Review Form 1.7

Journal Name:	**European Journal of Nutrition & Food Safety**
Manuscript Number:	**Ms_EJNFS_112884**
Title of the Manuscript:	**Occurrence of potentially pathogenic bacteria in commercially sold seafood from Misamis Occidental, Philippines**
Type of the Article	

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PART 1: Review Comments

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Compulsory REVISION comments 1. **Is the manuscript important for scientific community?** (Please write few sentences on this manuscript) 2. **Is the title of the article suitable?** (If not please suggest an alternative title) 3. **Is the abstract of the article comprehensive?** 4. **Are subsections and structure of the manuscript appropriate?** 5. **Do you think the manuscript is scientifically correct?** 6. **Are the references sufficient and recent? If you have suggestion of additional references, please mention in the review form.** **(Apart from above mentioned 6 points, reviewers are free to provide additional suggestions/comments)**		
Minor REVISION comments 1. **Is language/English quality of the article suitable for scholarly communications?**		
Optional/General comments	**Findings** The findings revealed that seafood samples analyzed microbiologically were loaded with potentially pathogenic bacteria The findings of the present study showed that the organism was not recovered from the gills and flesh of the fish but was isolated from their intestine only **Highlights** • This study aimed to investigate the occurrence of potentially pathogenic Vibrio spp., Staphylococcus aureus, and Salmonella spp. in 200 seafood samples purchased from several retail outlets in Ozamiz City, Philippines • The findings revealed that seafood samples analyzed microbiologically were loaded with potentially pathogenic bacteria • The Total aerobic plate count (TAPC) value suggests that samples of shrimps, clams, head/gills, and intestines of fishes as well as squids examined in the study were considered as medium to high risk of transmitting pathogenic bacteria • This study revealed that seafood samples analyzed microbiologically could be a source of	

foodborne bacterial pathogens such as Vibrio sp

- Monitoring the contamination of potentially pathogenic bacteria in harvested seafood is vital for the sustenance of public health

Critically Analysis
INtroduction
Differs from previous work
Salmonella is associated with fish and fishery products, thereby causing a public health problem. Various authors have reported the incidence of enterotoxigenic S. aureus and Salmonella [16] in seafood
Spp
Confirmation of earlier findings
Among the Vibrio species isolated, V. parahaemolyticus was the predominant species (40.5%), followed by V. cholera (16%) and V. alginolyticus (09%). The findings of this study agree favorably with the results of several authors in a similar study conducted in Brazil, China, India, and Bursa province, Turkey [35] [36]
Spp
Confirmation of earlier findings
This result is in agreement with the work of Green [47], in which the organism was never recorded from the flesh of the fish but was documented from the viscera and epithelium. The findings of the present study showed that the organism was not recovered from the gills and flesh of the fish but was isolated from their intestine only.

Summary
INTRODUCTION
Seafood is a vital food component for a large portion of the world's population [1]. there are health risks linked to the ingestion of seafood.
Various outbreaks of bacterial diseases associated with seafood consumption have been reported in both developed and developing countries [3]
Comprise an important group of pathogenic bacteria that frequently cause human disease and even death when the contaminated food is consumed raw or improperly cooked [4].
Among more than 20 Vibrio species identified to be linked with human disease, V. cholerae, V. parahaemolyticus, and V. vulnificus are the pathogenic species of Vibrio that pose the greatest threat to human health [10]
Most of these Vibrio species secrete enterotoxins in water, foods, or the gastrointestinal tract [11].
This study aimed to examine the occurrence of potentially pathogenic Vibrio sp., S. aureus, and Salmonella sp. in fresh seafood sold from several retail outlets in the province of Misamis Occidental, Philippines, and to highlight that consumption of raw and improperly cooked sea foods may pose serious health hazards
Sample collection and processing
Two hundred (200) samples of commercially sold seafood such as clams (40), shrimps (40), squids (20), and fish [anchovy (50) and oil sardines (50)] were purchased from several retail outlets in the province of Misamis Occidental, Philippines.
The samples were aseptically removed from the container.
After skin ranging, sterile forceps and knife appropriate for opening the shell to remove the intestine and gills were used.
The shell was peeled and detached from the fresh part and intestine.
Samples of head/gills, intestines, and flesh were analyzed microbiologically, whereas only the flesh of squid was examined using standard microbiological techniques.
1g from each sample was weighed and dispensed into 9 ml of sterile distilled water and shaken vigorously to mix properly.
Ten-fold dilutions were made, and dilutions (10-6 and 10-7) were selected for microbial enumeration using the standard pour plate method by Fawole and Oso (2001) [20]
Enrichment procedures
Five (5) grams of each shellfish flesh was incised using a sterile scalpel after removal of the carapace.

	These 5 g meat samples were homogenized in 45 ml of 3% NaCl containing 1% alkaline peptone water (APW, pH: 8.6) using a sterile blender. The shellfish homogenates were incubated at 37°C for 18 h [21] **Isolation procedures and identification of the bacterial colonies** Analysis of Vibrio spp. in seafood samples was performed in triplicate. Standard procedures of the US The Food and Drug Administration (FDA) was used to detect the contamination of potentially pathogenic Vibrio species [22] with some modifications. Pure isolated colonies were subjected to Gram staining and grown at different salt concentrations by placing the colonies into tubes containing peptone water and 0%, 3%, 6%, and 10% NaCl, and these tubes were incubated at 37°C for 24 h [25]. To isolate and identify S. aureus, 10 g of each seafood sample was homogenized with 90 ml of a sterile 0.1% peptone water solution. Serial dilutions of the homogenate were performed using sterile peptone water and plated in triplicates on Baird Parker agar (Merck 1.05406). For the detection of Salmonella sp., the EN ISO 6579 reference method was used This process currently involves non-selective pre-enrichment in buffered peptone water followed by selective enrichment in Rappaport–Vassiliadis soya broth and Muller– Kauffmann tetrathionate/novobiocin broth followed by selective plating of these on Salmonella– Shigella agar. Any suspect salmonella strains that are isolated are confirmed by biochemical and serological test methods **Bacterial counts and data analysis** Following proper incubation, dilutions of 30–300 colonies were selected and counted. The number of colony-forming units per g (cfu/g) of seafood was computed by multiplying the number of bacteria by the dilution. Total aerobic plate count (TAPC), Vibrio sp., S. aureus, and Salmonella sp. Counts that were attained for cfu/g of food were converted into log values. Seafood was classified as having a no-to-low risk of transmitting pathogenic bacteria if the TAPC was 5.0 log cfu/g and if the counts of Vibrio spp., S. aureus, and Salmonella sp. If the value is at least 5.0 log cfu/g for the total bacterial counts and at least 3.0 log cfu/g for Vibrio sp., S. aureus, and Salmonella sp., the seafood is classified as medium-to-high risk of transmitting pathogen [31] [32]. Tables and figures should be placed inside the text. Tables and figures should be presented as per their appearance in the text. No tables or figures should be given without discussion or reference within the text **Occurrence of Vibrio spp** Seafood samples analyzed microbiologically showed varying degrees of bacterial contamination. Gastroenteritis is a common cause of foodborne diseases in many Asian countries such as China, Japan, and Taiwan and is recognized in the United States as the leading cause of human gastroenteritis [38] This species is associated with the consumption of insufficiently or raw cooked seafood, poor handling of seafood during preparation, and improper post-harvest storage conditions [39].This pathogen is commonly isolated from a variety of seafood including oyster, crab, scallop, lobster, shrimp, and fish [40]. Hassanin (2007) reported higher infection rates for V. alginolyticus in shrimps in Egypt This bacterium is one of the most popular Vibrio species occurring in marine environments and seafood (Neetoo et al 2022). 12% of the foodborne outbreaks related to the consumption of fish are due to bacteria, including Salmonella **Bacterial counts in seafood** The clam and shrimp samples obtained the highest TAPC values of 7.61 and 7.48, respectively These molluskan bivalves and decapod crustaceans are filter feeders, and they may be apt to hoard microbes in nearby waters, which may contain these microorganisms. They are typically grownand collected in near-shore; shallow estuarine waters [52] and are expected to harbor high concentrations of pathogenic organisms. The TAPC value suggests that samples of shrimps, clams, head/gills, and intestines of fishes as well as squids examined in the study were considered as medium to high risk of transmitting pathogenic bacteria. This toxin might lead to stomach cramps, diarrhea, and fever and is called foodborne infection	

	Findings The findings revealed that seafood samples analyzed microbiologically were loaded with potentially pathogenic bacteria. The findings of the present study showed that the organism was not recovered from the gills and flesh of the fish but was isolated from their intestine only **Conclusion** Seafood is considered an essential element in the diet and plays a vital role in the development of foodborne diseases. This study revealed that seafood samples analyzed microbiologically could be a source of foodborne bacterial pathogens such as Vibrio sp. The contamination of these bacteria could be attributed to cross-contamination from the surroundings and handling by the sellers and constitute a potential health hazard to the public. Fish and other seafood have to be properly cooked before consumption, and proper quality control measures have to be adopted in the culturing, processing, harvesting, and consumption of seafood. Monitoring the contamination of potentially pathogenic bacteria in harvested seafood is vital for the sustenance of public health. Further studies have to be conducted to include other seafood products in the microbial analyses and to examine other pathogens probably in seafood **Confirmation of earlier findings** Among the Vibrio species isolated, V. parahaemolyticus was the predominant species (40.5%), followed by V. cholera (16%) and V. alginolyticus (09%). **The findings of this study agree favorably with the results of several authors in a similar study conducted in Brazil, China, India, and Bursa province, Turkey** [35] [36] This result is in **agreement with the work of Green [47], in which the organism was never recorded from the flesh of the fish but was documented from the viscera and epithelium. The findings of the present study showed that the organism was not recovered from the gills and flesh of the fish but was isolated from their intestine only**. **Contributions** Seafood is considered an essential element in the diet and plays a vital role in the development of foodborne diseases. This study revealed that seafood samples analyzed microbiologically could be a source of foodborne bacterial pathogens such as Vibrio sp. S. aureus and Salmonella. The contamination of these bacteria could be attributed to cross-contamination from the surroundings and handling by the sellers and thus constitute a potential health hazard to the public. **Future work** Further studies have to be conducted to include other seafood products in the microbial analyses and to examine other pathogens probably in seafood.	

PART 2:

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

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Reviewer Details:

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