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Bacterial Isolates from Seafood in Scotland

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***Abstract*:** *Vibrio spp., Enterobacteriaceae representatives and Listeria monocytogenes were isolated from commercially prepared smoked and fresh Atlantic salmon, smoked and fresh haddock, live mussels and oysters. Isolates were identified phenotypically and by sequencing the 16S rDNA gene. Vibrio spp. occurred in high densities (>10^6 CFU g^{-1}) in mussels. Enterobacteriaceae representatives were recorded at 2.2 x 10^6 CFU g^{-1} and 2.0 x 10^6 CFU g^{-1}in fresh salmon and smoked haddock, respectively. Total heterotrophic counts in fresh salmon, live mussels and oysters reached 10^7, 10^7 and 10^6 CFU g^{-1}, respectively. Listeria monocytogenes was recorded at 5.0 x 10^4 CFU g^{-1}in mussels. The prominent characteristics of fish spoilage isolates were demonstrated by the ability of the isolates to reduce trimethylamine oxide (TMAO) to trimethylamine, and to produce H_2S. The spoilage organisms revealed the ability to produce hydrolytic enzymes.*

Keywords: *Enterobacteriaceae*, filter feeders, fish fillets, fish spoilage bacteria, *Listeria monocytogenes*, *Vibrio* spp.

I Introduction

Fishery products are significant for human nutrition worldwide [1], but may also serve as a source of foodborne pathogens [2]. Chemicals, metals, marine toxins, and infectious agents, .i.e. bacteria, viruses, and parasites, have been detected in seafood, and may be associated with mild gastroenteritis to life-threatening diseases [3]. Microbial contamination of fish tissues may reflect the presence of pollutants in the aquatic environment [4]. This contamination by pathogens poses a potential risk to human health [5]. Specifically, the consumption of raw or undercooked seafood is especially problematic to human health. Filter feeders, such as mussels, and oysters, are particularly troublesome in terms of microbial contamination insofar as the animals collect bacteria in their filtering systems. Thus, they entrap pathogenic and non-pathogenic bacteria and viruses that occur in the aquatic environment [6].

The presence of putative pathogens, such as *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens* and *Edwardsiella tarda*, have been documented in conger eel, swordfish, sole, grouper, and whiting [2]. This study attempts has focused on potential pathogenic and spoilage microorganisms isolated from seafood in Central Scotland.

II Materials And Methods

Sample collection

The seafood samples were obtained from Edinburgh and Stirling, Scotland, UK from March to April, 2010. Thus, fresh salmon and haddock fillets, chosen from within the middle of stacks of fillets in chilled display cabinets within fishmongers, were collected by use of disposable plastic gloves and packed in sealed aluminium foil packs. Enquiries of the fishmongers confirmed that in all cases the fish had been landed from fishing boats within the previous 24 h, although it was unknown how long the fish had been on the boats after capture. Filleting of the whole fish occurred in the shops within the same day as purchase, and the fillets were immediately displayed in refrigerated cabinets. Smoked haddock fillets were obtained by the fishmongers from a commercial smoke house in Leith, Edinburgh. The smoked salmon was pre-packed, and was sourced from farmed Atlantic salmon that had been smoked in the Highlands of Scotland. There was a lack of clarity about the origin of bags of live mussels and oysters, which were obtained by the fishmongers from wholesalers. All animals were sourced from Scotland. There was not any stated shelf life for any of the fresh fish or shellfish purchases.

Samples from fishmongers in Stirling were packed in polyethylene wraps, and stated to be fresh with a 24 h use-by date. The samples from Edinburgh were transported to the laboratory within one hour of collection, and were maintained in a cold box at 4°C. The samples from Stirling were transported at room temperature, and were processed microbiologically within 20 min of collection. In total, 4 samples of cold smoked salmon, 4 samples of fresh salmon, 4 bags of mussels, one sample of cold smoked haddock, one sample of fresh haddock, and one bag of oysters were examined.

III Microbiological Examination

The samples were analysed for the presence of *Vibrio* spp., *Enterobacteriaceae* representatives on eosin methylene blue agar, total aerobic heterotrophic counts, and *Listeria monocytogenes* after [7; 8; 9; 10],

respectively. The microbiological procedures involved blending [using Stomacher Lab-Blender-80] 10 g quantities of each seafood sample in 90 ml of [30% (w/v) alkaline peptone water for vibrio, 0.5% (w/v) peptone water for *Enterobacteriaceae* and sterile 0.85% (w/v) saline for total heterotrophic counts broth for 2 min. The recovery of *Listeria* spp. involved pre-enrichment on *Listeria* primary selective enrichment broth base (Oxoid, CM0863) supplemented with *Listeria* primary selective enrichment supplement (Oxoid, SR0142E) (UVM I) following incubation at 30 °C for 24 h and *Listeria* secondary selective enrichment broth base CM0863 supplemented with *Listeria* secondary selective enrichment supplement (Oxoid, SR0143E) (UVM II) followed by cultures on PALCAM agar following incubation at 30 °C for 48 h as recommended by [10].The number of colonies was recorded before a random assortment was picked, and purified by streaking and restreaking on tryptone soya agar (TSA; Oxoid) plates supplemented with 1% (w/v) sodium chloride [= TNA] with incubation at 30 °C for 48 h [11].*Carnobacterium maltaromaticum* isolates [from smoked salmon] were grown routinely on de Man Rogosa and Sharpe agar (MRS; Oxoid) at 30 °C for 48 h. Stock cultures were stored in tryptone soya broth (TSB; Oxoid) supplemented with 1% (w/v) sodium chloride [= TNB] and 20% (v/v) glycerol at -70 °C [12].

IV Identification Of The Bacteria

All cultures were examined for micromorphology using Gram-stained smears, motility, colonial morphology, catalase and oxidase production, and the oxidative-fermentative metabolism of glucose using standard procedures by [13], before recourse to use of the API 20E, API 50CH ((BioMerieux® Marcy-l'Etoile, France) and MICROBACT™ Listeria 12L (Thermoscientific) rapid identification systems. Isolates were identified by 16S rDNA amplification, sequencing and species attribution [14].

V Bio-Activity Of The Cultures

Cultures were examined for DNase, Trimethylamine oxide (TMAO) reduction and H_2S production, lecithinase and lipase, gelatinase, esterase and coagulase activities after [15; 16; 17; 18; 19; 13], respectively. Haemolytic activity to 5% (v/v) defribrinated sheep blood in blood agar base (Oxoid) supplemented with 1% (w/v) sodium chloride was recorded after incubation at room temperature for 4 days.

VI Results

Isolation of microorganisms from seafood:

The mean presumptive numbers of *Vibrio*, *Enterobacteriaceae*, aerobic heterotrophs, and *L. monocytogenes* were from 2.8×10^3 to 2.1×10^6 CFU g^{-1}, 5.0×10^2 to 2.2×10^6 CFU g^{-1}, 1.6×10^3 to 5.8×10^7 CFU g^{-1} and 1.0×10^4 to 5.0×10^4 CFU g^{-1}, respectively (Tables 1, 2, 3 and 4).

Table 1. Presumptive *Vibrio* counts of the seafood samples

Date of seafood collection	seafood samples	Colony count CFU g^{-1} on TCBS at 30 °C	Colony count CFU g^{-1} on TCBS at 20 °C
01 March 2010	smoked salmon	0	0
	fresh salmon	0	1.2×10^5
05 March 2010	Mussels	0	3.2×10^5
	smoked salmon	0	0
	fresh salmon	0	0
15 March 2010	Mussels	0	0
	smoked salmon	0	0
	fresh haddock	0	0
22 March 2010	Mussels	0	2.1×10^6
	Oysters	0	1.2×10^6
12 April 2010	Mussels	2.8×10^4	1.9×10^6
	smoked salmon	0	0
	fresh salmon	0	0
15 April 2010	smoked haddock	2.8×10^3	0
	fresh haddock	0	0

Table 2. Total *Enterobacteriaceae* numbers in the seafood

Date of seafood collection	seafood samples	Colony count CFU g^{-1} on EMBA at 37 °C
01 March 2010	smoked salmon	0
	fresh salmon	2.2×10^6
05 March 2010	Mussels	0
	smoked salmon	0
	fresh salmon	0
15 March 2010	Mussels	0
	smoked salmon	0
	fresh salmon	0
22 March 2010	Mussels	0
	Oysters	0
12 April 2010	Mussels	0
	smoked salmon	0
	fresh salmon	0
15 April 2010	smoked haddock	2.0×10^6
	fresh haddock	5.0×10^2

Table 3. Total aerobic heterotrophic counts of the seafood

Dates of seafood collection	seafood samples	Colony count CFU g^{-1} on TSA at 30 °C	Colony count CFU g^{-1} on TSA at 20 °C
01 March 2010	smoked salmon	0	0
	fresh salmon	5.8×10^7	3.8×10^7
05 March 2010	Mussels	2.6×10^5	6.1×10^5
	smoked salmon	2.6×10^5	1.1×10^5
	fresh salmon	5.8×10^5	3.2×10^6
15 March 2010	Mussels	3.0×10^3	6.5×10^5
	smoked salmon	0	0
	fresh salmon	0	0
22 March 2010	Mussels	1.1×10^5	1.1×10^7
	Oysters	1.1×10^5	1.1×10^6
12 April 2010	Mussels	9.2×10^4	6.2×10^6
	smoked salmon	1.6×10^3	0
	fresh salmon	1.3×10^5	9.7×10^5
15 April 2010	smoked haddock	1.1×10^6	2.1×10^6
	fresh haddock	1.8×10^6	2.0×10^6

Table 4. Total *Listeria monocytogenes* counts of the seafood samples

Date of seafood collection	seafood samples	Colony count CFU g^{-1} on PALCAM at 30 °C	Colony count CFU g^{-1} on PALCAM at 20 °C
01 March 2010	smoked salmon	0	0
	fresh salmon	0	0
05 March 2010	mussels	0	0
	smoked salmon	0	0
	fresh salmon	0	0
15 March 2010	Mussels	0	0
	smoked salmon	0	0
	fresh salmon	0	2.5×10^6
22 March 2010	Mussels	1.0×10^4	5.0×10^4
	Oysters	0	0
12 April 2010	Mussels	0	0
	smoked salmon	0	0
	fresh salmon	0	0
15 April 2010	smoked haddock	0	0
	fresh haddock	1.7×10^5	1.6×10^4

Typically on PALCAM agar, *Listeria* colonies demonstrated black haloes with greenish surroundings that were ≤ 1 mm in diameter; they were round, smooth edged, flat and creamy. Colonies comprised non-sporeforming Gram-positive, tiny rods occurring singly, and in pairs and clusters. Cultures were catalase positive, but oxidase negative. Beta haemolysis was recorded on sheep blood agar. Hydrolysis of aesculin was positive. Acid was produced from xylose, arabitol, ribose, trehalose, M-D- gluconate and M-D-mannose.

The distribution, frequency, number and percentage of bacterial isolates from the seafood:

In Table 5, it may be observed that fresh haddock was the most contaminated species in terms of the diversity of bacteria, followed by fresh salmon and smoked haddock. In total, 19 genera of bacteria were recognised (Table 5). The proportions and prevalence of these taxa have been included in Table 5.

Table 5. Number and percentage of bacterial isolates in the seafood

Bacterial Isolate	No. (%)	Seafood species/ No. (%)					
		Fresh salmon	Smoked salmon	Fresh haddock	Smoked haddock	Mussels	Oysters
Acinetobacter	2 (3.2)	0 (0.0)	0 (0.0)	1 (6.3)	1 (7.7)	0 (0.0)	0 (0.0)
Aerococcus	3 (4.8)	1 (7.7)	0 (0.0)	1 (6.3)	0 (0.0)	1 (12.5)	0.(0.0)
Aeromonas	9 (14.5)	1 (7.7)	1 (12.5)	3 (18.8)	3 (23.1)	1 (12.5)	0.(0.0)
Bacillus	1 (1.6)	0 (0.0)	0 (0.0)	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)
Brochothrix	2 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	1 (25.0)
Carnobacterium	3 (4.8)	1 (7.7)	2 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Corynebacterium	3 (4.8)	2 (15.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)
Moraxella	5 (8.1)	0 (0.0)	1 (12.5)	0 (0.0)	3 (23.1)	1 (12.5)	0 (0.0)
Micrococcus	1 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)
Pseudomonas	5 (8.1)	1 (7.7)	1 (12.5)	1 (6.3)	0 (0.0)	1 (12.5)	1 (25.0)
Pseudomonads	1 (1.6)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Psychrobacter	1 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)
Shewanella	7 (11.3)	2 (15.4)	0 (0.0)	4 (25.0)	1 (7.3)	0 (0.0)	0 (0.0)
Staphylococcus	1 (1.6)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Citrobacter	2 (3.2)	1 (7.7)	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)
Enterobacter	2 (3.2)	1 (7.7)	0 (0.0)	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)
Escherichia coli	6 (9.6)	2 (15.4)	1 (12.5)	2 (12.5)	1 (7.7)	0 (0.0)	0 (0.0)
Serratia	1 (1.6)	0 (0.0)	0 (0.0)	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)
Vibrio	5 (8.1)	0 (0.0)	1 (12.5)	1 (6.3)	0 (0.0)	2 (25.0)	1 (25.0)
Listeria	2 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (25.0)	0 (0.0)
Total	62(100.0)	13(21.0)	8 (12.9)	16(25.8)	13(21.0)	8(13.0)	4(6.5)

All total heterotrophs were isolated using TSA, *Enterobacteriaceae* representatives were isolated using EMBA, presumptive vibrios were isolated using TCBS and *Listeria* spp. isolated using PALCAM agar.

Detection of specific spoilage bacteria:

Table 6 shows the prominent characteristics of fish spoilage bacteria, by their ability to reduce trimethylamine oxide (TMAO) red to trimethylamine (TMA) yellow, to produce H_2S when decomposing thiosulphate and or cysteine to form black colonies due to preciptation of FeS. The production of TMA was evidenced by the redox indicator in the medium being changed from red to yellow, and a black precipitate of FeS was formed as H_2S was produced from thiosulphate and/or cysteine. The *Sh. baltica* OS185 and *Aeromonas* spp. HB-6 were the strongest TMA producers as they were able to reduce TMAO to trimethylamine and to produce H_2S within two days of incubation. Other isolates of *Sh. baltica*, *Sh. putrefaciens*, *A. hydrophila* HX 201006-3, *A. salmonicida* subsp. *achromogenes*, *A. hydrophila* strain, *C. freundii*, *E. cloacae* were also strong producers of TMA and H_2S within three days of incubation. One strain each of *Sh. baltica*, *Sh. putrefaciens*, *V. metschnikovii*, *E. coli*, pseudomonad and *Serratia* spp. I-113-31 were late H_2S producers (Table 6).

Table 6. Detection of specific spoilage bacteria using trimethylamine oxide medium (TMAO)

Bacterial isolate	Bacterial code	Red to yellow	H_2S (Black)	
Aeromonas sp. HB-6	SHB 20 °C	+	+	
Aeromonas hydrophila HX201006-3	SHTA 30 °C	+	+	
Aeromonas salmonicida subsp. *achromogenes*	ZFHTA 30 °C	+	+	
Vibrio metschnikovii	FHTI 30 °C	+	(+)	
Aeromonas hydrophila	ZFHTA 20 °C	+	(+)	
Citrobacter freundii	SHG 37 °C	+	+	
Enterobacter cloacae	ZFSG 37 °C	+	+	
Escherichia coli	ZFSH 37 °C	+	(+)	
Pseudomonad	FSB 30 °C	+	(+)	
Serratia sp. I-113-31	ZFHG 37 °C	+	(+)	
Shewanella baltica OS678	ZFHB 20 °C	+	(+)	
Shewanella baltica OS185	FSA 20 °C	+	+	
Shewanella baltica	ZFSB 30 °C	+	(+)	
Shewanella baltica	FHB 30 °C	+	+	
Shewanella putrefaciens	ZFHB 30 °C	+	+	

(+) represents late producers of H_2S

Virulent characteristics of spoilage bacteria:

Table 7 shows the virulent characteristics of spoilage bacteria, revealing *Aeromonas* spp. and *Shewanella* spp. as high producers of lecithinase, lipase, haemolysin, gelatinase and elastinase. The ability of the microorganisms to degrade haemolysin (β- haemolysis) reveals that haemolysin causes a cytolytic effect creating pores in host membranes resulting in cell lysis [20]. Haemolysin is an exotoxin that acts destructively on the blood cell membrane and leads to cell rupture. Haemolysis, which results from the lysis of erythrocyte membranes with release of haemoglobin, consists of β-haemolysis, i.e. complete degradation, and α-haemolysis i.e. the incomplete

degradation of haemoglobin [21]. Proteases (gelatin) are associated in the growth and spread of the bacterium and contribute to the development of the disease by defeating host host defenses and providing nutrients for the host [22; 23; 24]. Lipases have hydrolytic effect on the lipids of the membrane of the host cells, causes intestinal damage, invasiveness and establishment of infections ([25; 26]. [27] in their study reported that phospholipases associated with fish disease are the lecithinases C and A1. Furthermore, that phospholipases act as both haemolysin and glycerolphospholipid: cholesterol acyltransferase (glycerolphospholipid: cholesterol acyltransferase, GCAT) is present in all *Aeromonas* species, and its role in fish virulence may be due to a combination with other factors [28; 29; 30].

Table 7. Virulent characteristics of spoilage microorganisms

Spoilage Microorganisms	Lecithinase	Lipase	Haemolysin	Gelatinase	Elastinase	
Aeromonas sp. HB-6		+	+	B	+	+
Aeromonas hydrophila HX201006-3		-	+	B	-	+
Aeromonas salmonicida subsp. *achromogenes*		-	+	B	-	+
Vibrio metschnikovii		-	-	A	-	-
Aeromonas hydrophila		+	+	-	-	-
Citrobacter freundii		-	-	-	-	-
Enterobacter cloacae		-	-	-	-	-
Escherichia coli		-	-	-	-	-
Pseudomonad		-	-	-	+	+
Serratia sp. I-113-31		+	+	-	-	-
Shewanella baltica OS678 ZFHB 20 °C		-	-	-	-	-
Shewanella baltica OS185 FSA 20 °C		+	+	B	+	+
Shewanella baltica ZFSB 30 °C		+	+	B	+	+
Shewanella baltica FHB 30 °C		-	-	-	+	-
Shewanella putrefaciens ZFHB 30 °C		+	-	-	+	-

VII Discussion

Seafood permits the transmission of many bacterial pathogens [31]. In particular, the microorganisms potentially pathogenic to man, include *Salmonella* spp., *E. coli*, *St. aureus*, *L. monocytogenes*, *Aeromonas* spp., *V. cholerae* and *V. parahaemolyticus;* all of which have been recovered at various times from seafood, namely fresh, frozen and smoked products (fish, shellfish, crustaceans, molluscs) [32; 6; 33;34]. It is likely that these bacteria may have been contaminants on fish, possibly introduced during harvesting and filleting operations [35; 36; 37]. Moreover, this study revealed the presence of potential pathogenic and spoilage microorganisms, such as *Listeria monocytogenes* and *Aeromonas* spp., *Vibrio* spp., *Enterobacteriaceae* representatives, *Shewanella* spp. and pseudomonads. Spoilage microorganisms, including those that produce hydrogen sulphide, for example *Sh. baltica*, *Sh. putrefaciens* and *Serratia* spp., have been detected from swordfish and tuna alongside non-H_2S producers, such as *Ps. fluorescens*, *Ps. fragi* and *Ac. radioresistens* [38].

Various authors have isolated bacterial pathogens from seafood by using a range of conventional methods ([39; 40; 41; 42; 43]. In this study, the homogenates of the seafood were inoculated onto/into general purpose media and selective isolation media for the recovery of specific groups of bacteria, with their identification including the use of 16SrDNA sequencing (www.ncbi.nlm.nih.gov/genbank/).

Among the organisms recovered in this study, a range of *Enterobacteriaceae* representatives were recovered. The presence of these organisms in fish is regarded as a pointer to possible sewage pollution. Also, some taxa are opportunistic pathogens of fish [44]. It remains a possibility that biogenic amines, such as putrescine, tyramine and histamine, may be produced in fish tissues; the presence of which accounts for some human illnesses [45]. Certainly, the occurrence of *E. coli* may well indicate the possibility of faecal contamination. Notwithstanding, it is argued that measures should be taken to ensure that seafood is not a means of transmission of *E. coli* namely:

1) to uphold the microbiological quality of the harvesting sites;
2) taking care of post harvest capture;
3) ensuring proper hygiene conditions in the handling processes;
4) with regard to processed food, careful measures should be put in place to avoid recontamination and above all, consumption of raw or undercooked seafood should be avoided [46].

Shewanalla spp. were recovered in this study, but this is not surprising as [47] demonstrated the presence of cells on newly caught fish. It should not be overlooked that the organisms may be opportunistic human pathogens, being associated with bacteraemia and skin and soft tissue infections [48; 49]. However and in agreement with this study, [38] demonstrated *Shewanella* spp., as a spoilage microorganism by its strong production of TMA and H_2S.

The presence of staphylococci on seafood is not unusual. Indeed, [50] reported the isolation of *Staphyococus lentus, S. sciuri* and *S. xylosus* from fish and seafood samples (22% of the total) from Thailand. However, *Staphylococcus* is not usually regarded as an indigenous component of the microflora of fish, and may well reflect contamination. Yet, the organism may be associated with environments containing high quantities of sodium chloride ([51; 52], such as fish smokers [53]. It is apparent that the detection of staphylococci in fish suggests:

(a) post harvest contamination due to poor hygiene, or

(b) disease in fish [54; 55].

[56] reported that some type of smoked fish may be associated with populations of both *L. monocytogenes* and *S. aureus*. In this connection, it was noted that *S. haemolyticus* was recovered from smoked salmon in this study.

It is not surprising that vibrios were common in marine fish and shellfish [6; 57]. Certainly, [58] reported that *V. parahaemolyticus* were detected from seafood samples (11.4% of the total), which were collected from seven fishing companies and local fishermen in Nigeria. In parallel, [59] noted the presence of *V. parahaemolyticus* in 10.3% of blue mussels collected from July 2002 to September 2004 at 102 production sites authorized by the Norwegian Food Safety Authorities (NFSA). It was suggested by [60] that the high level (10^3-10^4 g^1) of *Vibrio* spp. in some raw seafood reflected insufficient control in the storage temperatures from the time of harvesting.

Listeria spp., especially *L. monocytogenes*, were reported in farmed mussels in the North Aegean Sea ([61]. Of relevance, [62] noted that contamination with listeria could occur as a result of improper harvesting, handling, processing and sanitation [63]. It is appreciated that *L. monocytogenes* is a facultative anaerobic opportunistic intracellular bacterial pathogen, whose primary route of transmission to humans is the consumption of contaminated food [64]. The invasive form of listeriosis is observed primarily in high-risk groups, namely the elderly, individuals with lowered immunity, pregnant women and new borns [65]. The outcome of listeriosis in pregnant women is abortion. In healthy people, it has been reported that *L. monocytogenes* causes a non-invasive febrile gastroenteritis resulting from the consumption of contaminated smoked trout [66].

VIII Conclusion

The results from the current study suggest that potential pathogenic and spoilage microorganisms are present in seafood. Seafood safety, which differs according to products, is predisposed to a number of factors such as fish origin, product characteristics, handling and processing practices and preparation before consumption [67]. Adequate cooking of the seafood samples will inactivate the microorganisms, improper handling and cross contamination or raw seafood eating habits, might pose a health hazard, especially to susceptible populations such as the immunosuppressed, pregnant women, children and elderly people especially as regards *L. monocytogenes*[36]. Hazard Analysis and Critical Control Points (HACCP) should be used in all stages of food production and preparation processes including packaging and distribution to identify potential food safety hazards.

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