***Original Research Article***

**Comparative Analysis of Bacterial Contamination in Three Different Species of Commercially Sold Oysters in Mindanao, Philippines**

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ABSTRACT

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| Since oysters are filter feeders, they collect harmful bacteria from their environment, which can be a serious health risk when eaten raw. Commercially sold oysters (*Magallana bilineata*) from Pagadian City, (*Magallana gigas*) from Dapitan City, and (*Magallana* spp.) in Ozamiz City were collected and utilized in the study to measure and compare the levels of bacterial contamination across these locations. This study used a descriptive-comparative research design to determine and compare the levels of bacterial contamination in commercially sold oysters from public markets and vendors. A total of eighteen kilograms of oyster samples were collected during the dry season from public markets and vendors and processed using convenience sampling. Standard microbiological techniques that included extraction, homogenization, isolation, and biochemical testing of the oyster were applied. The results showed that oysters from Dapitan City have the highest microbial load among the three locations, with the highest average total *Vibrio* and *Salmonella* spp. counts of 6.95x10¹⁰ CFU/mL and 7.00x10¹⁰ CFU/mL, whereas Ozamiz City oysters have the least, at 5.02x10¹⁰ CFU/mL and 2.97x10¹⁰ CFU/mL, respectively. Dapitan City oysters showed the highest presence of *Vibrio parahaemolyticus* of the three locations. Statistical analysis revealed no significant difference in bacterial contamination across the three areas, which have p-values for both variables exceeding 0.05. The reduction of the microbial load and proper processing of oysters are essential in maintaining the health and safety of consumers. |

*Keywords: Contamination; food-borne; food safety; raw; shellfish*

1. INTRODUCTION

The aquaculture industry is vital in food production in various countries, particularly Southeast Asia. The average consumption per person of seafood, such as shellfish and fish, was more substantial in Asia than in other parts of the world (Wai et al., 2021). A significant portion of the global seafood trade recognizes oysters as essential to food security and nutrition, making them one of the most well-known and extensively farmed marine species (Estrada, 2023). The slipper oyster, or *Magallana bilineata*, as named by Röding (1798), or *Crassostrea bilineata* in particular, was commonly found in the Philippines and was the preferred type of oyster to cultivate, as it provides high-quality meat with a fast growth rate (Bermeo et al., 2022). On the other hand, the Pacific oyster, or *Magallana gigas*, which was named by Thunberg (1793), was the most commonly farmed and harvested species globally, introduced to at least 52 countries, including the Philippines, and it has a faster growth and high filtration rate (Ruesink et al. 2005, as cited in Smithsonian Environmental Research Center, 2024). Oysters were filter feeders; as a result, they were more prone to accumulation of contaminants (Yip, 2023).

The buildup of contaminants in bivalve mollusks, specifically oysters, depends on multiple biological and environmental conditions (Ribeiro et al. 2020). The risk of foodborne diseases increases significantly when these shellfish originate from contaminated areas were handled under precarious hygiene-sanitary conditions (Pereira et al. 2006, as cited in Nuñal et al. 2023). Furthermore, improper handling, storing, and processing oysters can compromise their safety due to the risk it poses of contamination with harmful pathogens (Estrada, 2023). Pakingking et al. (2022) showed high levels of fecal coliforms and *E. coli* in the agricultural areas of Capiz province. The study results indicate that Philippine oyster farms do not comply with international safety standards.

A study conducted by Nuñal et al. (2023) on shellfish from various growing areas revealed the presence of *Vibrio parahaemolyticus*, *E. coli*, and *Salmonella* spp. at high levels. In the Philippines, oysters and mussels carry significant pathogen loads, making them unsafe for raw consumption. According to Nuñal et al. (2023), the increase in microbial levels in shellfish upon consumption is due to the possibility that bacteria may grow during its transport. The detection of *Vibrio parahaemolyticus* at different stages of seafood production has caused serious concerns, affecting both public health and the seafood industry's sustainability.

Several pathogenic bacteria, including *Staphylococcus aureus*, *Vibrio* spp. and *Salmonella* species, were known to contaminate oysters. They were well known for producing serious infections and sometimes even death when consumed raw or undercooked seafood, particularly slipper oysters (*Magallana bilineata*) (Song et al., 2020). *Vibrio* spp. was the bacteria that naturally resided in the coastal water (Centers for Disease Control and Prevention, 2024). People become infected after exposure to the bacteria, either through an open wound that has been in contact with infected water or raw or undercooked shellfish, or, most commonly, by directly eating the latter. According to the CDC, about a dozen *Vibrio* spp. that can make people sick, and around 80,000 people are infected with *Vibrio* bacteria in the U.S. every year. Symptoms include diarrhea, stomach cramps, nausea, and fever. Vibrio spp. and Salmonella spp. cause foodborne diarrhea outbreaks involving ingesting undercooked seafood, particularly oysters (Bush and Vasquez-Pertejo, 2024). 65% of 200 oyster samples were found to be significantly contaminated with *Vibrio* species in research conducted by Dumaloan-Canini et al. (2024). Of them, *V. parahaemolyticus* was found to be 40%, followed by *V. cholerae* (16%) and *V. alginolyticus* (9%).

Recent research has identified possible health concerns linked to eating raw or undercooked Pacific oysters(*Magallana gigas*), mainly due to their ability to collect harmful microorganisms from their surroundings. In another study conducted by Nuñal et al. (2023) which examined the bacterial quality of ready-to-eat *M. gigas* oysters purchased from supermarkets and a local farm revealed that although most of the oyster samples met acceptable safety standards, some showed elevated levels of coagulase-positive *Staphylococcus*, which may indicate contamination. While tests did not detect *Salmonella* or harmful *Vibrio* species through traditional culture methods, molecular techniques found the presence of *Vibrio alginolyticus*, a bacterium that can cause illness in humans. These results highlight the need for careful inspection and strict hygiene practices during oyster harvesting and processing to reduce the risk of foodborne infections (Costa et al., 2023).

This study aimed to conduct a comparative analysis of the bacterial contamination of *Vibrio* spp. and *Salmonella* spp. in commercially sold oysters across Zamboanga del Sur, Misamis Occidental, and Zamboanga del Norte. Specifically, it aimed to compare the concentration of *Vibrio* spp. and *Salmonella* spp. in commercially sold oysters sourced from Pagadian City, Zamboanga del Sur, Dapitan City, Zamboanga del Norte, and Ozamiz City, Misamis Occidental. Moreover, it is also aimed to determine the risk levels based on bacterial contamination across the three cities. Furthermore, the study aimed to determine the count of *Vibrio parahaemolyticus* in oyster samples. Lastly, the study aimed to evaluate the significant differences in the bacterial contamination levels of *Vibrio* and *Salmonella* spp. across the three cities.

The scarcity of local data prevents the identification of contamination levels between *Vibrio parahaemolyticus* and *Salmonella* spp. in oysters from Pagadian City, Zamboanga del Sur, and Dapitan City, Zamboanga del Norte, and Ozamiz City, Misamis Occidental. Focusing on the comparative analysis of *Vibrio parahaemolyticus* and *Salmonella* spp. contamination levels, the study aimed to provide critical insights that can inform public health policies and food safety regulations. Understanding the prevalence of these pathogens in commercially sold oysters will help identify high-risk areas, ultimately contributing to improved safety measures in the aquaculture industry. Additionally, the findings could guide local farmers in adopting better hygiene practices, ensuring the quality of seafood products, and enhancing consumer safety. Given that seafood plays a significant role in nutrition and food security, the results of this research can significantly impact community health and economic stability in the region.

This study was conducted during the dry season from December 2024 to March 2025 focusing exclusively on the three areas and species acquired in each area: slipper oyster species (*Magallana bilineata*) in Pagadian City, Zamboanga del Sur, Pacific oyster (*Magallana gigas*) in Dapitan City Zamboanga del Norte and (*Magallana* spp.) in Ozamiz City, Misamis Occidental. The study utilized 6 kilos of oyster samples from each city totaling 18 kilos in all areas. Environmental factors such as seasonal variations in water quality were not accounted for, potentially influencing bacterial contamination levels at different times. The study has specifically only focused on the presence of *Vibrio parahaemolyticus* and *Salmonella* species. When interpreting the results and their implications for public health and food safety practices, these factors were considered.

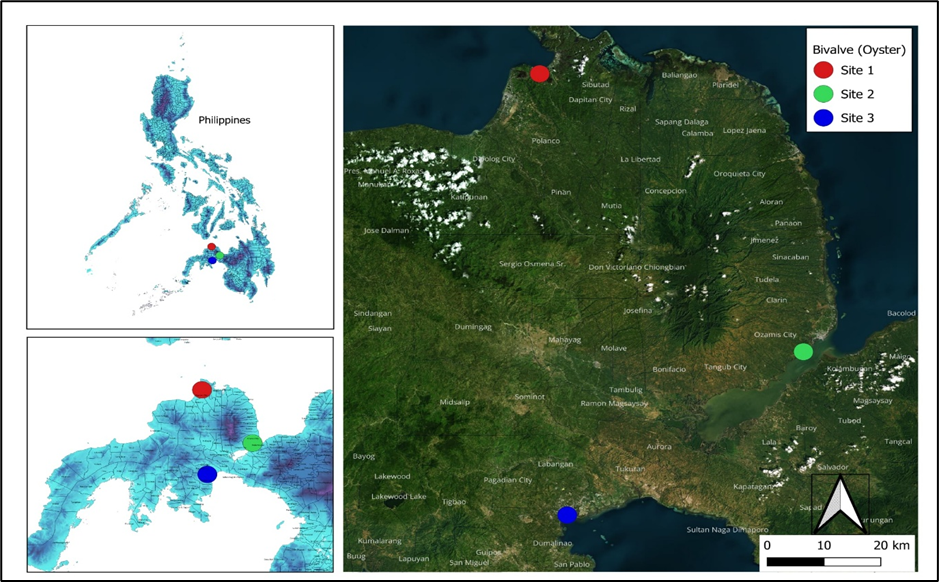
2. material and methods

**2.1 Research Design**

This study utilized a descriptive-comparative research design to comprehensively compare and determine the levels of bacterial contamination as well as to compare the concentration between Vibrio spp. and Salmonella spp. present in commercially sold oysters from three different provinces.

**2.2 Research Setting**

This study was conducted on the three key provinces in Mindanao, Philippines: Pagadian City, Zamboanga del Sur; Ozamiz City, Misamis Occidental; and Dapitan City, Zamboanga del Norte as shown in Figure 1. These sites were selected based on the accessibility and availability of the slipper Oyster (*Magallana bilineata*) and Pacific Oyster (*Magallana gigas*) in markets and their role as significant sources of oysters within the region. Within these provinces, samples were collected in areas like public and wet markets or from vendors outside of this area, where oysters are frequently sold.



**Fig 1. Map of the three study sites**

**2.3 Sample Species Identification**

Before the collection of the samples, proper species identification was conducted. Three oyster samples from the three provinces were sent to the Bureau of Fisheries and Aquatic Resources (BFAR)- Region X, Cagayan de Oro City office for the morphological analysis of the oyster samples. As seen in Figure 2, the results of the analysis identified that the three oyster samples were: *Magallana* spp., *Magallana bilineata* (Röding, 1798), and *Magallana gigas* (Thunberg, 1793).

A close up of a shell

AI-generated content may be incorrect.A close up of a shell

AI-generated content may be incorrect.A close up of a shell

AI-generated content may be incorrect.

(C)

(B)

(A)

**Fig 2. (A) *Magallana* spp.; (B) *Magallana bilineata* (Röding, 1798); and (C) *Magallana gigas* (Thunberg, 1793)**

**2.4 Sample Collection, Storage and Transport**

The researchers bought two (2) kg of oysters from three (3) different seafood stalls of the exact source of commercially sold slipper oyster (*Magallana bilineata*) for Pagadian City, Pacific oyster (*Magallana gigas*) for Dapitan City, and *Magallana* spp. for Ozamis City. Thus, a total of 18 kg of oysters were utilized in the study. These samples were purchased randomly from different vendors in the public market or outside of the vicinity of the three provinces. Furthermore, the purchasing and processing of samples were done weekly per province. This ensured that all purchased samples were used as fresh and accurately representative of current conditions.

The samples purchased were placed in a sterile sealed plastic bag to prevent contamination. The samples were contained in a cooler which contained ice, ensuring proper temperature (7° C to 10° C) to prevent changes in bacterial level. The samples were then transported to the laboratory within 2-3 hours after collection. Upon arrival at the laboratory, all samples were rinsed with distilled water (Pakingking et al., 2023).

**2.5 Sample Preparation, Bacterial Isolation and Identification**

**2.5.1 Isolation of *Vibrio parahaemolyticus***

A sterile knife was used to open the shell aseptically (Shuping et al. 2023). The flesh was detached from the shell and placed in a sterile Erlenmeyer flask (Pakingking et al. 2023). The samples were homogenized by mixing 25 grams of oyster meat and 225 mL of alkaline peptone water (APW) to create a 1:10 dilution, and serial dilutions were followed. Consequently, the APW homogenates were incubated at 37°C for 18 hours (Villicaña et al. 2019; Dumaloan-Canini et al., 2024).

All procedures were performed in triplicate to ensure accuracy and reliability of results.

The detection of *V. parahaemolyticus* was based on the standard procedures of the U.S. Food and Drug Administration Bacteriology Analytical Manual, followed by some modifications (Dumaloan-Canini et al., 2024; Tan et al. 2020). Using an inoculating loop, a 3-mm loopful from the prepared APW tubes containing the highest dilutions of sample was streaked to Petri dishes containing thiosulfate citrate bile-salt sucrose (TCBS) agar and was incubated at 35-37°C overnight (FDA, 2004; Castello et al., 2023).

For presumptive identification of *V. parahaemolyticus*, it appeared as round, opaque, and greenish or bluish colonies in the TCBS agar. After the incubation period, colonies with typical characteristics were counted (Stratev et al. 2021). Using these characteristics, other species just like *V. cholerae*, *V. alginolyticus*, and *V. furnissii*, which exhibit yellow colonies on TCBS agar were ruled out. The suspected colonies were then subcultured on another Petri dishes containing the same culture media, and bacterial smears were then prepared from the suspected colonies and stained with Gram stain (Al-Garadi et al. 2024).

The isolates which appeared as pink-colored, curved, rod-shaped, and gram-negative were identified as *V. parahaemolyticus*. The colonies were subjected to the salt tolerance test available in the laboratory for further identification of *V. parahaemolyticus*.

**2.5.2 Isolation of *Salmonella* species**

To detect *Salmonella* spp., a pre-enrichment procedure was done by mixing 25 grams of oyster meat and 225 mL of buffered peptone water in a sterile blender (Dumaloan-Canini et al., 2024). Following the Bacterial Analytical Manual for *Salmonella* spp., selective enrichment was performed by transferring 0.10 mL of the blended samples to 10 mL of Rappaport-Vassiliadis (RV) medium and incubating for 24 ± 2 h at 42 ± 0.2°C. The colony that grew on the RV medium was then inoculated to the selective medium called the Salmonella-Shigella (SS) agar and was incubated at 35°C (Dumaloan-Canini et al., 2024). In this selective agar, the *Salmonella* species appears as colorless colonies with black centers (Neyaz et al. 2024). Moreover, biochemical tests were performed for further identification.

**2.6 Biosafety and Biosecurity Management**

The Biosafety Level 2 (BSL-2) containment guidelines were strictly followed for handling the Risk Group 2 pathogens such as Vibrio parahaemolyticus and Salmonella species. A biosafety cabinet class II was used during the conduct of the study. These measures align with the international biosafety protocols and the national standards of the Philippines (U.S. Department of Health and Human Services, 2020). In the laboratory safety manual, the usage of personal protective equipment (PPE), including gloves and laboratory gowns, was strictly employed to prevent contamination of the sample being processed. Moreover, work surface areas were disinfected regularly with a disinfectant (Reckitt). Lastly, the bacteria-containing plates were sterilized using an autoclave set at 121°C and 15 psi for 15 minutes. After which, the disinfected Petri dishes were disposed of on a yellow bag with a biohazard symbol. The sterilized wastes were then collected by the sanitary management of Misamis University.

**2.7 Data Analysis**

The colony forming unit (CFU) count in Equation 1 was utilized to quantify the isolated bacteria in the population of the confirmed colonies. The CFU per gram was calculated by multiplying the number of colonies by the dilution factor divided by the volume plated.

Equation (1)

The microbial count of *Vibrio* spp*.* and *Salmonella* spp*.* was determined for each sample obtained from the individual stalls in the three cities; the CFU/g was converted into log10 values using the formula in Equation 2 (Dumaloan-Canini et al., 2024).

log10 (CFU) Equation (2)

The classification of the risk in the bacterial contamination levels in each province was based on whether the log values were ≥ 5.0 log10 CFU/g for *Vibrio* spp. and a value of ≥ 3.0 log10 CFU/g for *Salmonella* spp. was classified as medium-to-high risk; However, if the log CFU values fall ≤ 5.0 log10 CFU/g for *Vibrio* spp. and a value of ≤ 3.0 log10 CFU/g for *Salmonella* spp. the risk level was classified as no-to-low risk (Dumaloan-Canini et al., 2024).

To determine whether there is no significant difference in the levels of bacterial contamination across cities, IBM SPSS Statistics V21 was utilized to compute the Analysis of Variance (ANOVA) using the obtained CFU/g values.

3. results and discussion

**3.1 Bacterial Concentration of Oyster Samples Across the Three Stalls in Each City**

The analysis of the oyster samples across Dapitan City, Ozamiz City, and Pagadian City revealed the presence of *Vibrio* spp. and *Salmonella* spp., detected using thiosulfate citrate bile-salt sucrose (TCBS) and Salmonella-Shigella (SS) agars, respectively. The eighth serial dilution was selected from the 9th and 10th dilutions since it provided a countable colony of 30-300 colony-forming units.

The study revealed that the Pacific oysters (*Magallana gigas*) from Dapitan City posed the greatest potential risk in terms of concentration of *Salmonella* spp*.* contamination, while Ozamiz City oysters showed the least risk, with one stall showing no contamination and lower bacterial loads in the others. This emphasized the importance of monitoring, handling practices, and sanitary regulations in reducing seafood-borne pathogens.

The average concentrations of *Vibrio* spp. and *Salmonella* spp. presented in Table 1 were calculated using the data obtained from three sampling stalls (Stalls A, B, and C) per location. Microbial concentrations (CFU/mL) were measured for each stall through standard plate count methods. The individual microbial concentrations from each stall were then summed and divided by the number of samples to obtain the mean concentration for each bacterium per location. This arithmetic averaging method allows for a standardized comparison of microbial loads across different sampling sites and is a commonly used approach in microbial contamination assessment (Agostini et al., 2025)

**Table 1. Microbial concentrations of *Vibrio* spp. and *Salmonella* spp. in commercially**

**sold oysters from the three cities**

|  |  |  |  |
| --- | --- | --- | --- |
| **Location** | **Bacterium** | **Average Concentration (CFU/g)** | **Most Contaminated Stall** |
| Dapitan City | *Vibrio* spp.  *Salmonella* spp. | 6.95 x 1010  3.59 x 1010 | Stall A  Stall A |
| Ozamiz City | *Vibrio* spp.  *Salmonella* spp. | 5.02 x 1010  2.97 x 1010 | Stall A  Stall C |
| Pagadian City | *Vibrio* spp.  *Salmonella* spp. | 6.36 x 1010  3.83 x 1010 | Stall A  Stall C |

The concentration of *Vibrio* spp. was highest in Dapitan City, followed by Pagadian City, and lowest in Ozamiz City. The study of Guedes et al. (2023) revealed that Pacific oysters (*Magallana gigas*) harbor *Vibrio alginolyticus* but not *Salmonella* spp*.* In contrast, in the present study, *V. alginolyticus* was not detected but is part of the general enumeration of another *Vibrio* species*.* Notably, *Salmonella* spp. was detected in stalls A and C in Dapitan City where Pacific oysters (*Magallana gigas*) were studied*,* the same species examined by Guedes et al. (2023). Despite this similarity, the present study revealed higher overall concentration of *Vibrio* spp. and high levels of *Salmonella* spp. surpassing stalls from Pagadian City and Ozamiz City. This variation may be influenced by environmental factors such as water temperature and salinity, and differences in post-harvest handling and sanitation practices (Su & Liu, 2007; Caburlotto et al., 2010). The consistent detection of higher levels in Stall C in Pagadian City and Ozamiz City suggests localized issues related to hygiene or sourcing. Meanwhile, the lower levels in Dapitan indicate relatively better handling or storage conditions.

In Pagadian City, where *Magallana bilineata* was collected, the samples contained slightly low concentrations of *Vibrio* spp*.* and *Salmonella* spp (Stall C). While Ozamiz City, where an unidentified *Magallana* species was collected, had the lowest concentration of *Vibrio* spp. and *Salmonella* spp. The absence of *Salmonella* s*pp*. in both Stall A and Stall B in Pagadian City and Ozamiz City may indicate enhanced handling and processing practices, possibly reflecting improved water quality at the harvesting locations. Variations in oyster sources, such as harvesting from waters with lower fecal coliform counts, may also contribute to these differences (Nuñal et al. 2023). The detection of contamination in most Dapitan stalls indicates a potential widespread issue, possibly linked to a common source or inadequate handling practices. However, the absence of contamination in Stall B suggests variability in hygiene standards or sourcing among vendors.

Variations in temperature, sewage contamination, industrial discharge, water quality, and geographic location affect the bacterial load.An increase in the growth of *Vibrio* spp*.* is more common in warmer marine environments, particularly in the summer when the water temperature rises above 15° C (Su & Liu 2007). Moreover, locations nearsewage discharge points showed significantly higher levels of *Salmonella* spp*.* in oysters (Brands et al. 2022).

Shellfish sold in wet markets have a high microbial load due to poor drainage, dirty surfaces, and ambient temperatures. Bacteria flourish in wet markets and outdoor seafood markets because they frequently lack refrigeration, have poor sanitation, and reuse tainted water for rinsing. Cross-contamination between seafood items was a result of inadequate infrastructure (Solo-Gabriele et al. 2021).

During the collection of samples in Dapitan City, it was observed that the stalls selling the oyster samples display their products on trays that were visibly not cleaned properly. As mentioned by Mante (2021), any *Salmonella* present can be transferred from previously contaminated items. These surfaces can act as reservoirs, allowing bacteria to persist and spread to other seafood items that were otherwise initially uncontaminated. If trays and tools (e.g., knives, cutting boards, and tongs) were not cleaned with hot water and effective disinfectants, *Salmonella spp.* containing biofilms can form. Bacterial growth will then be difficult to eliminate and can continuously reintroduce bacteria into seafood.

According to a study of Ehuwa et al. (2021), microbiological concentrations are greatly influenced by the vendor's handling procedures, which include cleanliness, the use of gloves and ice, and appropriate seafood storage. Contamination risk was increased by reusing water, keeping seafood at room temperature, or practicing poor personal hygiene. Higher levels of *Salmonella* infection were found in samples from vendors who did not use gloves or their equipment.

**3.2 Bacterial Contamination Levels of Commercially Sold Oyster in the Three Cities**

The CFU/mL counts were converted into log10 values to determine the level of risk across the different stalls from each area. The microbial assessment highlighted a consistent and elevated level of contamination of *Vibrio* spp*.* across the different stalls in Dapitan City, Ozamiz City, and Pagadian City. The microbial load of *Vibrio* spp*.* is presented in Table 2, which varied from 10.23 ± 0.19 log₁₀ CFU/g (Ozamiz Stall C) to 11.08 ± 0.12 log₁₀ CFU/g (Pagadian Stall A). These values exceed the safety thresholds set by the international guidelines. All stalls were designated under “medium to high risk,” with Pagadian Stall C with the highest microbial load which could be due to high filter-feeding capability of oysters that allowed them to readily accumulate pathogens like *Vibrio* spp*., Salmonella* spp*.,* etc. The abundance of *Vibrio* spp*.* might be due to being a halophilic bacterium that thrives in brackish water, marine environments, and freshwater, which supported the growth of this organism (Centers for Disease Control and Prevention, 2024).

On the other hand, *Salmonella* spp*.* that were detected range from 10.35 ± 0.39 to 10.64 ± 0.09 log₁₀ CFU/g, still classified under the “Medium to High Risk” category, which posed a significant public health concern. The five stalls that tested negative for *Salmonella* spp*.* were classified as “no-to-low risk.” Although *Salmonella* spp. are not halophilic bacteria like the *Vibrio* spp., they have the ability to adapt to various environmental conditions wherein they form biofilms, which increases their survivability (Petrin et al. 2022).

**Table 2. Risk Level of *Vibrio spp.* and *Salmonella spp.* Contamination on Oysters from**

**Dapitan City, Ozamis City, and Pagadian City**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Area** | **Stall** | ***Vibrio* spp. (log10 CFU/g ± SD)** | **Risk Level** | ***Salmonella* spp. (log10 CFU/g ± SD)** | **Risk Level** |
| Dapitan City | A | 10.92 ± 0.10 | Medium-to- High risk | 10.64± 0.09 | Medium-to- High risk |
|  | B | 10.85 ± 0.11 | Medium-to- High risk | 0 | No-to-low risk |
|  | C | 10.65 ± 0.27 | Medium-to- High risk | 10.62 ± 0.03 | Medium-to- High risk |
| Ozamiz City | A | 10.92 ± 0.07 | Medium-to- High risk | 0 | No-to-low risk |
|  | B | 10.69 ± 0.12 | Medium-to- High risk | 0 | No-to-low risk |
|  | C | 10.23 ± 0.19 | Medium-to- High risk | 10.35 ± 0.39 | Medium-to- High risk |
| Pagadian City | A | 11.08 ± 0.12 | Medium-to- High risk | 0 | No-to-low risk |
|  | B | 10.65 ± 0.09 | Medium-to- High risk | 0 | No-to-low risk |
|  | C | 10.64 ± 0.43 | Medium-to- High risk | 10.55 ± 0.20 | Medium-to- High risk |

Dapitan City had the highest number of *Salmonella*-positive samples, with two stalls testing positive. Moreover, the highest count of *Vibrio* spp*.* was detected in Stall A Pagadian, whereas *Salmonella* spp*.* was undetected in the sample. A study conducted by Dumaloan-Canini et al. (2024) revealed that 65% of their seafood samples were heavily contaminated with *Vibrio* spp*.* and 9% were contaminated with *Salmonella* spp*.* Another study that was conducted by Nuñal et al. (2023) supported the findings based on their results, which also showed high levels of *V*. *parahaemolyticus, V. cholerae, E. coli,* etc., in their oyster samples. They further elaborated and confirmed that due to the oyster’s ability to accumulate pathogens, by the time they reach the consumers, they already contain higher loads of bacteria, which was an indication that these oysters may not be safe for raw consumption. A recent study from 2022 conducted by Lopatek et al. has similar results to the current study wherein, from their sample (raw bivalve mollusk), 3.1% of *Salmonella* spp*.* was detected in 26.1% of *Vibrio parahaemolyticus.* This highly suggests that *Vibrio* spp*.* was more prevalent and stable in oysters.

Nuñal et al. (2023) stated that in the Philippines, both *Vibrio* spp*.* and *Salmonella* spp. were among the leading causes of foodborne disease outbreaks, with oysters accounting for 12.9% of these cases. A study conducted by Chen et al. (2024) investigated the health burden of foodborne gastroenteritis caused by non-typhoidal *Salmonella enterica* and *Vibrio parahaemolyticus* in Zhejiang Province, China. The researchers employed sentinel hospital surveillance and community surveys to determine the incidence of acute gastrointestinal infections (AGI) caused by these bacteria. Their results found that both *Salmonella* spp*.* and *Vibrio parahaemolyticus* play a vital role in the contribution to the prevalence of foodborne gastroenteritis in the province. These findings strongly highlight the danger of consuming raw seafood as they can undergo more bacterial multiplication during transport and storage.

**3.3 Microbial counts of *Vibrio parahaemolyticus* and other *Vibrio* species**

The total counts of *Vibrio* spp*.* among the three areas were presented in Table 3. The highest average total *Vibrio* count obtained from the samples were detected in Pagadian City, followed by Dapitan City and then Ozamis City, with the CFU/mL being 6.31 × 10¹⁰, 6.04 × 10¹⁰, and 5.02 × 10¹⁰, respectively. This high level of microbial load suggested that environmental factors such as water contamination were present in these areas.

**Table 3. Total Microbial Count of *Vibrio* Species in Oysters from Dapitan City, Ozamiz**

**City, and Pagadian City**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Area** | **Stall** | ***Vibrio* *parahaemolyticus* (CFU/g)** | **Other *Vibrio* species (CFU/g)** | **Total *Vibrio* Count (CFU/g)** |
| Dapitan City | A | 4.80 x 1010 | 3.70 x 1010 | 8.50 x 1010 |
|  | B | 6.47 x 1010 | 0.73 x 1010 | 7.20 x 1010 |
|  | C | 0.32 x 1010 | 2.10 x 1010 | 2.42 x 1010 |
|  |  |  |  | Average: 6.04 x 1010 |
| Ozamiz City | A | Negative | 8.30 x 1010 | 8.30 x 1010 |
|  | B | 3.60 x 1010 | 1.36 x 1010 | 4.96 x 1010 |
|  | C | Negative | 1.80 x 1010 | 1.80 x 1010 |
|  |  |  |  | Average: 5.02 x 1010 |
| Pagadian City | A | 10.4 x 1010 | 1.70 x 1010 | 12.1 x 1010 |
|  | B | 3.40 x 1010 | 1.30 x 1010 | 4.70 x 1010 |
|  | C | 1.12 x 1010 | 1.00 x 1010 | 2.12 x 1010 |
|  |  |  |  | Average: 6.31 x 1010 |

A similar study conducted by Sorio & Peralta (2018) has shown that the consistent detection of *Vibrio cholerae* and *Vibrio parahaemolyticus* in all oyster samples suggested that closeness to human settlements and its resulting waste discharge plays a significant role in bacterial contamination. The microbiological safety of bivalve mollusks was strongly influenced by the quality of their surrounding waters. When these areas were exposed to sewage discharges, whether treated or untreated, from residential, agricultural, or urban sources, the risk of contamination significantly increased (Souza et al., 2011). Hence, creating proper safeguards is essential in producing seafood that is safe for consumption which is achieved through understanding the environmental factors relating to microbiological pollutants in estuarine waters (Silva et al., 2020).

**3.4 Differences of the bacterial contamination levels across the three provinces**

The comparative analysis of bacterial contamination levels in commercially sold oysters across the three areas was analyzed using one-way analysis of variance (ANOVA) and revealed in Table 4 that there were no significant differences in *Vibrio spp*. contamination across the provinces, F= 2.57, p = .20, and no significant differences in *Salmonella spp.* contamination, F = 0.38, *P* = .70. Since the *P*-values for both variables were greater than .05, the null hypothesis was retained, indicating that the levels of bacterial contamination did not significantly differ among the provinces.

**Table 4. Analysis of Variance on Bacterial Contamination of *Vibrio* spp. and**

***Salmonella* spp. Across Three Cities**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | ***F*** | ***P*-value** | **Decision and Conclusion** |
| *Vibrio* spp. across three provinces | 2.574 | .20 | Since the *P*-value is > .05, we fail to reject the null hypothesis |
| *Salmonella* spp. across three provinces | 0.382 | .70 | Since the *P*-value is > .05, we fail to reject the null hypothesis |

The statistical analysis implied that the bacterial contamination levels across the different areas were uniform. Moreover, it can be interpreted that the locations may not have a strong influence on the levels of contamination of commercially sold oysters. A recent study conducted by Indrastuti et al. (2021) found out that there were no statistically significant differences in terms of the total coliform levels in various topographies in Yogyakarta, Indonesia. The different areas may have similar food-handling practices for oysters. In addition, the limited number of samples may have reduced the ability to detect any noticeable differences between areas. The findings of this study highly suggest that the level of contamination of each area was influenced by anthropogenic factors (such as poor handling practices, improper washing and storage, etc.) regardless of the location.

4. Conclusion

The study revealed the high levels of bacterial contamination in oysters from Dapitan City, Ozamis City, and Pagadian City. All stalls across the different locations exhibited medium to high risk levels for both *Vibrio* spp*.* and *Salmonella* spp*.* Statistical analysis revealed that there is no significant difference in bacterial contamination across the three areas, having p-values for both variables exceeding .05.

Based on the findings of this study, it is recommended that the local government units and health authorities in Dapitan City, Ozamis City, and Pagadian City should intensify monitoring and regulatory measures related to the handling, transport, and selling of oysters in their public markets. Implementing stringent sanitary measures and periodic microbial quality testing of seafood commodities can help avoid any potential consumer risk to health. Additionally, educational campaigns among oyster vendors and consumers regarding the risks of eating raw or contaminated oysters need to be initiated. Procedures of post-harvest treatment like depuration or high-pressure processing could also be investigated to minimize levels of bacterial contamination and provide microbiological safety for oysters sold in the three cities.

Ethical approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

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