

Monitoring of *Vibrio* spp. in marine waters for the cultivation of bivalve mollusks in the South bay of Santa Catarina's Island, Brazil.

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Introduction

In Brazil, bivalve mollusks production mainly takes place in the Santa Catarina State, in south Brazil due to excellent geographical conditions for marine organism cultures, as the presence of a large number of bays facilitates the establishment of mollusk farms (5, 6, 14). *Vibrios* are very common in marine and estuarine water environments and some may cause infections in humans which were exposed to seafood or sea water. Several *vibrios* species are pathogenic to humans and may be present in shellfish raw or partially cooked (11, 19, 20). The concentration of *Vibrio* spp. in oysters is directly related to water temperature, with a higher concentration being present in oyster in warm water, and outbreaks usually occur during summer months (4, 18, 19, 24).

This study aimed to monitor the presence of pathogenic *vibrios* in marine waters for the cultivation of bivalve mollusks in the South Bay of Santa Catarina Island, Brazil, during the period of one year.

Material and methods

We evaluated 156 samples of saline water from different regions of cultivation of bivalve mollusks located in the South Bay of Santa Catarina's Island, Brazil (Figure 1), between September 2008 and August of 2009. The samples were collected in sterile 1-liter bottles, directly into the local culture, to 50 cm depth, the currents in the opposite direction, along the crops. Later they were packed in boxes containing ice water in isothermal packaging sealed and transported to the Microbiology Laboratory, Department of Food Science and Food Technology, Federal University of Santa Catarina, where they were analyzed in a maximum of three hours.

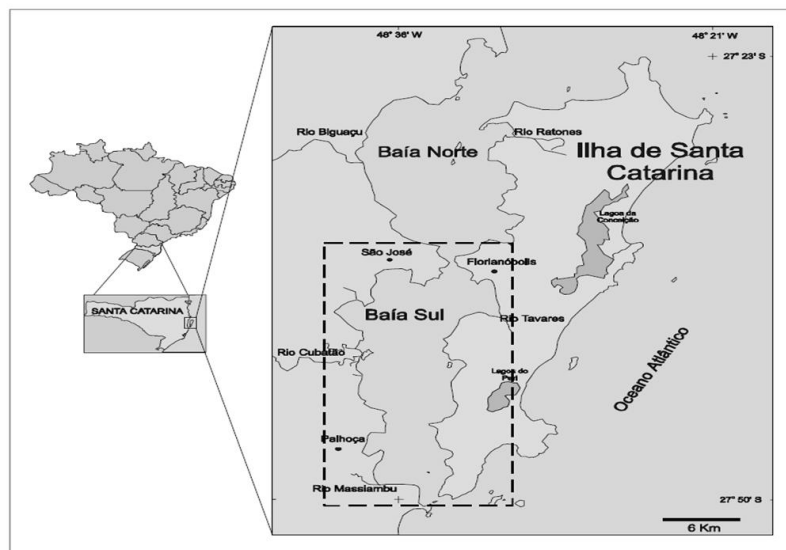


Figure 1. The South Bay of Santa Catarina's Island

At the time of sample collection, were examined in situ physic-chemical parameters of temperature, salinity and transparency of the sea waters. The temperature was checked using portable mercury thermometer graduated to 0.5 ° C, transparency using Secchi Disk and salinity was checked using the brand Alfakit hydrometer portable, model 211. All tests were performed in triplicate.

Counting of *Vibrio parahaemolyticus* and *Vibrio vulnificus* by the multiple tube technique, as described in BAM / U.S. FDA (2001) and *Vibrio cholerae* and *Vibrio alginolyticus* as the method described in Standard Methods for the examination of Water and Wastewater - APHA / AWWA / WEF (2005).

The influence of physical-chemical microbiological counts in marine waters was evaluated by analyzing nonparametric Spearman rank correlation, while the correlation between the physical and chemical parameters and incidence of *Vibrio* spp. was assessed by Pearson correlation. We used the program Statistica ® 7.0.

Results and discussion

Of the 156 samples analyzed, 101 (64.7%) samples had one or more species of vibrios (Table 1). The species most frequently isolated in samples of seawater was *Vibrio parahaemolyticus*, followed by *V. alginolyticus* and *V. vulnificus*, while *V. cholerae*, the only species of vibrios surveyed that has its origin in waters contaminated by sewage, was isolated in only three of 156 samples. The counts of *V. parahaemolyticus* ranged from <1.8 to 4.8x 10 MPN/100ml, and the average score in the samples was 5.7 MPN/100ml, while the counts of *V. vulnificus* ranged from <1.8 to 9.4 x 10 MPN/100ml, and the average score of 4.1 MPN/100ml. Although the observed counts are considered low, it is important to note that in filter feeding shellfish such as oysters and mussels, these microorganisms are concentrated in their intestines and other tissues, reaching up to 10⁶ bacteria per gram of clam (Strom and Paranjpye, 2000). In work done by Hoi et al (1998) evaluated 115 samples saline water from regions of cultivation of marine mollusks in Denmark, as the presence of *V. vulnificus*, found 39 (33.9%) samples positive, a percentage far greater than in this study. However, while only 14.7% of samples were positive for *V. vulnificus*, even at low concentrations, these data should serve as a warning to a need for constant monitoring of this species in regions of cultivation of bivalve mollusks, due to the ability of this microorganism in causing serious infections and often fatal (Paranjpye and Strom, 2000) .

Table 1-Distribution of *Vibrio* spp. in 156 samples of marine waters from areas for the cultivation of bivalve mollusks in the South Bay of Santa Catarina's Island, between the years 2008 and 2009.

Microorganism	Positive samples		Negative samples	
	No.	%	No.	%
<i>Vibrio</i> spp.	101	64,7	55	35,3
<i>V. parahaemolyticus</i>	58	37,2	98	62,8
<i>V. alginolyticus</i>	40	25,6	116	74,4
<i>V. vulnificus</i>	23	14,7	133	85,3
<i>V. cholerae</i>	03	1,9	153	98,1

Several studies have suggested a strong influence of temperature on the concentration of vibrios in marine waters, however this study could not establish this correlation nor with *V. parahaemolyticus* (Spearman R: 0.14, p> 0.05) or with *V. vulnificus* (Spearman R: - 0.03, p> 0.05). This is likely to have occurred due to the high temperature of marine waters, observed over the years in the study region, with average temperature around 23°C, whereas the minimum temperature recorded was 16.3°C and maximum 27°C, enabling continuous detection of different

species of vibrios, unlike what occurs in cooler regions, like Europe, where detection is significantly higher in summer (Su and Liu, 2007). According to Strom and Paranjpye (in 2000), *V. vulnificus* thrives in areas where the temperature exceeds 18°C, a condition that most samples of this study was collected, since only seven of 156 samples were collected at temperatures below 18°C.

We observed a negative correlation between the counts of *V. parahaemolyticus* and salinity (Spearman R: - 0.21, $p < 0.05$), however this did not occur to *V. vulnificus* (Spearman $r = 0.07$, $p > 0.05$). The measure of water clarity was not correlated with the level of contamination, or by *V. parahaemolyticus* (Spearman R: 0.08, $P > 0.05$) nor by *V. vulnificus* (Spearman R: - 0.02, $p > 0.05$). The incidence of different species of marine vibrios in water does not correlate with any physical-chemical parameters measured ($p > 0.05$).

Conclusions

The results of this study suggest the need to improve strategies to control diseases transmitted by consumption of bivalve mollusks, monitoring not only indicators of fecal contamination, such as fecal coliform and *Escherichia coli*, but also the pathogenic vibrios, thus contributing towards the growth and maintaining this important economic activity in the state of Santa Catarina and especially food security of consumers of bivalve mollusks.

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