Detection of *Salmonella* and *Vibrio* species in some seafood in Alexandria

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Abstract: Sea foods are prone to bacterial contamination and could cause health risk to consumers. The present work aimed to study the occurrence of *Salmonella* and *Vibrio* in some seafood from different markets in Alexandria. The study was carried out on 150 seafood samples (shrimp, oyster (Gandofli) and mussel (Om El Kholoul)). For detection of *Salmonella;* samples were cultured on CHROM agar *Salmonella* Plus medium and Xylose lysine desoxycholate (XLD) agar plates. Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar was used for *Vibrio* isolation. *Salmonella* was isolated from 10.0% of samples, distributed as 7 (14.0%), 4 (8%) and 4 (8%) from shrimp, oyster and mussel respectively. *Vibrios* were isolated from 52.0% of tested seafood with the highest percentage (88.0%) from oyster. The most frequently isolated *Vibrio* spp. were *V. alginolyticus* (52.5%), *V. parahaemolyticus* (14.1%) and *V. mimcus* (11.5%). Seven different *Salmonella* serotypes were identified (Typhimurium, Derby, Typhi, Paratyphi A, Paratyphi B, Infantis, and Abortus equi). Our results confirm that bacterial contamination in seafood products is common, and suggest that routine examination of such products for pathogenic agents would be advisable.

1. Introduction:

Fish and seafood constitute an important food component for a large section of world population. They come after meat and poultry as staple animal protein foods where fish forms a cheap source of protein. (BAM online)

Consumption of raw or undercooked seafood is recognized as a health risk to consumers. Sea foods are prone to bacterial contamination, especially filter feeders such as mussels, and oysters, which concentrate these bacteria in their filtration systems and, therefore, are ideally suited to trap all bacteria and viruses, pathogenic or otherwise, that live in the water. (Huss 1997; Popovic *et al.* 2010)

Seafood may be a vehicle for most of known bacterial pathogens, as *Salmonella* and *Vibrio spp.* (Huss 1997). Pathogenic *Vibrios* have been a public health concern for seafood consumers and have been cause of import bans, detentions and rejections in international fish trade (WHO 2001). *Vibrio parahaemolyticus* and *Vibrio vulnificus* are part of the natural flora of estuarine and coastal marine environments worldwide and have been isolated from sea - and brackish water of both tropical and temperate regions, sediments, and a variety of seafood especially shellfish and bivalve mollusks (Kirs *et al.* 2010). Although the vast majority of environmental *V. parahaemolyticus* isolates are non virulent, it is a leading cause of gastroenteritis linked to seafood consumption in the United States(U.S.) (Iwamoto *et al.* 2010). *Vibrio vulnificus* poses a significant health threat to humans who suffer from

immune disorders, liver disease, or hemochromatosis. It enters human hosts via wound infections or consumption of raw shellfish (primarily oysters), and infections frequently progress to septicemia and death in susceptible individuals (Harwood *et al.* 2004).

Contamination of seafood with *Salmonella* is a major public health concern. The presence of *Salmonella* in seafood has been reported in Vietnam, India, Sri Lanka, Thailand, Taiwan and Japan (Ponce *et al.* 2008). Heinitz *et al.* 2000 highlighted that the incidence of *Salmonella* in seafood is highest in the central Pacific and African countries and lowest in Europe including Russia, and North America (12% versus 1.6%). During a 9-year study (1990–1998), the Food and Drug Administration noted an overall incidence of *Salmonella* in 7.2% of 11,312 samples from imported and 1.3% of 768 samples from domestic U.S. seafood. In Croatia, *Salmonella* spp. were recorded as the primary microbial pathogens responsible for the majority of food-borne illnesses (Muli *et al.* 2004).

Little is known regarding the prevalence of pathogenic bacteria in locally consumed seafood. Hence, the main aim of this study was to investigate the occurrence of *Salmonella* and *Vibrio*; the primary microorganisms causing food-borne infections in seafood, from local markets serving consumers in Alexandria.

2. Material and Methods

This study was carried out during the period from October 2009 until December 2009. A total of 150

seafood samples were collected from 11 localities in Alexandria, Egypt. (El Hadara - Mahata Misr-El Ebrahemeia - Bahary- Khorsheid- Karmouz-EL Manshia - El Asafra- Sedi Bishr -El Mandara- Moharram Beik).

Sampling:

The collected seafood samples included; shrimp, oyster (Gandofli), and mussel (Om El Kholoul) (50 samples each). Each sample was collected in a sterile container, labeled and transferred to the laboratory for examination within 1-2 hours. The samples were scrubbed, rinsed with tap water then Gandofli and Om Elkholoul were aseptically opened and flesh was collected.

Bacteriological examination:

I-   Detection of *Salmonella* by culture technique: (Rall *et al.* 2005)

Pre-enrichment: Seafood (25 g) samples were homogenized with 225 ml of buffered peptone broth in a stomacher for one minute. The samples were pre-enriched in buffered peptone broth; incubated for 18-20h at 35ºC to provide available nutrients required for the survival and repair of stressed and injured *Salmonella* cells.

Selective Enrichment: About 0.1 mL of the pre-enriched sample was transferred to 10 ml of Rappaport-Vassiliadis broth (RV). In addition 1ml of the pre-enriched sample was transferred to 10 ml of tetrathionate broth (TT); both media were incubated at 42ºC for 24 hours.

Plating on Solid Selective Media: Each selective enrichment broth was shaken and then a loopful from each of them was streaked onto plates of: CHROM agar Salmonella Plus medium and Xylose lysine desoxycholate (XLD) agar. All plates were incubated at 35°C for 24 hours and then examined for typical *Salmonella* colonies.

Identification of *Salmonella* spp.: *Salmonella* were identified morphologically by microscopic examination and biochemically. Suspected Salmonella colonies; pink or reddish color with black centre on XLD or those appeared as mauve colonies on CHROM agar Salmonella Plus medium. Isolates were inoculated on nutrient agar slants and incubated at 37°C for 24 hours. About 3 to 4 suspected colonies were emulsified in one drop of sterile normal saline, on a glass slide. A drop from polyvalent Salmonella O -antisera poly A–I & Vi were added, and agglutination was observed. Colonies that showed positive agglutination were further sent to a reference laboratory (Cairo MIRCEN) for serotyping.

III- Detection of *Vibrio* spp.: (BAM online)

1. **Enrichment:** Samples were kept refrigerated (4˚C) until cultured. Aseptically 25 g of each tested seafood sample were weighed and placed into a sterile stomacher bag. Large samples were cut into smaller pieces before blending. A small amount of alkaline peptone water (APW) was added to the bag and blended thoroughly. After blending, additional APW was added to bring the total amount added to 225 ml (10^{-1} dilution). Two tenfold dilutions (10^{-2} and 10^{-3}) of the blended samples were prepared in APW and incubated at 35˚C for 6 to 8 hours.

2. **Culture on a selective medium:** Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar plates were streaked using one large loopful from the surface and topmost portion of the APW after incubation. TCBS plates were incubated for 18 to 24 hours at 35˚C.

3. **Identification of *Vibrio* spp.:** Colonies from TCBS plates suspicious of being *Vibrio* spp. (yellow or green colonies) were differentiated and identified according to various biochemical characteristics including: oxidase test, subculture to peptone water with sodium chloride (NaCl) of different concentrations (0-3-6-8-10%), arginine dehydrolase, lysine and ornithine decarboxylase tests, growth at 42˚C, Voges proskauer test, urease test, and gelatinase test.

Statistical analysis (Armitag *et al*. 2002):

The results of the present study were tabulated and statistical analyses were conducted using PC with the software: Statistical Package for the Social Sciences (SPSS) version 15 and Excel.

Statistical significance was set at 5% ($P < 0.05$).
The Z- test was done.

3. Results:

The present study showed that out of the 150 seafood samples examined, *Salmonella* were recovered from 10% of samples (14.0%, 8% and 8% of shrimp, oyster and mussel respectively). *Vibrio* was recovered from 52% of samples (88%, 36% and 32% of oyster, mussel and shrimp respectively). (Table 1)

Seven different *Salmonella* serotypes were recovered from the examined 150 seafood samples. The *Salmonella* serotypes were Typhimurium (40.3%), Derby (26.7%), Typhi, Paratyphi A, Paratyphi B, Infantis, and Abortus equi (6.6% each) (Figure 1&Table 2).

The isolated *Vibrio* spp. were *alginolyticus* (52.5%), *parahaemolyticus* (14.1%), *mimcus* (11.5%), *furnissii* (9%), *fluvialis* (5.1%), *hollisae* (4%), *vulnificus* (2.5%) and *cholera* (1.3%) (Figure 2 & Table 2).

Table 1: Bacteria isolated from 150 tested seafood samples

Tested Seafood	No. of samples	Bacterial isolates				Z test
		Salmonella spp.		Vibrio spp.		
		No.	%	No.	%	
Shrimp	50	7	14.0 %	16	32.0%	2.189*
Oyster	50	4	8.0%	44	88.0%	13.36*
Mussel	50	4	8%	18	36.0%	3.59*
Total	150	15	10.0%	78	52.0%	

*=Significant (p<0.05)

Table 2: Distribution of identified *Salmonella* serotypes and *Vibrio* species in tested seafood

		Tested Seafood							
		Shrimp		Oyster		Mussel		Total	
		No.	%	No.	%	No.	%	No.	%
Salmonella	Typhimurium	1	14.3	1	25	4	100	6	40.3
Serotypes	Derby	3	42.8	1	25	0	0	4	26.7
	Typhi	1	14.3	0	0	0	0	1	6.6
	Paratyphi A	1	14.3	0	0	0	0	1	6.6
	Paratyphi B	0	0.0	1	25	0	0	1	6.6
	Infantis	0	0.0	1	25	0	0	1	6.6
	Abortus equi	1	14.3	0	0	0	0	1	6.6
	Total	7	100.0	4	100	4	100.0	15	100.0
Vibrio spp.	V. alginolyticus	4	25.0	29	65.9	8	44.4	41	52.5
	V. parahaemolyticus	0	0.0	5	11.4	6	33.3	11	14.1
	V. mimicus	1	6.3	6	13.6	2	11.1	9	11.5
	V. furnissii	5	31.3	2	4.5	0	0.0	7	9.0
	V. fluvialis	3	18.8	1	2.3	0	0.0	4	5.1
	V. hollisae	3	18.8	0	0.0	0	0.0	3	4.0
	V. vulnifcus	0	0.0	0	0.0	2	11.1	2	2.5
	V. cholera	0	0.0	1	2.3	0	0.0	1	1.3
	Total	16	100.0	44	100.0	18	100.0	78	100.0
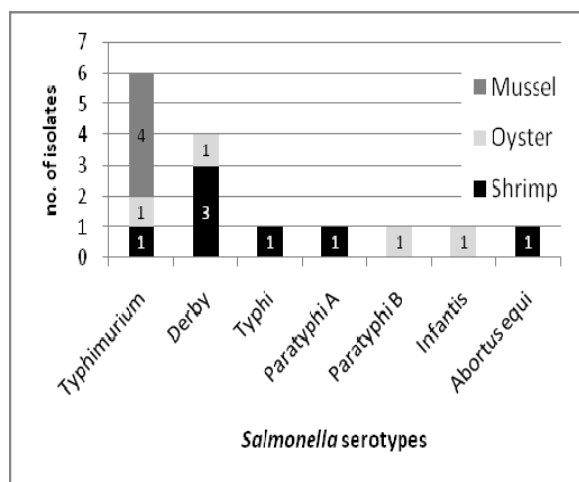
Figure 1: Distribution of isolated *Salmonella* serotypes from 150 tested seafood samples.
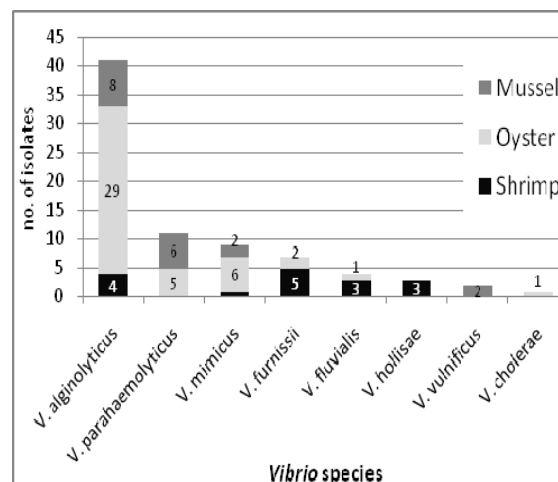
Figure 2: Distribution of isolated *Vibrio* species from 150 tested seafood samples.4.

4. Discussion:

Seafood products harvested from contaminated waters or which have been improperly preserved after harvesting are known to play an important role in infections by *Vibrio spp.* especially crustaceans (Baffone *et al.* 2000). This coincides with the results of the present study, where *Vibrio* were recovered from 52% of samples with oyster showing significantly higher rate of contamination (88%). Oyster shellfish poses a particular problem because they are filter feeders which could be a cause for the abundance of *Vibrio*.

Alexandria (located in north cost of Egypt-Mediterranean Sea) shares similar climate of Eastern/Southern costs of Europe. Compared to other studies, nearly similar results were detected by a study in Turkey determining the presence of *Vibrios* in 66% of samples (Colakoglu *et al.* 2006).

V. alginolyticus was the most frequently isolated *Vibrio* spp. (52.5%) in the present study, followed by *V. parahaemolyticus* (14.1%) and *V. mimcus* (11.5%). These percentages are similar to a study reported by Baffone *et al.* 2000, recording *V. alginolyticus* (81.48%), and *V. parahaemolyticus* (14.8%), with greater isolation frequency (18.9%) from mussels.

V. alginolyticus has been reported to be the most common species in Europe and North America (Toti *et al.* 1996). On the other hand, varying rates were demonstrated in other studies. Multiple shrimp farm environments from the east and west coast of India were studied for abundance of *Vibrio* spp. (Gopal *et al.* 2005).The study revealed the dominance of *V. alginolyticus* (19%), followed by *V. parahaemolyticus* (13%), *V. cincinnatiensis* (7%) in west coast samples, compared with east coast samples which accounted for *V. alginolyticus* (4%) and *V. parahaemolyticus* (3%). While in Iran, a report showed that only 2.1% of studied shrimp samples harbored *Vibrio* spp. Isolated *Vibrio* spp. were *V. parahaemolyticus*, *V. damsela*, *V. alginolyticus* and *V. fluvialis*, that the authors described as indigenous to the marine environment and shrimps (Hosseini *et al.* 2004). Yang *et al.* 2008, revealed 251 isolates of *V. parahaemolyticus* from 1293 seafood samples (19%) collected from the 25 sites in China, during July to October in 2007, while Ji *et al.* 2011, reported 58.6% of 239 samples from different sources were positive for *V. vulnificus* in 10 Chinese cities from June to September 2009.

In the present study, *Salmonella* was recovered from 10% of seafood samples, 14% of shrimp samples, and 8% of each of oyster and mussel samples. Nearly similar results were obtained by Heinitz *et al.*(1998) and Brands *et al.*(2005), who noted an overall *Salmonella* prevalence of 7.2% for imported seafood and 7.4% for oysters in the USA (Heinitz and Johnson 1998; Brands *et al.* 2005). In India, Kumar *et al.* 2008, detected *Salmonella* in 18.9% of naturally contaminated shrimp samples and 21.4% of crab, clam, mussel and oyster samples. Much higher results were obtained by Shabarinath *et al.* 2007, who examined 100 fish and shellfish samples obtained from the market and fish-landing centre in India, where *Salmonella* was detected in 70% of fish, 59% of shrimp and 30% of oyster samples.

The potential source of *Salmonella* contamination in seafood farms is likely due to poor water quality, farm runoff and fecal contamination from wild animals or livestock. In addition to poor distribution, retail marketing, handling, and preparation practices, high stocking densities and high water temperature may be responsible for increased *Salmonella* contamination of shrimp. (Feldsine *et al.* 2003; Zhao *et al.* 2003)

In the present study, the relatively low recovery of *Salmonella* obtained from samples could be attributed to the fact that mussel are cleaned from dirt and plankton and salted by addition of sodium chloride before they are sold. That comes in concordance with the results of Mansour *et al.* 1998, who suggested that risk of food poisoning due to *Salmonella* from consumption of Om El Kholoul is reduced by addition of salt and lemon before consumption.

Regarding the localities from which different seafood samples were obtained, the highest percent of *Salmonella* was from the samples collected from Karmouz (15%), followed by Khourshid (12.5%). EL-Manshia and Mahat-Misr both come next (12%), then EL-Ebrahemia, EL-Asafra and Sedi-Bishr (10%) followed by Bahary (6.7%), where improper cleaning of preparation and storage areas and unclean utensils were noticed. Several authors reported the occurrence of *Salmonella* at wholesale markets, importers and distributors, where they isolated *Salmonella* from utensils (2%) and floor swab samples (4%). (BAM online; Sivapalasingam *et al.* 2004)

As regards the isolated Salmonella, seven different Salmonella serotypes were identified (Typhimurium, Derby, Typhi, Paratyphi A, Paratyphi B, Infantis, and Abortus equi). S. Typhimurium and S. Derby were the predominant serotypes in the present study (40.3% and 26.7% respectively). In concordance to our findings, Kumar *et al*. 2009, reported that S. Weltevreden, S. Rissen, S. Typhimurium and S. Derby were found to be the most predominant serovars in seafood. Nevertheless the most prevalent, *S.* Weltevreden was not detected in any of the tested samples throughout our study

period. Similarly the most frequent serotypes in imported seafood were S. Weltevreden (1st), S. Senftenberg (2nd), S. Lexington, and S. Paratyphi-B (3rd, equal numbers for each serotype), the top 20 list included S. Enteritidis (5th), S. Newport (6th), S. Thompson (7th), S. Typhimurium (12th), and S. Anatum (13th), commonly involved in food borne illness in the U.S. (Heinitz *et al*. 2000).

S. Typhimurium is the most *Salmonella* serotype that has ubiquitous host range (Busani *et al.* 2004). In the present study, the presence of *S.* Typhimurium was maximum in mussel (4 out of 6), while the highest incidence of *S*. Derby was noted in shrimp (3 out of 4). Isolation of *Salmonella* serovars from live molluscan shellfish from marine environments has been reported from Galicia region of Spain (Martinez-Urtaza *et al.* 2003)

Owing to the potential hazard of some pathogenic bacteria, namely *Salmonella and Vibrio*, it is clearly necessary to put more emphasis on food hygiene. Therefore surveillance of potential contaminant bacteria in harvested seafood is crucial for sustenance of public health.

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