistically different from those of April, May and July ($p<0.05$).

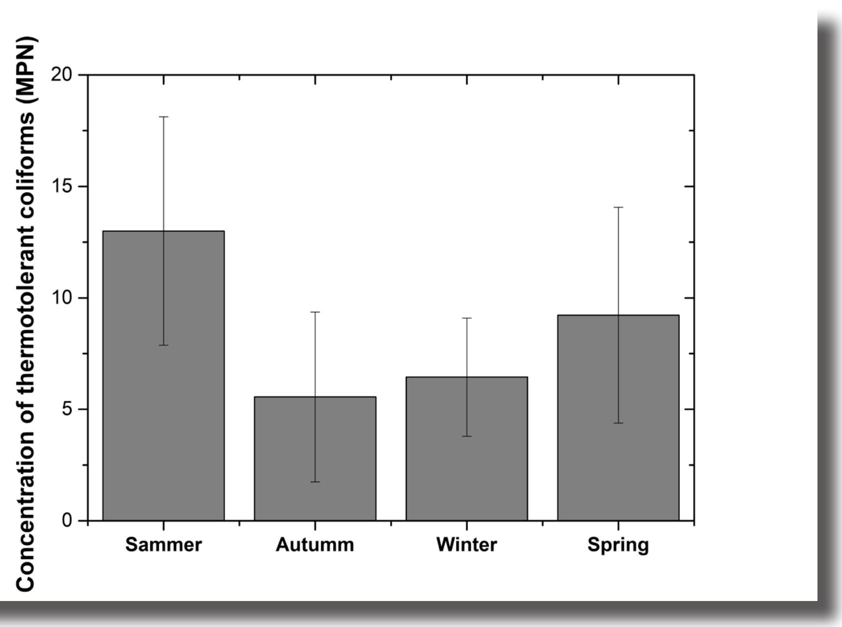

Figure 4 - Analysis of the means of thermotolerant coliforms (standard deviation bars, N=30) in oyster tissue by seasonal variation. The bars show their respective. There was no significant difference between seasons ($p<0.05$).

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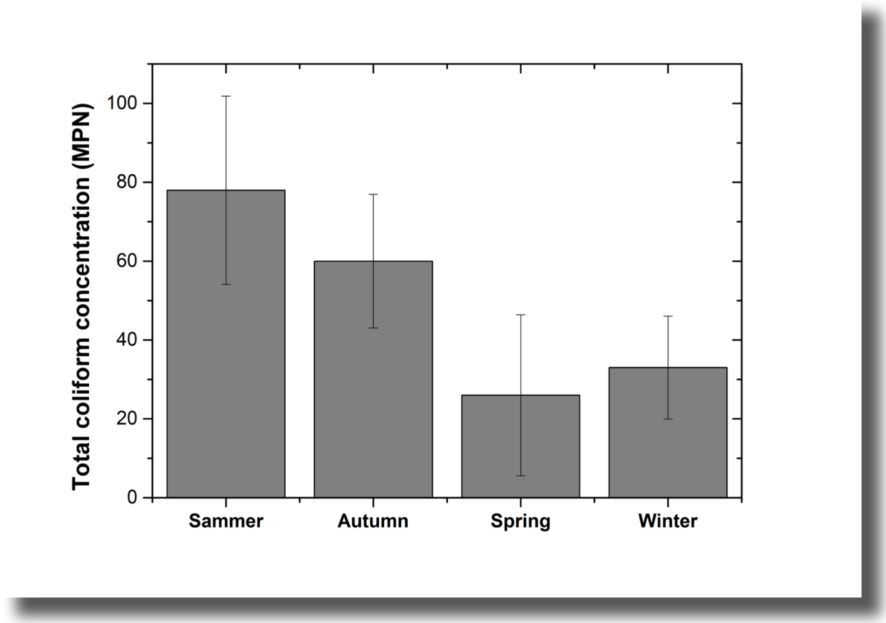

Figure 5 - Analysis of total coliform means (standard deviation bars, N=30) in oyster tissue by seasonal variation. The bars show their respective standard deviations. There was a statistical difference between the winter seasons when compared to summer ($p<0.05$).

Table 1 presents the counts of mesophilic bacteria that ranged from 1.45±0.22 to 3.32±0.28 log CFU g^{-1}; with average values of 2.24±0.86 log CFU g^{-1} for the oysters studied.

Table 1 - Counts of mesophilic, psychrotrophic bacteria, fungi and yeasts found in *Saccostrea cucullata* oysters in the Cananéia estuary.

Microbiological analysis	June	July	January	February
Mesophilic	1.45±0.22	1.65 ±0.35	3.32±0.28	2.55 ±0.43
Psychrotrophic	1.34±0.29	1.52±0.42	2.99±0.55	3.12±0.45
Fungi and yeasts	2.65±0.31	1.88±0.28	3.57±0.22	3.07±0.41

The count of psychrotrophic bacteria in oyster samples varied between 1.34±0.29 and 3.12±0.45 log CFU g^{-1} (Table 1).

The data presented in Table 1 show that the fungal and yeast counts of oyster samples ranged from 2.65±0.23 to 3.57±0.22 log CFU g^{-1}.

The maximum *S. aureus* count was 1.24 log CFU g^{-1}, and 83.5% of the samples tested negative for this microorganism. The overall average count found for this analysis was the lowest among the microorganisms evaluated in this study for the collection point studied throughout the period. *Salmonella* spp. was detected in no sample.

DISCUSSION

The results presented in the study provide important information about the microbial quality and food safety of the exotic oyster *S. cucullata* from extraction in the natural environment. The discussion of these results highlights the presence and levels of specific micro-organisms, such as the coliform group, psychrotrophic bacteria, fungi and yeasts, *Staphylococcus aureus* and *Salmonella* spp.

Regarding psychrotrophic bacteria, the study found that counts varied on average by 2.24 log CFU g^{-1} (Table 1). All samples analyzed showed acceptable results for mesophilic and psychrotrophic bacteria, with values below 7.0 log UFC g^{-1}, according to the International Commission on Microbiological Specifications for Foods[25]. Psychrotrophic bacteria are known to be able to grow at low temperatures, which is concerning in the context of oyster safety[9,21]. The observed variation in counts suggests potential differences in storage conditions or contamination levels between samples. The average count provides a general indication of microbial load, and it is important to note that these counts must be within acceptable limits to ensure the safety and quality of oysters for consumers[7].

The data in Table 1 also shows the fungal and yeast counts in the oyster samples with an average value of 2.79 log CFU g^{-1}. Fungi and yeast can play a role in food spoilage and can be indicators of poor hygiene and handling practices[6]. The observed variation in counts may be attributed to differences in environmental conditions, post-harvest handling, or storage practices. Monitoring and controlling fungal and yeast contamination in oysters is crucial to ensuring product quality and extending its shelf life.

The study also investigated the presence of *Staphylococcus aureus*, a potential foodborne pathogen. The low average count found in this study, the lowest among the microorganisms evaluated, suggests that contamination by *Staphylococcus aureus* was generally low, as it is not a bacterium found in the environment with varying salinity[7,12]. However, it is important to note that even low levels of this pathogen can pose a risk to consumers, as it can cause pneumonia, meningitis, urinary tract infection and sepsis[26]. Emphasizing the importance of implementing strict hygiene practices and monitoring procedures throughout the production and distribution chain.

Furthermore, it is noteworthy that *Salmonella* spp. was not detected in any of the samples tested, its absence is a positive result and indicates that the oyster samples studied were free of this specific pathogen during the study period. This may have occurred due to the variation in salinity that occurs in the estuary[9].

The results obtained regarding concentrations of total coliforms and thermotolerant coliforms provide valuable information about seasonal variations and potential risks associated with water quality. The observed decrease in total coliform concentrations from June to September (Figure 2) suggests improved microbial conditions during this period. This reduction can be attributed to factors such as increased sun exposure, higher water temperatures and better environmental conditions, generally associated with the summer months. A similar result was found by Mignani *et al.*[14] studying the concentration of coliforms in oysters of the species *Crassostea brasiliana*.

In contrast, the decrease in thermotolerant coliform concentrations from April to September (Figure 3) indicates a similar trend, but with a broader period, mainly due to these months being those with the lowest number of tourists in the city of Cananéia. This finding suggests a prolonged period of improved microbial quality in the water. The reduction of thermotolerant coliforms is especially important, as they are considered indicators of fecal contamination and may represent a greater risk to human health[20,21].

The seasonal pattern observed in thermotolerant coliform concentrations is notable. Increased concentrations during the summer and spring months imply a greater risk of fecal contamination during these seasons. This can be attributed to factors such as increased recreational activities, population density, and potential runoff from agricultural or urban areas[14,22]. These findings highlight the importance of implementing appropriate monitoring and management strategies to mitigate risks associated with waterborne pathogens during peak periods.

Interestingly, concentrations of total coliforms showed a different pattern compared to thermotolerant coliforms; their increase

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during the summer months and their significant decrease during the winter (Figure 4) suggest complex dynamics of microbial populations in the water. The increase in total coliforms during the summer can be attributed to several sources, including non--fecal sources such as soil, vegetation, and wildlife[12,13]. The decrease in winter may be associated with factors such as reduced microbial activity due to lower temperatures and less human and animal interaction with bodies of water.

In general, these results highlight the importance of monitoring and managing water quality, suggesting the use of bivalve molluscs as excellent bioindicators of the microbiological quality of water in polluted environments, especially during seasons with greater risks of contamination. Understanding the seasonal dynamics of coliform concentrations can guide the development of appropriate strategies to ensure the safety of water resources for human use, recreational activities, and ecosystem health[22,23,24]. Furthermore, more studies are needed to deepen our understanding of these dynamics and further strengthen water contamination prevention and control measures.

CONCLUSION

The results presented in the study provide important scientific evidence regarding the microbial quality and safety of oyster samples. The presence and levels of psychrotrophic bacteria, fungi and yeasts, *Staphylococcus aureus* and the absence of *Salmonella* spp. were evaluated, offering valuable insights into the potential risks associated with oyster consumption. These findings highlight the need for ongoing monitoring, good purification practices, and effective control measures to ensure the safety and quality of oysters for consumers.

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CRediT author statement

Conceptualization: Barbieri, E; Rezende, KFO. Methodology: Barbieri, E; Rezende, KFO. Validation: Barbieri, E. Statistical analysis: Barbieri, E. Formal analysis: Barbieri, E; Vásquez-Ponce, F. Investigation: Barbieri, E; Araújo, ALS. Resources: Barbieri, E. Writing-original draft preparation: Barbieri, E; Vásquez-Ponce, F. Writing-review and editing: Barbieri, E; Vásquez-Ponce, F. Visualization: Barbieri, E; Araújo, ALS; Rezende, KFO; Vásquez-Ponce, F. Supervision: Barbieri, E. Project administration: Barbieri, E.

All authors have read and agreed to the published version of the manuscript.

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