Formatted: Font: (Default) Arial, 18 pt, Bold, Kern at 14 pt

Occurrence of potentially pathogenic bacteria in commercially sold seafood from Misamis Occidental, Philippines

ABSTRACT

Seafood is a nutritious food that is an enviable componentestablishes one of the enviable components of a healthy diet.Nevertheless, there areis health risks associatedlinked with the consumption of seafood.
This study aimed to investigate the occurrence of potentially pathogenic *Vibrio* spp., *Staphylococcus aureus*, and *Salmonella*sppsp. in 200 seafood samples purchased from several retail outletsretailed outlet in Ozamiz City, Philippines.Different samples analyzed using standard microbiological techniques were the clam, shrimp, squid, and and fish (anchovy and oil sardines).The *Vibrio* species identified and isolated were *V. parahaemolyticus* (47%), *V. cholerae* (16%) and *V. alginolyticus* (9%).Thirty percent (30%) of the samples analyzed were positive for *S. aureus* and 9% of the samples were contaminated with *Salmonella* sp.The total aerobic plate count mean $\log_{10}$cfu/g of clams, shrimps, head/gills, intestine of fishfishes (oil sardine, anchovy), and squids were considered as medium to high risk of transmitting pathogenic bacteria.Moreover, the clams and the head/gills of oil sardines were considered to be atas medium to high risk of transmitting pathogenic *V. parahaemolyticus*.The findings revealed that seafood samples analyzed microbiologically were loaded with potentially pathogenic bacteria.This result is of public health concern becauseas these organisms are documented as causes of food-borne diseases.
Keywords:clam, fish, food-born pathogen, shrimp, squid

1.INTRODUCTION

Seafood is a vitalvital food component for a large portion of the world's populationsection of world populace[1].However, there areis health risks linked to the ingestion of seafood.One of the main risks involves the consumption of improperly cooked or raw seafood that might be contaminated by foodborne pathogens present in the marine environment [2].Various outbreaks of bacterial diseasesdisease associated with seafood consumptionthe consumption of seafood have been reported infor both developed and developingcountries [3].From these seafood-borne bacteria, *Vibrio* spp. *S. aureus*, and *Salmonella*sp. comprise comprise an important group of pathogenic bacteria that frequently causecauses human disease and even death when the contaminated food is consumed raw or improperly cooked [4].

In the Asian region, *Vibrio*spp. has been recognized as the leading cause of foodborne outbreaks in various countries, including including Japan, India, China, Korea, and Malaysia [5] [6].Investigations have showns**how** that many outbreaks may lead to acute gastroenteritis, including including diarrhea, headache, vomiting, nausea, and fever, and are and fever and were caused by ingestion of infected seafood [7].For food samples, temperature abuse may be instigated by improper storage or a long holding time on the exhibit rack at the retail level without proper temperature control [8] [9].Among more than 20 Vibrio species identified to be linked with human disease, *V. cholerae*, *V. parahaemolyticus*, and 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 in the gastrointestinal tract [11].

Aside fromfor*Vibriospp.*, several several bacteria in seafood may cause infection into humans.Examples of these bacteria that may cause foodborne illness are *Salmonella*, *Staphylococcus, Shigella,* and *Listeria* [12].*S. aureus* is one of the most popular sources of gastroenteritis worldwide, which is caused bydue to the ingestion of food that containshaspreformed

toxins.Humans are common carriers of *S. aureus* in ~~the~~ nose, throat, and skin infections.Therefore, the organism can be ~~just~~ transmitted to seafood during handling [13].

Seafood is not the usual habitat of *Salmonella*, but ~~still,~~ the occurrence of *Salmonella* in seafood is escalating.Hence, fishery products have been recognized as major spreaders~~a major spreader~~ of foodborne pathogens [14][15].*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.Infection with these bacteria may affect only the intestines or may spread from the intestinal tract to the blood vessels and then to other body sites.

Although~~Though~~ several technological alternatives that could augment the quality of the food supply have been implemented~~practiced, yet~~, instances of food-borne diseases caused by consumption of seafood still emerge.In Iligan City, Philippines [17], an ~~an~~ outbreak of cholera ~~had~~ killed two people.Health authorities have begun a series of tests~~test~~ of water samples in the villages.Other reports said some of the~~those~~ infected individuals were associated with the consumption of improperly cooked seafood~~seafoods~~, particularly oysters~~oyster~~.
In the provinces of Zamboanga del Sur, Lanao del Norte, and Misamis West~~Occidental~~ Philippines, massive mortality~~mortalities~~ were observed among pond-cultured shrimp due to *Vibrio harveyi*, which ~~that~~ was isolated purely from the lymphoid organs and hepatopancreas of affected shrimp, and ~~and~~ histopathological observations indicated a severe bacterial infection [18].Disease problems have been recorded~~recorded~~ in shrimp– -finfish polyculture in Misamis West~~Occidental~~ due to bacteria [19].Nevertheless, studies on the presence of potentially pathogenic bacteria in seafood~~on seafoods~~ are scarce.This study aimed to examine the occurrence of potentially pathogenic *Vibrio* sp.*, S. aureus*, and *Salmonella*sp. in~~ in~~ fresh seafood sold from several retail~~retailed~~ outlets in the province of Misamis Occidental, Philippines, and ~~and~~ to highlight that consumption of raw and ~~ingestion of~~ improperly cooked sea foods may pose serious health hazards.~~hazards~~

2. MATERIAL AND METHODS/EXPERIMENTAL~~METHODS / EXPERIMENTAL~~DETAILS/METHODOLOGY~~DETAILS / METHODOLOGY~~

2.1 Sample collection and processing

Two hundred (200) samples of commercially sold seafood such as clams (40), shrimps (40), squids (20), and~~ and~~ fish [anchovy (50) and oil sardines (50)] were purchased from several retail~~retailed~~ outlets in the province of Misamis Occidental, Philippines.Samples were placed in sterile, labeled, sealed plastic bags and ~~were~~ transported to the laboratory under refrigerated conditions~~the refrigerated condition~~ for analysis.The samples were aseptically removed from the container.After ~~the~~ skin ranging, sterile forceps~~a sterile forceps~~ and knife appropriate for opening the shell to remove the intestine and gills were used.For shrimp samples, the shell was peeled and detached from the fresh~~freshly~~ part and intestine.For fishes, samples of head/gills, intestines, and flesh were analyzed microbiologically, whereas ~~while~~ only the flesh of squid was examined using standard microbiological techniques.For the clams, the flesh and intervalve water were used for bacterial analyses.Approximately~~About~~ 1g from each sample was weighed and dispensed into 9 ml~~9ml~~ of sterile distilled water and ~~will be~~ 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].

2.2 Enrichment procedures

Five (5) grams of each shellfish flesh was~~were~~ 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~~with the use of~~ a sterile blender.The shellfish homogenates were incubated at 37°C for 18 h [21].

2.3 Isolation procedures and identification of the bacterial~~bacterial~~ colonies

Analysis of *Vibriospp*. in seafood samples was performed in triplicate~~triplicates~~.Standard procedures of the US~~U.S.~~The Food~~Food~~ and Drug Administration (FDA) was~~were~~ used to detect the contamination of potentially pathogenic *Vibrio* species [22] with some modifications.Following incubation, ~~the~~ shellfish homogenates were inoculated using an inoculating loop on thiosulfate citrate bile salt~~Thiosulfate Citrate Bile salts~~ sucrose agar media (TCBS, Hi-Media, India) and incubated at 37°C for 18 h [23].For each seafood sample, 25g was~~were~~ homogenized in 225 ml~~225ml~~ sterile 0.1% peptone water using a sterile blender.A 10-fold~~10 fold~~ serial dilution was prepared in sterile 0.1% peptone water as described in the Bacteriological Analytical Manual [24].The spread~~Spread~~ plate method was carried out using 10 and ten dilutions on TCBS agar.The TCBS plates were incubated at 37°C for 18–24 h,~~18-24 h~~ and counts were made for each colony type.Discrete colonies were aseptically subcultured~~sub-cultured~~ into fresh agar plates to obtain pure~~get pure cultures of~~ colonies.

Colonies recognized as discrete were carefully examined macroscopically for cultural characteristics.Pure isolated colonies were subjected to Gram staining and growngrowing at different salt concentrations by placingputting the colonies into tubes containingwhich contain peptone water and 0%, 3%, 6%, and and 10% NaCl, and these tubes were incubated at 37°C for 24 h [25].In additionAlso, all bacterial colonies from different samplesgrown; growing on TCBS plates were chosen to be streaked on the surface of trypticase soya agarTrypticase Soya Agar slants (TSA; Oxoid, UK) added with 2% NaCl and incubated for 24 hhours at 37°C.Further identification of Vibrio sp. was performedcarried out using physiological, morphological, and and different biochemical tests available in the laboratory.The isolates were identifiedIdentification of the isolates was performed by comparing their characteristics with those of recognized taxa, as illustrated and described by [26] [27] [28] [29].To isolate and identify *S. aureus*, 10 g of each seafood sample wassamples were homogenized with 90 ml of a sterile 0.1% peptone water solution.Serial dilutions of the homogenate were performed usingwith sterile peptone water and plated in triplicates on Baird Parker agar (Merck 1.05406).After incubation at 35 °C35 ºC for 48 h, the distinctive black colonies with apparent halowith apparent halo colonies of *S. aureus* were subjected to Gram staining procedure to clarify bacterial morphology.Staphylococci are spherical Gram-positive bacteria that occur in microscopic clusters).*S. aureus* forms a relatively large yellow colony on rich medium, whereas whileS. *epidermidis* has a comparatively small white colony [30].

For the detection of *Salmonella* sp., the ENEN 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-Vassiliadis Soya Broth and Muller– -Kauffmann tetrathionate/novobiocin broth followed by selective plating of these on Salmonella– -Shigella agar.Incubation was made at 35°C, and examine after 24-48 h for colonies resembling *Salmonella sp*. or *Shigella sp*. *Salmonella* will produce hydrogen sulfide (H_2S) gas but will not ferment lactose. The resulting bacterial colonies will appear colorless with black centers.Any suspect salmonella strains that are isolated are thenthen confirmed by biochemical and serological test methods.

2.4 Bacterial counts and data analysis

Following proper incubation, dilutions ofwith 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 counts that were attained for cfu/g of food were converted into $\log_{10}$ values.Seafood was classified as having a no-to-low risk of transmitting pathogenic bacteria if the TAPC was lesser than 5.0 $\log_{10}$cfu/g and if the counts of Vibrio spp., S. aureus, and Salmonella sp. were less than 3.0 $\log_{10}$cfu/g.Furthermore, if the value is at least 5.0 $\log_{10}$cfu/g for the total bacterial counts and at least 3.0 $\log_{10}$cfu/g for *Vibrio* sp., *S. aureus*, and *Salmonella* sp., the seafood is classified as medium-to-high risk of transmitting pathogen [31][32].

ProvideGive adequate information to allow the experiment to be reproduced.PreviouslyAlready published methods should be mentioned with references.Significant modifications of the publishedpublished methods and new methods should be described in detail.This section includeswill include sub-sections.Tables and& figures should be placed inside the text.Tables and figures should be presented as per their appearance in the text.It is suggested thatathe discussion about the tables and figures should appear in the text before the appearance of the respective tables and figures.No tables or figures should be given without discussion or reference withininside the text.

Comment [Trinka1]: Passive voice: This action (computed) seems to have a 'doer' or 'agent'. Consider using the active voice. For example, the sentence 'The risk of osteoporosis was decreased by a higher vitamin C intake' is in the passive voice; it can be changed to the active voice like this: 'A higher vitamin C intake decreased the risk of osteoporosis.'

3. RESULTS AND DISCUSSION

3.1 The Occurrence of *Vibrio spp.*

Seafood samples analyzed microbiologically in this study showed varying degreesdegree of bacterial contamination.The results on the contamination of *Vibrio* spp. are summarized in Tables 1a andTable 1a & 1b.Of the 200 seafood samples tested, 65.5% were contaminated with potentially pathogenic *Vibrio* spp.The high incidence of *Vibrio* spp. could be due to the nature of these bacteria, which are which is known as halophilic waterborne and commonly dwellwater-borne that commonly dwells in environmental water sourcessource worldwide.It has been found that brackish water, as well as marine environments , and freshwaterand freshwater, may support the growth of these organisms, which which are pathogenic to humans [33].

Table 1a Distribution and frequency of occurrence of *Vibrio* spp., isolates from fresh seafood samples.

Samples	No.Tested (%)	No.Positive for Vibrio spp
FISH		
Anchovy	50 (25)	39 (78)
Sardines	50 (25)	42 (84)
SHRIMP	40 (20)	18 (45)
CLAM	40 (20)	22 (55)

SQUID	20 (10)	10 (50)
Total	200 (100)	131 (65.5)

Table 1b.Distribution and frequency of occurrence of *Vibrio* spp.~~,~~ isolates from fresh seafood samples.

Seafood Samples	Number (%) of positive samples			
	V. parahaemolyticus	*V. chloreachlorea~~e~~*	*V. alginolyticus*	Total Positive for *VibrioVibrios* (%)
FISH				
Anchovy				
Gills	14 (28)	06 (12)	04 (08)	24 (48)
İntestine	05 (16)	04 (08)	02 (04)	11 (22)
Meat	04 (08)	00 (00)	00 (00)	04 (08)
SARDINES				
Gills	16 (40)	04 (08)	02 (04)	22 (44)
İntestine	8 (20)	02 (04)	02 (04)	12 (24)
Meat	06 (12)	02 (04)	00 (00)	08(16)
SHRIMP	10 (25)	06 (15)	02 (05)	18(45)
CLAM	12 (30)	06(15)	04(10)	22(55)
SQUID	06 (30)	02 (10)	02 (10)	10(50)
Total number (%) of positive samples	81 (40.5) /200	32 (16)/200	18 (09) /200	131(65.5)

The samples that harbored most of the *Vibrio* spp. were the fish in which the gills obtained most of the isolates.This could be due to the gill physiology, whichgills physiology which serves as a sieve that filters water that comes into the fish from their water ecosystem.Fish could be predisposed to gill disease because the gillgill is used for the exchange of gasses, and and the fish will be hassled because of the intricacyintricacy in the exchange due to the heavyheavy load of microbes in water, causing then the gill is to be infected [34].

Among the *Vibrio* species isolated, *V. parahaemolyticus* was the predominant species ((40.5%), followed 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 and Bursa province, Turkey [35][36].On the other hand, varying rates have beenwere reported in some other studies.In Nigeria, *V. chlorease~~chloreae~~* was the most frequently isolated *Vibrio* sp. followed by *V. parahaemolyticus*[37].

The predominant *V. parahaemolyticus* is ~~a~~ salt tolerant but very sensitive to heat and is destroyed by cooking.However, gastroenteritis~~this~~ 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~~linked~~ with the consumption of insufficiently or raw cooked seafood, poor handling of seafood during preparation, and and improper post-harvest storage conditions [39].This pathogen is commonly isolated from a variety of seafood including oyster, crab, scallop, lobster, shrimp, and and fish [40].

In this~~the present~~ study, 16% of the samples tested were positive for~~showed positive of~~*V. cholera~~cholerae~~.This bacterium favors alkaline surroundings to thrive well [41].Perhaps the presence of this bacterium was linked to the low salinity in various locations due to the influx of freshwater into~~to~~ the ocean.This species can cause cholera and is typically a waterborne illness.Food-borne cases, primarily primarily fish outbreaks, are are also known carriers~~carrier~~ of this microorganism.

In this study, the prevalence of *V. alginolyticus* in seafood was 9%.This finding was ~~nearly~~ close to the result of Baffoneet al.et al. ((2006), who reported 8.16% in clams in Italy.In contrast, Hassanin (2007) reported~~cited~~ higher infection rates for *V. alginolyticus* in shrimps in Egypt.This bacterium is one of the most popular *Vibrio* species occurring in ~~the~~ marine environments and seafood (Neetooet al.~~et al~~ 2022). *V. alginolyticus* is an opportunistic pathogen, and its pathogenicity is thought to be similar to that of *V. parahaemolyticus*[42].In India and Taiwan, this *Vibrio* species is linked with a white spot in shrimp while the zoonotic risk of this pathogen has been implicated in soft tissue, wound and ear infections in humans~~human~~[43].*Vibrio* spp. and several pathogenic species are ~~known to be~~ commonly associated with outbreaks of *Vibrio* infections due to ingestion of raw or improperly cooked seafood, water contaminated with human feces or sewage, inappropriate post-harvest storage conditions, or ~~or~~ poor handling of seafood during preparation.

3.2 Occurrence~~The occurrence~~ of S. aureus and Salmonella sp.

The incidence of *S. aureus* in this study was found to be higher than *that of Salmonella~~Salmonella~~* sp. *S. aureus* was detected in 30% of the 200 raw samples of seafood, whereas the incidence of *Salmonella*sp was 9%.The head/gills of fish~~fishes~~ (oil sardines & anchovy) were heavily loaded with *S. aureus*.Four isolates of *S. aureus* were obtained from shrimp samples, three ~~isolates~~ from clams, and two ~~and two isolates~~ from squids (Table 2).

It is well documented that freshly caught seafood is~~are~~ free from *S. aureus*, and contamination occurs~~takes place~~ upon handling [44].Other conditions~~,~~ such as poor personal hygiene, inadequate refrigeration, and delay in processing are associated with staphylococcal contamination.*S. aureus* is a major cause of food poisoning and~~as well as of~~ a range of extraintestinal infections~~extraintestinal infection~~[45].Staphylococcal food poisoning is caused by a preformed enterotoxin produced by the growth of~~growth of the~~ bacteria in food that is ingested [46].

The overall prevalence of *Salmonella* sp. in 100 seafood samples tested was in agreement with~~to~~ that reported elsewhere.The findings of the~~this~~ present study showed that the organism was not recovered from the gills and flesh of the fish~~,~~ but was isolated from their intestine only.This result is in agreement with the work of Green [47], in~~in~~ which the organism was never recorded from the flesh of the fish~~,~~ but was documented from the viscera~~viscera~~ and epithelium.Several scientists believe that fish are possible carriers of *Salmonella*, which accumulate ~~which are accumulated~~ in their intestines for somewhat short periods of time, and ~~and~~ some believe that fish become~~get~~ actively infected by *Salmonella*[48][49].Furthermore, the mollusk and ~~the~~ crustacean samples also harbored colonies of *Salmonella*.

The possible cause of *Salmonella* contamination in seafood might be ~~due to~~ farm runoff and fecal contamination from nearby domesticated or wild animals and poor water quality.In addition to poor distribution, retail marketing, handling, and high stocking, preparation practices, densities, and ~~and~~high water temperature may be responsible for increased Salmonella contamination~~contamination of Salmonella~~ in shrimp [50](Loest et al.~~et al~~ 2022).Other investigations of~~concerning~~ live bivalve mollusks confirmed that this bacterium could endure coastal water salinity.The bacteria may be found in 0%–2.5~~found in 0%-2.5~~% of live shellfish, and ~~and~~ intake of these foods may cause salmonellosis, characterized by enteric fever along with gastroenteritis and diarrhea.Similarly, the United States~~U.S.~~Food and Drug Administration~~'s (FDA)~~ data revealed that Salmonella was the most common contaminant of fish and fishery products~~the most common contaminant of fish and fishery products was *Salmonella*~~[51].Approximately~~About~~ 12% of the foodborne outbreaks related to the consumption~~consumption~~ of fish are due to bacteria, including ~~including~~*Salmonella*.

Comment [Trinka2]: Passive voice: This action (caused) seems to have a 'doer' or 'agent'. Consider using the active voice. For example, the sentence 'The risk of osteoporosis was decreased by a higher vitamin C intake' is in the passive voice; it can be changed to the active voice like this: 'A higher vitamin C intake decreased the risk of osteoporosis.'

Table 2.Distribution and frequency ~~of occurrence~~ of *Salmonella spp~~sp~~*. and *S. aureus* isolates from fresh seafood samples.

Seafood Samples	Number (%) of positive samples/number~~samples / number~~ of samples tested	
	Salmonella sp.	*S. aureus*
FISH		
Anchovy		
Gills	00 (00) /50	08 (16)/50
İntestine	02 (04) /50	06 (12)/50
Meat	00 (00) /50	04 (08)/50
Sardines		
Gills	00 (00) /50	12(24)/50
İntestine	04 (08) /50	04/(08)50
Meat	00 (00) /50	08(16)/50
SHRIMP	04 (10) /40	06(15)/40
CLAM	06(15)/40	08(20)/40
SQUID	02 (10) /20	04(20)/20
Total number (%) of positive samples/total~~Total~~number of samples tested	18 (09) /200	60 (30) /200

3.3Bacterial counts in seafood~~Counts in Seafood~~

The mean log_{10}cfu/g of *Vibrio* spp., *S. aureus,and Salmonella~~Salmonella~~*sp. and total aerobic plate counts (TAPC) values are~~were~~ presented in Table 3.The clam and shrimp samples~~sample of clams and shrimps tested~~ obtained the highest TAPC values~~value~~ of 7.61 and 7.48, respectively~~ respectively~~.These molluskan~~molluscan~~ bivalves and decapod~~decapods~~ crustaceans are filter feeders, and they may be apt to hoard microbes in ~~the~~ nearby waters, which ~~which~~ may also contain these microorganisms.They are typically grown~~ up~~ and collected in near-shore; shallow estuarine waters [52] and are therefore expected to harbor high concentrations of pathogenic organisms.If this seafood is eaten after a very mild heat treatment or raw, it poses~~they pose~~ a significant health risk to ~~the~~ consumers.The seafood that~~which~~ may be linked with human infection caused by *V. parahaemolyticus* are bivalve mollusks, crustaceans,~~crustacean~~ and finfish, especially ~~especially~~ those served raw or superficially cooked and ~~which have been~~ exposed to temperature abuse[53].Of the fishes, the head/gills of oil sardines and anchovy harbored considerable TAPC values~~value~~ of 7.42 and 6.78, respectively.~~.~~

The TAPC value suggests that samples of shrimps, clams, head/gills, and ~~and~~ intestines of fishes as well as squids examined in the study were considered as ~~a~~ medium to high risk of transmitting pathogenic bacteria (not within acceptable limits, i.e. <5.0 log10 cfu/g for total counts).Moreover, the clams and ~~the~~ head/gills of oil sardines were also considered to be at~~as~~ medium to high risk of transmitting pathogenic *V. parahaemolyticus*.From the zoonotic point of view, contamination of potentially pathogenic bacteria in all samples of seafood tested suggests a possible risk to the health of people consuming raw or improperly cooked seafood.Naturally,~~Naturally~~, fish ~~fish~~ and other seafood have immunity to~~for~~ infections, but their immunity decreases when the concentration of ~~the~~ microorganisms is on the increase [54].Bacteria in seafood improperly cooked could be transmitted to humans~~man~~ as they establish themselves in the intestine, particularly ~~particularly~~ those potentially pathogenic, causing problems, including ~~causing problems including~~ diseases of various kinds.These bacteria enter ~~into~~ the body and then discharge endotoxins that~~which~~ irritate the stomach and ~~the~~ bowels.This toxin might lead to stomach cramps, diarrhea, and ~~diarrhea and~~ fever and is called foodborne infection.

Comment [Trinka3]: Consider reviewing or deleting this word, depending on the context. Such words are called hedge words as they are used to reduce the certainty or directness of an argument. If used unnecessarily, it can lessen the impact of your message. Use such words selectively.

Comment [Trinka4]: Consider reviewing or deleting this word, depending on the context. Such words are called hedge words as they are used to reduce the certainty or directness of an argument. If used unnecessarily, it can lessen the impact of your message. Use such words selectively.

Table 3 Total aerobic plate counts, *Vibrio*spp.~~spp~~, *S. aureus*, and *Salmonella* spp. counts ~~counts~~mean log_{10}cfu/g (ml) ± standard deviation of seafood~~Seafoods~~ in Ozamiz City.

Seafood Item	n	TAPC	*Vibrio* isolates				
			V. parahaemolyticus	*V. chloreae~~chloreae~~*	*V. alginolyticus*		
Fish							
Anchovy	25						
head/gills		6.78±1.7	2.87±1.0	1.23±0.7	1.02±0.6	1.19±0.4	00
intestine		5.60±0.5	1.14±0.9	1.70±0.9	0.37±0.6	1.10±0.5	1.66±1.2
meat		3.64±2.0	1.05±1.1	00	00	1.20±0.1	00
Oil Sardines	25						
head/gills		7.42±2.1	3.04±0.4	1.21±1.0	1.03±0.1	2.19±0.5	00
intestine		5.90±1.9	1.06±0.5	1.07±1.6	1.87±0.6	1.19±0.5	1.15±0.3
meat		3.51±1.0	1.04±0.7	1.02±0.8	00	1.05±0.5	00
Shrimp	20	7.48±1.8	2.69±0.7	1.10±0.7	0.87±0.8	1.73±1.1	1.06±1.8
Clam	20	7.61±1.5	3.14±0.5	1.05±0.4	0.89±0.4	1.93±0.5	1.59±0.2
Squid	10	5.55±0.9	1.20±0.2	1.02±0.2	1.04±0.9	1.14±1.	1.01±0.

4.CONCLUSION

Seafood is considered ~~as~~ an essential element in the diet and plays a vital role in the development~~appearance~~ of foodborne diseases.This study revealed that seafood samples ~~that were~~ 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~~surroundings,~~ handling by the sellers and thus~~,~~ constitute a potential health hazard to the public.

Nevertheless, fish~~fishes~~ and other seafood have to be properly cooked before consumption, and proper quality control measures have to be adopted in the culturing~~culturing~~, processing, harvesting, and ~~and~~ consumption of seafood.Therefore, monitoring ~~of~~ the contamination of potentially pathogenic bacteria in harvested seafood is vital for the sustenance~~sustenance~~ of public health.Further studies have to be conducted to include other seafood products in the microbial analyses and to examine~~examine~~ other pathogens probably ~~present~~ in seafood.

REFERENCES

1. Gopal TS. Foods from the ocean for nutrition, health, and wellness. In Nutritional and Health Aspects of Food in South Asian Countries Academic Press.2020;113-122.
2. Song X, Zang J, Yu W, Shi, X, Wu, Y. Occurrence and identification of pathogenic *Vibrio* contaminants in common seafood available in a Chinese traditional market in Qingdao, Shandong Province. Frontiers in microbiology. 2020 11 (1488).
3. Ndraha N, Wong HC, Hsiao HI. Managing the risk of *Vibrio parahaemolyticus*infections associated with oyster consumption: A review. Comprehensive reviews in food science and food safety. 202019(3), 1187-1217.
4. Gautam RK, Venugopal V. Electron beam irradiation to control biohazards in seafood. Food Control. 2021 130, 108320.
5. Tunung R, Margaret SP, Jeyaletchumi P, Chai LC, Zainazor TC, Ghazali FM, Nakaghuchi Y, Nishibuchi M, Son R. Prevalence and quantification of *Vibrio* in raw salad vegetables at retail *Vibrio parahaemolyticus* from fresh, low-temperature preserved, dried and salted seafood products.2021.
6. Loo KY, Letchumanan V, Law JWF, Pusparajah P, GohBH, Ab Mutalib NS, Lee LH. Incidence of antibiotic resistance in Vibrio spp. Reviews in Aquaculture. 2020 12(4), 2590-2608.
7. Gourama, H., 2020 Foodborne pathogens. Food safety engineering, 25-49.
8. Canini, N. D., Bala, J. J. O., Maraginot, E. N., Mediana, B. C. B., Evaluation of Street Food Vending in Ozamiz City. Journal of Multidisciplinary Studies. 2013 1(1).
9. Zhou, B., Luo, Y., Huang, L., Fonseca, J. M., Yan, H., Huang, J., 2022 Determining effects of temperature abuse timing on shelf life of RTE baby spinach through microbial growth models and its association with sensory quality. Food Control, 133, 108639.
10. Mok, J. S., Ryu, A., Kwon, J. Y., Park, K., Shim, K. B. Abundance, antimicrobial resistance, and virulence of pathogenic Vibrio strains from molluscan shellfish farms along the Korean coast. Marine Pollution Bulletin. 2019 149, 110559.
11. Bonnin-Jusserand, M., Copin, S., Le Bris, C., Brauge, T., Gay, M., Brisabois, A., Midelet-Bourdin, G. Vibrio species involved in seafood-borne outbreaks (*Vibrio cholerae, V. parahaemolyticus and V. vulnificus*): Review of microbiological versus recent molecular detection methods in seafood products. Critical reviews in food science and nutrition. 2019 59(4), 597-610.
12. Owusu-Apenten, R., Vieira, E. Microbial Foodborne Disease Outbreaks. In Elementary Food Science 2022 (pp. 171-196). Cham: Springer International Publishing.
13. Dai, J., Huang, J., Wu, S., Zhang, F., Li, Y., Rong, D., Wu, Q. Occurrence, Antibiotic Susceptibility, Biofilm Formation and Molecular Characterization

of *Staphylococcusaureus* Isolated from Raw Shrimp in China. Foods. 2023; 12(14), 2651.
14. OlgunoğluİA.*Salmonella*in Fish and Fishery Products.University of Adiyaman Vocational School of Kahta Turkey.www.intechopen.com. ISBN. 2012: 978-953-307-782-6.
15. Parlapani, F. F., Boziaris, I. S., DeWitt, C. A. M.Pathogens and their sources in freshwater fish, sea finfish, shellfish, and algae. In Present Knowledge in Food Safety. 2023 (pp. 471-492). Academic Press.
16. Sorsa, M., Mamo, G., Abera, L. Major fish-borne bacterial and parasitic zoonoses in Ethiopia: A review. Int. J. Fauna Biol. Studies. 2019: 6, 50-58.
17. Man, D.On cholera outbreak. The Mindanao Examiner Regional Newspaper. 2008.
18. Pena, L. D. D. L., Lavilla-Pitogo, C. R. Mortality in pond-cultured shrimp *Penaeus monodon* in the Philippines associated with *Vibrio harveyi* and white spot syndrome virus. 2003: *38*(2), 59-61.
19. Fitzsimmons, K. Bolivar R. JunRey Sugue. Survey of Tilapia-Shrimp Polycultures inthe Philippines (10NSR3E). Final Report. 2003: 14-15.
20. Fawole, M.O., Oso B.A.Laboratory manual of Microbiology: Revised edition pectrumbooks Ltd. Ibadan. 2001: 127.
21. Wafaa M.K.B., Walaa A.H., Amani F.A. Detection of *Salmonella* and *Vibrio* species in some seafood in Alexandria. J. American Science. 2011; 7(9): 663-668.
22. Nordstrom, J. L., Kaysner, C. A., Blackstone, G. M., Vickery, M. C. L., Bowers, J. C., & DePaola, A. Effect of intertidal exposure on Vibrio parahaemolyticus levels in Pacific Northwest oysters. *Journal of food protection*. 2004; *67*(10), 2178-2182.
23. Wafaa M.K.B., Walaa A.H., Amani F.A. Detection of *Salmonella* and *Vibrio* species in some seafood in Alexandria. J. American Science. 2011; 7(9): 663-668.
24. BAM., 1998. Bacteriological Analytical Manuel 8 Ed, Garthersbury, M.D U.S.A.
25. Lahfi S.K., Kuhne M. Occurrence of *Vibrio* spp. in blue mussels (*Mytilus edulis*) from the German Wadden Sea. International Journal of Food Microbiology. 2007;116: 297-300.
26. Jolt J.G., Krieg N.R., Sneath P.H.A., Stanley J.T., Williams S.T. Bergey's manual ofsystematic bacteriology, 9 ed. Williams Wilkins Co. Baltimore, Maryland, pp: 786. Journal of Food Microbiology. 1994; 126: 71-75.
27. Cheesbrough M. District laboratory practice in tropical countries. Cambridge University Press. 2006; 434.
28. FAO/WHO.FAO/WHO guidance to Biotechnology. 2007; 24: 2045-2054. Governments on the application of HACCP in small and/or less-developed food businesses.
29. Oyeleke S.B., Manga S.B. Essentials of Laboratory Practicals in Microbiology. Tobest publisher, Minna, Nigeria. 2008; 36-75.
30. Todar K. Online Textbook of Bacteriology. Retrieved from www.textbookof bacteriology.net. 2012; 1.
31. Mensah P., Manu D.Y., Darko K.O., Ablordey A. Street foods in Accra, Ghana: how safe are they?.Bulletin of the World Health Organization. 2002; 80 (7), 546–554.
32. Jans, C., Sarno, E., Collineau, L., Meile, L., Stärk, K. D., & Stephan, R. Consumer exposure to antimicrobial resistant bacteria from food at Swiss retail level. *Frontiers in microbiology*. 2018; *9*, 362.
33. Harrison, J. P., Hoellein, T. J., Sapp, M., Tagg, A. S., Ju-Nam, Y., Ojeda, J. J.Microplastic-associated biofilms: a comparison of freshwater and marine environments. Freshwater microplastics: emerging environmental contaminants?2018; 181-201.
34. Megarajan, S., Ranjan, R., Xavier, B., Ghosh, S.Training Manual on Cage Culture of Marine Finfishes. 2016; 7-12.

35. Dash, H. R., Mangwani, N., Chakraborty, J., Kumari, S., & Das, S. Marine bacteria: potential candidates for enhanced bioremediation. Applied microbiology and biotechnology. 2013; 97(2), 561-571.
36. Mus, Tulay Elal., Figen Cetinkaya., Umut C.Occurrence of *Vibrio*, *Salmonella* and *Staphylococcus aureus* in retail fresh fish, mussel and shrimp. *ACTA VET. BRNO* 2014,83:75–78; doi:10.2754/avb201483020075
37. Bonnin-Jusserand, M., Copin, S., Le Bris, C., Brauge, T., Gay, M., Brisabois, A., Midelet-Bourdin, G. Vibrio species involved in seafood-borne outbreaks (*Vibrio cholerae, V. parahaemolyticus and V. vulnificus*): Review of microbiological versus recent molecular detection methods in seafood products. Critical reviews in food science and nutrition 2019; 59(4), 597-610.
38. Letchumanan, V., Loo, K. Y., Law, J. W. F., Wong, S. H., Goh, B. H., Ab Mutalib, N. S.,Lee, L. H.,*Vibrio parahaemolyticus*: The protagonist of foodborne diseases. Progress In Microbes & Molecular Biology 2019; 2(1).
39. Bedane, T. D., Agga, G. E., Gutema, F. D., Hygienic assessment of fish handling practices along production and supply chain and its public health implications in Central Oromia, Ethiopia. Scientific Reports 2022. 12(1), 13910.
40. Roobab, U., Fidalgo, L. G., Arshad, R. N., Khan, A. W., Zeng, X. A., Bhat, Z. F., Aadil, R. M. High-pressure processing of fish and shellfish products: Safety, quality,and research prospects. Comprehensive reviews in food science and food safety 2022; 21(4), 3297-3325.
41. Kostiuk, B., Becker, M. E., Churaman, C. N., Black, J. J., Payne, S. M., Pukatzki, S., Koestler, B. J. *Vibrio cholerae* Alkalizes Its Environment via Citrate Metabolism to Inhibit Enteric Growth In Vitro. Microbiology Spectrum 2023; 11(2), e04917-22.
42. Cai, H., Yu, J., Li, Q., Zhang, Y., Huang, L. Research Progress on Virulence Factors of *Vibrio alginolyticus*: A Key Pathogenic Bacteria of Sepsis.2022.
43. Wodajo, W. Review on Zoonotic Bacterial Diseases of Fish. ARC Journal of Animal and Veterinary Sciences. 2020; 6(1), 1-9.
44. Stratev, D., Stoyanchev, T., Bangieva, D. Occurrence of *Vibrio parahaemolyticus* and *Staphylococcus aureus* in seafood. Italian Journal of Food Safety. 2021; 10(4).
45. Zhang, P., Miao, X., Zhou, L., Cui, B., Zhang, J., Xu, X., Wang, X. Characterization ofoxacillin-susceptible mecA-positive *Staphylococcus aureus* from food poisoning outbreaks and retail foods in China. Foodborne Pathogens and Disease. 2020 17(11), 728-734.
46. Pal, M., Ketchakmadze, D., Durglishvili, N., Ketchakmadze, K. *Staphylococcus aureus*: A major pathogen of food poisoning: A rare research report. Nutrition Food Process. 2022; *5*(1), 1-3.
47. Green, B. W. Effect of channel catfish stocking rate on yield and water quality in an intensive, mixed suspended-growth production system. North American journal ofaquaculture. 2010; 72(2), 97-106.
48. Mol S., Cosansu S., Alakavuk D.U., Ozturan S. Survival of *Salmonella enteritidis* during salting and drying of horse mackerel (*Trachurus trachurus*) fillets. International Journal of Food Microbiology. 2010; 139: 36–40.
49. OlgunoğluİA. *Salmonella* in Fish and Fishery Products.University of Adiyaman Vocational School of Kahta Turkey.www.intechopen.com. ISBN 2012; 978-953-307- 782-6.
50. Loest, D., Uhland, F. C., Young, K. M., Li, X. Z., Mulvey, M. R., Reid-Smith, R., Carson, C. A. Carbapenem-resistant *Escherichia coli* from shrimp and salmon available for purchase by consumers in Canada: a risk profile using the Codex framework. Epidemiology & Infection. 2022; 150,

e148.
51. Shen, A. Q., 2023 Multistate Outbreak of Salmonella Thompson Infections Linked to Seafood Exposure—United States, 2021. MMWR. Morbidity and Mortality Weekly Report, 72.
52. Bauer, R. T., 2023 Symbioses. In Shrimps: Their Diversity, Intriguing Adaptations and Varied Lifestyles (pp. 515-581). Cham: Springer International Publishing.
53. Adeyemo, O. K., Foyle, L. Public Health, Zoonoses, and Seafood Safety. *Fundamentals of Aquatic Veterinary Medicine* 2021; 181-204.
54. Rohani, M. F., Islam, S. M., Hossain, M. K., Ferdous, Z., Siddik, M. A., Nuruzzaman, M.,Shahjahan, M. Probiotics, prebiotics and symbiotic improved the functionality of aquafeed: Upgrading growth, reproduction, immunity and disease resistance in fish. Fish & shellfish immunology. 2022; 120, 569-589.

TRINKA

REVISION SUMMARY

File name: Ms_EJNFS_112884.docx
(Run on 28 Jan 2024, 04:26:44 PM GMT)

Trinka scanned and edited your text for language errors and identified the areas of improvement. Here are the details.

This file had 5271words and 264revisions. Our initial assessment showed 58% as the language score for your uploaded file. The distribution of errors and improvements is shown below. A total of 4 suggestions have been added as comments in the document.

Category	**Revisions**
Abbreviations	0
Adjectives/Adverbs	2
Articles	34
Capitalization & Spacing	4
Conjunctions	0
Difficult-to-read Sentence	0
Enhancement	0
Fragment	0
Idioms	0
Number Style	0
Plain Language	0
Prepositions	15
Pronouns& Determiners	0
Punctuation	50
Redundancy & Conciseness	0
Run-on Sentence	0
Sensitive Language	0
Singular-Plural nouns	15
Spelling & Typos	1
Style	0
Subject-Verb Agreement	7
Symbols/Notations	0
Syntax	3
Tense	4

Verbs	8
Word Form	5
Word Order	1
Word/Phrase Choice	17
Writing Advisor	0
Other	98
Style Guide - None	0
Total	**264**