Food Hygiene Department, Port-Said Lab.,
Animal Health Research Institute, Dokki, Giza, Egypt.

INCIDENCE OF HALOPHILIC *VIBRIO* SPECIES IN SOME SEAFOODS AND THEIR PUBLIC HEALTH SIGNIFICANCE IN PORT-SAID CITY

(With 4 Tables)

By

H.EL-S.M. FARAG and NAHLA T. KORASHY

Microbiology Department, Port-Said Lab., Animal Health Research Institute, Dokki, Giza, Egypt.

(Received at 21/9/2006)

مدي وجود أصناف من بكتيريا الفيبريو المحبة للملوحة في بعض المأكولات البحرية وعلاقتها بالصحة العامة في مدينة بورسعيد

حسن السيد محمد فرج ، نهلة طه عبد الجواد قرشي

في دراسة لتحديد مدي وجود أصناف بكتيريا الفيبريو المحبة للملوحة في بعض المـــأكولات البحرية تم فحص مائة وخمسون عينة صالحة ظاهريا من ام الخلول (*Donax trunculus anatinus*)، بكلويز (*Tartufo di mare*) سـمك بلطـي (*Oreochromis niloticus*) ، سمك سردين (*Sardinella gibbosa*) وحبار (*Sepia* spp.) بواقع ٣٠ عينة من كل نوع والتي تم جمعها عشوائيا في الفترة من شهر مايو الي اغسطس ٢٠٠٦ من أسـواق مدينـة بورسعيد بهدف عد وعزل وتصنيف بكتريا الفيبريو المحبـة للملوحـة بالاضـافة لتحديـد العترات التي لها القدرة علي احداث اصابة مرضية من غير التي ليست لها هـذه القـدرة. واظهرت النتائج ان نسبة العينات الايجابية لبكتريا الفيبريو المحبة للملوحة كانت ٩٦,٦٧ % (٢٩)، ٧٣,٣٣ % (٢٢)، ٦٠,٠٠ % (١٨) ٨٠,٠٠ % (٢٤) و ٧٠,٠٠ % (٢١) بينما كان متوسط العد الكلي لهذه البكتريا $X8,3 ^{10}3$ ، $X3,5 ^{10}3$ ، $X1,4 ^{10}3$ ، $X3,8 ^{10}3$ ، و $X1,8 ^{10}3$ خلية/ جرام في كل من ام الخلـول (*Donax trunculus anatinus*)، بكلـويز (*Tartufo di mare*) سـمك بلطـي (*Oreochromis niloticus*) سـمك سردين (*Sardinella gibbosa*) وحبار (*Sepia* spp.) على التوالي. وتم عد وتصـنيف عترات بكتريا الفيبريو المحبة للملوحة المعزولة فوجد أن عدد العترات كان ٦٩، ٥٢، ٤١، ٥٩ و ٥٠ عتـرة فـي كـل مـن ام الخلـول (*Donax trunculus anatinus*)، بكلـويز (*Tartufo di mare*) سـمك بلطـي (*Oreochromis niloticus*)، سـمك سردين (*Sardine Sardinella gibbosa*) وحبار (*Sepia* spp.) على التوالي بينما كانت الانواع المعزولة من جميع العينات فيبريو الجينولينكس، فيبريو بـاراهيموليتكس ، فيبريـو فالنيفيكس، فيبريو فورنوزاي، فيبريو فليوفيالس، فيبريو ميمـيكس وفيبريـو ميتشـينكوفاي. وبدراسة العترات لتحديد مدي قدرتها علي احداث الاصابة فتم اختبـار العتـرات لظـاهرة كاناجاوا (Kanagawa) وكانت النسبة الايجابية في العينات موضـع الدراسـة ١٣,٠٤ %

(٩)، ٩,٦٢ % (٥)، ٤,٨٨ % (٢)، ٨,٤٧ % (٥) و ٤,٠٠ % (٢) علـــي التـــوالي وتـــم مناقشة العلاقة بين عدد العترات المعزولة من جانب وايجابية العترات لظـــاهرة كاناجـــاوا (Kanagawa) في العيناتِ موضع الدراسة من جانب اخر.

SUMMARY

One hundred fifty apparently healthy samples of some seafoods (30 each of Om EL-Khloul *"Donax trunculus anatinus"*, baclawese *"Tartufo di mare"*, boltifish *"Oreochromis niloticus"*, sardine *"Sardinella gibbosa"* and cuttlefish *"Sepia* spp.*"*) were randomly purchased during the period from May to August 2006 from Port-Said markets. The samples were examined for enumeration and isolation of halophilic *Vibrio* species as well as the incidence of Kanagawa positive phenomena in the isolated strains and their public health significance was determined. The incidence of positive samples for halophilic *Vibrio* species was 96.67% (29), 73.33% (22), 60.00% (18) 80.00% (24) and 70.00% (21) while the mean values of the total halophilic *Vibrio* counts were 8.3 X 10^3, 3.5 X 10^3, 1.4 X 10^3, 3.8 X 10^3 and 1.8 X 10^3 CFU/g of Om EL-Khloul *"Donax trunculus anatinus"*, baclawese *"Tartufo di mare"*, boltifish *"Oreochromis niloticus"*, sardine *"Sardinella gibbosa"* and cuttlefish *"Sepia* spp.*"* respectively. The numbers of halophilic *Vibrio* isolates were 69, 52, 41, 59 and 50 while the incidence of Kanagawa positive phenomena in these isolates was 13.04% (9), 9.62% (5), 4.88% (2), 8.47% (5) and 4.00% (2) in the examined samples of Om EL-Khloul *"Donax trunculus anatinus"*, baclawese *"Tartufo di mare"*, boltifish *"Oreochromis niloticus"*, sardine *"Sardinella gibbosa"* and cuttlefish *"Sepia* spp.*"* respectively. The bacterial isolates in the examined samples were identified as *Vibrio alginolyticus, Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio furnissii, Vibrio fluvialis, Vibrio mimicus and Vibrio metschnikovii.* The relationship between the number of the isolated strains and Kanagawa positive phenomena in the examined samples were discussed.

Key words: *Seafoods, Vibrio spp., fish, halophiles.*

INTRODUCTION

Seafoods have been a popular part of the diets in many parts of the world and in some countries constitute the main supply of animal protein due to their palatability and digestibility. Today more people are turning to fish as a healthy alternative to red meat. With the increase of

seafoods consumption, the epidemiological data confirmed the role of seafoods as a carrier of foodborne infection or intoxication including contamination by pathogenic halophilic *Vibrio* species (Huss, 1993; Oliver and Kaper, 1997).

Halophilic *Vibrio* species are opportunistic bacterial pathogens common inhabitant of water-based ecosystem virtually at all temperate regions such as marine, coastal and estuarine (brackish) environment and can be found in fresh water. Therefore they could be isolated from marine and estuarine environment and from various species of marine seafoods and fishery products dwelling in these environments (Takeda, 1983; Cavallo and Stabili, 2002; FDA/CFSAN, 2004).

Species of halophilic *Vibrio* are Gram-negative, asporogenous, straight rods or have a single rigid curve, facultative anaerobic, highly motile with a single polar flagellum of family *Vibrionaceae* including *V. parahaemolyticus, V. alginolyticus, V. fluvialis, V. metschnikovii,* and *V. vulnificus* (Kaneko and Colwell, 1975; Baumann and Schubert, 1984; Oliver and Kaper, 1997; Hurley *et al.,* 2006).

The pathogenicity of the halophilic *Vibrio* species depends upon its ability to secrete several extracellular virulent products such as hemolysin, enterotoxins, cytotoxins, lipase, proteases, lipopolysaccharidase, DNAase and enzymes that associated with the extensive tissues damage and seafood spoilage (Smith and Merkel, 1981; Inamura *et al.,* 1985; Kodama *et al.,* 1985; Kothary and Kreger, 1985; Chiang and Chuang, 2003).

These microorganisms are the most important and serious bacterial pathogens causing systemic infection and pathological conditions in large number of various species of primarily marine and estuarine fish culture and wild fish population of all age consequently cause economic loss. The hemorrhage at the base of fins, around vent and inside the mouth with diffused hemorrhage around body surface and external ulcers and inflamed intestine with petechiae on the viscera and musculature, distended abdomen with dark viscous fluid, with exophthalmia and behavioral changes were named *Vibriosis* (hemorrhagic septicemia) (Fryer *et al.,* 1972; Ghittino *et al.,* 1972; Bullock, 1987).

Non-cholera halophilic *Vibrio* infections were a major foodborne diseases causes worldwide health problem associated with the consumption of raw, undercooked and contaminated seafoods producing self-limiting gastroenteritis lasting 2-3 days and characterized by diarrhea, sometimes bloody stools, abdominal cramps, nausea, vomiting, headache and fever. While the extraintestinal infections in the form of

eye, ear and wound infection, necrotizing fascitis and septicemia specially with chronic liver diseases, adrenal insufficiency, portal hypertension and immunocompromised hosts (Lee *et al.*, 1997; Garcia *et al.*, 1998; Bag *et al.*, 1999; Ng *et al.*, 1999; Zanetti *et al.*, 1999; Morris, 2003; Yeung and Boor, 2004).

The objective of this study aimed to determine the safety of some seafoods in Port-Said city through the enumeration and detection of the different species of halophilic *Vibrio* and their public health significance.

MATERIALS and METHODS

1: Samples collection:

A total of 150 random samples of various species of seafoods (30 each of Om EL-Khloul *"Donax trunculus anatinus"*, baclawese *"Tartufo di mare"*, boltifish *"Oreochromis niloticus"*, sardine *"Sardinella gibbosa"* and cuttlefish *"Sepia* spp.*"*) were purchased during the period from May to August 2006 from Port-Said Markets. Each individual sample was placed separately into sealed sterile plastic bag, thoroughly identified and delivered to the laboratory in a refrigerated container. All specimens were processed within 4 hours of collection.

2: Bacteriological examination:

2-1: Preparation and enrichment of the samples:

A representative 50 g sample of seafoods were taken aseptically and homogenized with 450 ml of NaCl (3%) in a blender for 1 min. at 8000 rpm according to FAO (1992). Then tenfold serial dilutions were prepared using 3% NaCl till dilution 10^4 (FAO, 1992; Stavric and Buchanan, 1995).

2-2: Isolation and Enumeration of Vibrio species:

Three 1 ml portions of each of the original dilution (10^{-1}: 10^{-5} further dilution may be included in heavily contaminated samples) were inoculated into three tubes of MPN (Most probable number) series containing 10 ml alkaline peptone water (APW). All MPN tubes were incubated at 35°C for 12-16 hr. From the three highest dilutions showing growth (turbidity), loopfuls from top 1 cm of APW were streaked onto TCBS (Thiosulphate citrate bile salts sucrose) agar. All plates were inverted and incubated at 35°C for 18-24 hr. Enumeration of *Vibrio* species were applied by using MPN (Most probable number) table according to Sakazaki *et al.*, (1986) and Stavric and Buchanan (1995).

2-3: Biochemical identification of the isolates:

Three typical colonies per sample were picked from TCBS agar plates and streaked onto trypticase soya agar slant (TSA with 3% NaCl) and incubated at 35°C for 18-24 hr. The isolates were morphologically and biochemically identified by Gram stain, oxidase test, catalase test, motility, carbohydrates fermentation, TSI slant and other biochemical tests according to Overman *et al.*, (1985) and Elliot *et al.*, (1995).

3- Statistical methods

Minimum, maximum, mean, standard deviation and standard error of mean as well as frequency distribution were used to describe data. T-test was used to evaluate relationship between the number of the halophilic *Vibrio* isolates and Kanagawa phenomena positive. P value was considered significant if less than 0.05 and 0.01 at 95% and 99% respectively. These tests were analyzed using the Statistical Package for Social Scientists (SPSS) for windows 12.0 (SPSS Inc., Chicago, IL, and USA).

RESULTS

Table 1: Statistical analytical results of the total counts of halophilic *Vibrio* species (CFU/g) recovered from some seafoods.

			Type of samples				
			Om EL-Khloul *"Donax trunculus anatinus"*	Baclawese *"Tartufo di mare"*	Boltifish *"Oreochromis niloticus"*	Sardine *"Sardinella gibbosa"*	Cuttlefish *"Sepia* spp.*"*
Samples	Total	No.	30	30	30	30	30
		%	100	100	100	100	100
	(ND)	No.	1	8	12	6	9
		%	3.33	26.67	40.00	20.00	30.00
	(D)	No.	29	22	18	24	21
		%	96.67	73.33	60.00	80.00	70.00
Statistic for the counts of (D) samples		Min.	2.4×10^2	2.1×10^2	1.5×10^2	2.4×10^2	2.4×10^2
		Max.	4.0×10^4	2.4×10^4	4.6×10^3	2.4×10^4	4.6×10^3
		Mean	8.3×10^3	3.5×10^3	1.4×10^3	3.8×10^3	1.8×10^3
		S.E.	1.8×10^3	1.2×10^3	2.5×10^2	1.0×10^3	3.0×10^2
		S.D.	1.0×10^4	5.6×10^3	1.1×10^3	5.0×10^3	1.4×10^3

ND= Non-Detectable (<3). D= Detectable (>3). Min. = Minimum. Max. = Maximum.
SE = Standard Error SD = Standard Deviation.

Table 2: Frequency distribution of the examined seafoods based on their *Vibrio* organisms count (n=30 of each)

Count range	Type of samples									
	Om EL-Khloul *"Donax trunculus anatinus"*		Baclawese *"Tartufo di mare"*		Boltifish *"Oreochromis niloticus"*		Sardine *"Sardinella gibbosa"*		Cuttlefish *"Sepia* spp.*"*	
	No.	%	No.	%	No.	%	No.	%	No.	%
<3	1	3.33	8	26.67	12	40.00	6	20.00	9	30.00
10^2 - <10^3	4	13.33	7	23.33	5	16.67	3	10.00	5	16.67
10^3 - <10^4	15	50.00	12	40.00	13	43.33	17	56.67	16	53.33
≥10^4	10	33.33	3	10.00	--	--	4	13.33	--	--
Total	30.00	100.00	30.00	100.00	30.00	100.00	30.00	100.00	30.00	100.00

< 3 = Non detectable level.

Table 3: Incidence of halophilic *Vibrio* species recovered from some seafoods.

Incidence of halophilic *Vibrio* spp.			Type of samples					Total
			Om EL-Khloul *"Donax trunculus anatinus"*	Baclawese *"Tartufo di mare"*	Boltifish *"Oreochromis niloticus"*	Sardine *"Sardinella gibbosa"*	Cuttlefish *"Sepia* spp.*"*	
No. of examined samples			30	30	30	30	30	120
No. of positive samples			29	22	18	24	21	114
Isolates		No.	69	52	41	59	50	271
		%	100.00	100.00	100.00	100.00	100.00	100.00
Isolates type	*Vibrio alginolyticus*	No.	18	10	8	19	8	63
		%	26.09	19.23	19.51	32.20	16.00	23.25
	Vibrio parahaemolyticus	No.	13	6	5	11	11	46
		%	18.84	11.54	12.20	18.64	22.00	16.97
	Vibrio vulnificus	No.	5	1	1	3	1	11
		%	7.25	1.92	2.44	5.08	2.00	4.06
	Vibrio furnissii	No.	4	5	3	6	10	28
		%	5.80	9.62	7.32	10.17	20.00	10.33
	Vibrio fluvialis	No.	5	9	7	7	8	36
		%	7.25	17.31	17.07	11.86	16.00	13.28
	Vibrio mimicus	No.	11	13	10	9	7	50
		%	15.94	25.00	24.39	15.25	14.00	18.45
	Vibrio metschnikovii	No.	13	8	7	4	5	37
		%	18.84	15.38	17.07	6.78	10.00	13.65

Table 4: Incidence of Kanagawa phenomena positive halophilic *Vibrio* species recovered from some seafoods

Halophilic *Vibrio* species			Type of samples					Total
			Om EL-Khloul *"Donax trunculus anatinus"*	Baclawese *"Tartufo di mare"*	Boltifish *"Oreochromis niloticus"*	Sardine *"Sardinella gibbosa"*	Cuttlefish *"Sepia* spp.*"*	
Examined strain		No.	69	52	41	59	50	271
Kanagawa positive strain		No.	9	5	2	5	2	23
		%	13.04	9.62	4.88	8.47	4.00	8.49
Vibrio alginolyticus	Isolates	No.	18	10	8	19	8	63
	Kanagawa +ve	No.	2	1	1	2	1	7
		%	11.11	10.00	12.50	10.53	12.5	11.11
Vibrio para-haemolyticus	Isolates	No.	13	6	5	11	11	46
	Kanagawa +ve	No.	3	1	1	2	1	8
		%	23.08	13.37	20.00	18.18	9.09	17.39
Vibrio vulnificus	Isolates	No.	5	1	1	3	1	11
	Kanagawa +ve	No.	1	0.00	0.00	1	0.00	2
		%	20.00	0.00	0.00	33.33	0.00	18.18
Vibrio furnissii	Isolates	No.	4	5	3	6	10	28
	Kanagawa +ve	No.	0.00	1	0.00	0.00	0.00	1
		%	0.00	20.00	0.00	0.00	0.00	3.57
Vibrio fluvialis	Isolates	No.	5	9	7	7	8	36
	Kanagawa +ve	No.	0.00	0.00	0.00	0.00	0.00	0.00
		%	0.00	0.00	0.00	0.00	0.00	0.00
Vibrio mimicus	Isolates	No.	11	13	10	9	7	50
	Kanagawa +ve	No.	3	2	0.00	0.00	0.00	5
		%	27.27	15.38	0.00	0.00	0.00	10.00
Vibrio metschnikovii	Isolates	No.	13	8	7	4	5	37
	Kanagawa +ve	No.	0.00	0.00	0.00	0.00	0.00	0.00
		%	0.00	0.00	0.00	0.00	0.00	0.00

*Significant at $P < 0.05$ and $P < 0.01$ using t-test

DISCUSSION

Halophilic *Vibrio* spp. is a natural inhabitant of seafoods. These organisms are considered foodborne pathogens able to contaminate seafoods causing world health problems and economic loss in fish industry. Not all strains of halophilic *Vibrio* are considered pathogenic strains except that produce thermostable direct hemolysin (TDH) or TDH-Related hemolysin (TRH) or that produce both hemolysin (Honda and Iida, 1993; Bag *et al.*, 1999).

The obtained results in table (1) showed that the incidence of positive Om EL-Khloul *"Donax trunculus anatinus"*, baclawese *"Tartufo di mare"*, boltifish *"Oreochromis niloticus"*, sardine *"Sardinella gibbosa"* and cuttlefish *"Sepia* spp.*"* for halophilic *Vibrio* spp. was 96.67% (29), 73.33% (22), 60.00% (18), 80.00% (24) and

population where bivalves specially Om EL-Khloul *"Donax trunculus anatinus"* show more population due to they are filter feeder, filter large volume of water, present close in shore and are liable to contamination from different sources as sever effluent, discharge from shipping, house boat and accumulate and concentrate pathogenic microorganisms specially aquatic and are considered the reservoir of these microorganisms (FAO, 1990; NACM, 1992) besides the difference in salinity, temperature and pH between the different locality of seafoods collection (FDA/CFSAN, 2004).

The results given in table (3) reveal that the total number of halophilic *Vibrio* isolates in Om EL-Khloul *"Donax trunculus anatinus"*, baclawese *"Tartufo di mare"*, boltifish *"Oreochromis niloticus"*, sardine *"Sardinella gibbosa"* and cuttlefish *"Sepia* spp." samples were 69, 52, 41, 59 and 50 isolates respectively. The highest incidence of halophilic *Vibrio* isolates was recovered from Om EL-Khloul *"Donax trunculus anatinus"* but the lowest one was found in boltifish *"Oreochromis niloticus"*. These high results may be attributed to the significant relationship between the total counts and the number of isolates of halophilic *Vibrio* spp., besides the location, sample type, high levels of pollution and organic matter (Cooke *et al.,* 2002) and the ability of halophilic *Vibrio* spp. to grow well at warm months and at different degrees of salinity (Dalsgaard *et al.,* 1996).

Also table (3) showed that the isolated *Vibrio* strains from the examined samples were identified as *Vibrio alginolyticus, V. parahaemolyticus, V. vulnificus, V. furnissii, V. fluvialis, V. mimicus* and *V. metschnikovii* with an incidence of 23.25% (63), 16.97% (46), 4.06 % (11), 10.33% (28), 13.28% (36), 18.45% (50) and 13.65% (37) respectively. *Vibrio alginolyticus* which constitute the highest prevalence rate was recovered from 26.09% (18), 19.23% (10), 19.51% (8), 32.20% (19) and 16.00% (8) of Om EL-Khloul *"Donax trunculus anatinus"*, baclawese *"Tartufo di mare"*, boltifish *"Oreochromis niloticus"*, sardine *"Sardinella gibbosa"* and cuttlefish *"Sepia* spp." samples respectively. Meanwhile the lowest incidence of isolates was *V. vulnificus* which could be detected in 7.25% (5), 1.92% (1), 2.44% (1), 5.08% (3) and 2.00% (1) of Om EL-Khloul *"Donax trunculus anatinus"*, baclawese *"Tartufo di mare"*, boltifish *"Oreochromis niloticus"*, sardine *"Sardinella gibbosa"* and cuttlefish *"Sepia* spp." samples respectively. These results were lower than the results reported by Wong *et al.,* (1999) and Soliman *et al.,* (2002) but higher than the results recorded by Garcia and Antillon (1990); Scoglio *et al.,* (2001); Elhadi *et al.,* (2004) and Normanno *et al.,* (2006).

70.00 % (21) respectively. These results agree with the results recorded by Schintu *et al.*, (1994) and Venkateswaran *et al.*, (1996) except that of Om EL-Khloul *"Donax trunculus anatinus"* which had higher incidence. Also our results were higher than the results recorded by Depaola *et al.*, (2003); Parisi *et al.*, (2004) and Fuenzalida *et al.*, (2006). The high figures of our results than that recorded by some authors may be attributed to the variation in the locality of harvested seafoods which substantiate what have been reported by FAO (1990). Also table (1) showed that the mean values of the total halophilic *Vibrio* spp. counts of Om EL-Khloul *"Donax trunculus anatinus"*, baclawese *"Tartufo di mare"*, boltifish *"Oreochromis niloticus"*, sardine *"Sardinella gibbosa"* and cuttlefish *"Sepia* spp.*"* were 8.3 X 10^3, 3.5 X 10^3, 1.4 X 10^3, 3.8 X 10^3 and 1.8 X 10^3 CFU/g, respectively. These results agree with the results recorded by Venkateswaran *et al.*, (1996), meanwhile lower than the results recorded by Chan *et al.*, (1989) and higher than the results recorded by Depaola *et al.*, (2003); Sherif *et al.*, (2003) and Fuenzalida *et al.*, (2006). Higher counts may be attributed to abuse temperature during harvesting and storage besides the length of storage (Lorca *et al.*, 2001), post harvesting bad handling practices as using of polluted and non-hygienic water (Dalsgaard *et al.*, 1996; Depaola *et al.*, 2003). Also the tropic status of the harvested area cause seasonal variation in the counts of *Vibrio* spp. where the warmer months, location, pollution and time of collection show the greatest populations (Cooke *et al.*, 2002).

Regarding frequency distribution of the examined seafood samples presented in tables (2) it is evident that most of the examined Om EL-Khloul *"Donax trunculus anatinus"* (50%), baclawese *"Tartufo di mare"* (40%), boltifish *"Oreochromis niloticus"* (43.33%), sardine *"Sardinella gibbosa"* (56.67%) and cuttlefish *"Sepia* spp.*"* (53.33%) had *Vibrio* spp. count within the range of 10^3 - $<10^4$ CFU/g. Whereas 33.33%, 10 and 13.33% of the examined Om EL-Khloul *"Donax trunculus anatinus"*, baclawese *"Tartufo di mare"* and sardine *"Sardinella gibbosa"* respectively had $\geq 10^4$ CFU/g. On the other hand, 3.33, 26.67, 40, 20 and 30% of the above mentioned seafood samples had non detectable levels (< 3 CFU/g).

Higher results may be attributed to high initial counts of halophilic *Vibrio* spp. and bad hygiene of the post-harvested handling (Depaola *et al.*, 2003) as improper refrigeration and cross contamination (CDC, 1998). Also the season and the time of harvesting of the seafoods increase the population of *Vibrio* spp. where warm months and high temperature directly proportionally with the counts (FDA/CFSAN, 2004). On the other hand, the type of sample has direct effect on the

However, higher prevalence of halophilic *Vibrio* isolates in the examined different types of seafoods may be attributed to abuse time/temperature of storage of seafoods (Lorca *et al.*, 2001), post harvesting bad handling and cross contamination (CDC, 1998; Gopal *et al.*, 2005), differences in the water and salinity in the different localities (NACM, 1992; Depaola *et al.*, 2003), improper freezing after harvesting and during storage (Matches *et al.*, 1971), season and samples type (Cooke *et al.*, 2002) and high level of dissolved organic matter (FDA/CFSAN, 2004).

Pathogenic *Vibrio* spp. are characterized by positive Kanagawa phenomena as a result of the production of thermostable direct hemolysin (TDH) and/or TDH-Related hemolysin (TRH) (Honda and Iida, 1993), so the pathogenic halophilic *Vibrio* isolates present in table (4) were *Vibrio alginolyticus, V. parahaemolyticus, V. vulnificus and V. furnissii* which showed positive Kanagawa phenomena were present with an incidence of 11.11% (7), 17.39% (8), 18.18% (2) and 3.57% (1) in the examined seafoods samples. The high incidence of Kanagawa phenomena positive in the examined samples was *V. parahaemolyticus* but the low incidence was *V. furnissii,* so *V. parahaemolyticus* was considered a documented human pathogen while *V. furnissii* was considered an occasional human pathogen (Ji, 1989; McLaughlin, 1995).

The samples which showed high incidence of Kanagawa phenomena positive were Om EL-Khloul *"Donax trunculus anatinus"* while boltifish *"Oreochromis niloticus"* and cuttlefish *"Sepia* spp.*"* showed low incidence of Kanagawa phenomena positive. The recorded results in table (4) were higher than the results recorded by Honda *et al.*, (1988); Cooke *et al.*, (2002) and Depaola *et al.*, (2003). Statistically by using t-test, table (4) showed non-significant relationship between the number of the halophilic *Vibrio* isolates and Kanagawa phenomena positive so the variations between our results and the results recorded by other authors may be attributed to the variations in the virulence of the isolates where the virulent strain have gene responsible for production of TDH and TRH (Nishibuchi *et al.*, 1986).

Pathogenic halophilic *Vibrio* spp. has been recognized as a major cause of seafoods-borne illness (Gopal *et al.*, 2005). This illness has been increased in the recent year as a result of increasing the consumption of the seafoods and mostly present in the form of outbreaks or sporadic cases after consumption of raw (in some country), partially salted (as salted Om EL-Khloul), undercooked or improperly cooked seafoods and cross and post cooking contamination (Honda and Iida,

1993; FDA/CFSAN, 2004). The infection by pathogenic halophilic *Vibrio* spp. still a problem due to these bacteria are not killed by freezing but reduced (Matches *et al.*, 1971; Ward *et al.*, 1997) and the produced TDH and TRH toxin are heat stable not destroyed by some cooking procedures (Bradshaw *et al.*, 1974). In conclusion, to increase their shelf life and prevent the infections by these microorganisms, good seafood handling practices including icing or rapid immersion of the catch in water chilled to -1°C followed by uninterrupted frozen storage, good time/temperature storage besides prevention the cross and secondary contamination, strictly hygienic measurement for prevention and removal the source of pollution from the harvested sites and strictly prevention the consumption of raw or insufficient cooked seafoods specially in chronic liver diseases, adrenal insufficiency, portal hypertension and immunocompromised hosts

REFERENCES

Bag, P.; Nandi, S.; Bhardra, R.; Ramamurthy, T.; Bhattacharya, S.; Nishibuchi, M.; Hamabata, T.; Yamasaki, S.; Takeda, Y.; Nair, G. (1999): Clonal diversity among recently emerged strains of *Vibrio parahaemolyticus O3:K6* associated with pandemic spread. J. Clin. Microbiol. 37: 2354-2357.

Baumann, P. and Schubert, R. (1984): Family II. *Vibrionaceae*, p. 516-550. In N. R. Krieg and J. G. Holt (eds.), Bergey's Manual of Systemic Bacteriology, 1st ed. Williams & Wilkins Co., Baltimore/London.

Bradshaw, J.; Francis, D. and Twedt, R. (1974): Survival of *Vibrio parahaemolyticus* in cooked seafood at refrigeration temperatures. Appl. Microbiol. 27: 627-661.

Bullock, G. (1987): Vibriosis in fish. United State Department of the Interior, Fish and Wildlife Service, Division of Fisheries and Wetlands Research, Washington, D.C. 20240

Cavallo, R. and Stabili, L. (2002): Presence of *Vibrios* in seawater and Mytilus galloprovincialis (Lam.) from the Mar Piccolo of Taranto (Ionian Sea). Water Res. 36: 3719-3726.

CDC "Center for Disease Control" (1998): Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters-Pacific Northwest, 1997. MMWR 47 (22): 457-462.

Chan, K.; Woo, M.; Lam, L. and French, G. (1989): Vibrio parahaemolyticus and other halophilic *Vibrios* associated with seafood in Hong Kong. J. Appl. Bacteriol. 66 (1): 57-64

Chiang, S. and Chuang, Y. (2003): Vibrio vulnificus infection: Clinical manifestation. Pathogenesis and antimicrobial therapy. J. Microbiol. Immunol. Infect. 36: 81-88

Cooke, D.; O' Leary, P.; Hunsucker, J.; Sloan, E.; Bowers, J.; Blodgett, R. and DePaola, A. (2002): Vibrio vulnificus and *Vibrio parahaemolyticus* in U.S. retail shell oysters: a national survey June 1998 to July 1999. J. Food Prot. 65: 79-87.

Dalsgaard, A.; Möller, N.; Brin, B.; Hoei, L. and Larsen, J. (1996): Chemical manifestation and epidemiology of *Vibrio vulnificus* in Denmark (summer 1996). European Journal of Clinical Microbiology and Infectious Diseases. 15, 227-232.

Depaola, A.; Nordstrom, J.; Bowers, J.; Wells, J. and Cook, D. (2003): Seasonal abundance of total and pathogenic *Vibrio parahaemolyticus* in Alabama oysters. Appl. Environ. Microbiol. 69: 1521-1526.

Elhadi, N.; Radu, S.; Chen, C. and Nishibuchi, M. (2004): Prevalence of potentially pathogenic *Vibrio* species in the seafood marketed in Malaysia. J. Food Prot. 67: 1469-1475.

Elliot, E.; Kaysner, C.; Jackson, L. and Tamplin, M. (1995): Vibrio cholera, V. parahaemolyticus, V. vulnificus and other *Vibrio* spp. pp. 9.01-9.27. In FDA Bacteriological Analytical Manual, 8th ed. AOAC International, Gaithersburg, MD.

FAO "Food and Agriculture Organization" (1990): Specific Establishment And Commodity Inspection Techniques. Manual of Food Quality Control. 5.Food Inspection. Food and Agriculture Organization of The United Nation, Rome, Italy, Chap. 5 pp 51-115.

FAO "Food and Agriculture Organization" (1992): Vibrio parahaemolyticus. Manual of Food Quality Control. 4.Rev.1-Microbiological Analysis. Food and Agriculture Organization of The United Nation, Rome, Italy, Chap. 7 pp 69-77.

FDA/CFSAN "Food and Drug Administration/Center for Food Safety and Applied Nutrition" (2004): Vibrio. Bacteriological Analytical Manual Online, Chapter 9.

Fryer, J.; Nelson, J. and Garrison, R. (1972): Vibriosis in Fish. Prog. Fish Food Sci. 5: 129-133

Fuenzalida, L.; Hernandez, C.; Toro, J.; Rioseco, M.; Romero, J. and Espejo, R. (2006): Vibrio parahaemolyticus in shellfish and clinical samples during two large epidemics of diarrhea in southern Chile. Environ. Microbiol. 8(4): 675-683.

Garcia, C. and Antillon, F. (1990): Isolation of enteropathogenic *Vibrio* in bivalves and mud from the Nicoya Gulf, Costa Rica. Rev. Biol Trop. 38 (2B): 437-440.

Garcia, C.; Collazos, G.; Martinez, G. and Mayo, S. (1998): Vibrio vulnificus septicemia in Spain. An. Med. Interna. 15 (9): 485-486.

Ghittino, P.; Andruetto, S. and Vigliani, E. (1972): "Red mouth" enzootic in hatchery rainbow trout caused by *Vibrio anguillarum.* Riv. Ital. Piscic. Ittiopathol. 7: 41-45.

Gopal, S.; Otta, S.; Kumar, S.; Karunasagar, I.; Nishibuchi, M. and Karunasagar, I. (2005): The occurrence of *Vibrio* species in tropical shrimp culture environments; implications for food safety. Int. J. Food Microbiol. 102 (2): 151-159.

Honda, T. and Iida, T. (1993): The pathogenicity of *Vibrio parahaemolyticus* and the role of the thermostable direct hemolysin and related hemolysins. Rev. Med. Microbiol. 4: 106-113.

Honda, T.; Ni, Y. and Mwatani, T. (1988): Purification and characterization of a hemolysin produced by a clinical isolates of Kanagawa phenomenon-negative *Vibrio parahaemolyticus* and related to the thermostable direct hemolysin. Infect. Immunol. 56: 961-965.

Hurley, C.; Quirke, A.; Reen, F. and Boyd, E. (2006): Four genomic islands that mark post-1975 pandemic *Vibrio parahaemolyticus* isolates. BMC Genomic, 3 (7): 104.

Huss, H. (1993): Assurance of seafood quality. FAO Fisheries Technical Paper No. 334 Rome, FAO 169p.

Inamura, H.; Nakai, T. and Muroga, K. (1985): An extracellular protease produced by *Vibrio anguillarum.* Bull. Jpn. Soc. Sci. Fish. 51: 1915-1920

Ji, S. (1989): The first isolation of *Vibrio alginolyticus* from samples which caused food poisoning. Chin. J. Prevent. Med. 23: 71-73.

Kaneko, T. and Colwell, R. (1975): Incidence of *Vibrio parahaemolyticus* in Chesapeake Bay. Appl. Microbiol. 30: 251-257.

Kodama, H.; Moustafa, M.; Mikami, T. and Izawa, H. (1985): Characterization of extracellular substances of *Vibrio anguillarum* toxic for rainbow trout and mice. Microbiol. Immunol. 29: 909-920.

Kothary, M. and Kreger, A. (1985): Purification and characterization of an extracellular cytolysin produced by *Vibrio damsela.* Infect. Immunol. 49: 25-31

Lee, C.; Tong, K.; Howe, H. and Lam, M. (1997): Vibrio vulnificus infections: Case reports and literature review. Ann. Acad. Med. Singapore, 26 (5): 705-712

Lorca, T.; Pierson, M.; Flick, G. and Hackney, C. (2001): Levels of *Vibrio vulnificus* and organoleptic quality of raw shellstock oysters (*Crassostrea virginica*) maintained at different storage temperatures. J. Food Prot. 64: 1716-1721.

Matches, J.; Liston, J. and Deneault, L. (1971): Survival of *Vibrio parahaemolyticus* in fish Homogenate during storage at low temperature. Appl. Microbiol. 21: 951-952.

McLaughlin, J. (1995): Vibrio, pp. 465-474. In P.R. Murray, E. J. Baron, M. A. Pfaller, F.C. Tenover and R. H. Yolken (eds.), Manual of Clinical Microbiology, 6th ed. ASM press, Washington.

Morris, J. (2003): Cholera and other types of *Vibriosis*: a story of human pandemics and oysters on the half shell. Clin. Infect. Dis. 37:272-280.

NACM (1992): Microbiological criteria for raw *molitiscan* shellfish. J. Food Prot. 55: 463-480.

Ng, T.; Chiang, P.; Wu, T. and Leu, H. (1999): Vibrio parahaemolyticus bacteria: Case report. Changgeng Yi Xue Za Zhi., 22 (3): 508-514.

Nishibuchi, M.; Hill, W.; Zon, G.; Payne, W. and kaper, J. (1986): Synthetic oligodeoxyribonucleotide probes to detect Kanagawa phenomenon-positive *Vibrio parahaemolyticus*. J. Clin. Microbiol. 23:1091-1095.

Normanno, G.; Parisi, A.; Addante, N.; Quaglia, N. Dambrosio, A.; Montagna, C. and Chiocco, D. (2006): Vibrio parahaemolyticus, Vibrio vulnificus and microorganisms of fecal origin in mussels (*Mytilus galloprovincialis*) sold in the Puglia region (Italy). Int. J. Food Microbiol. 106(2): 219-222.

Oliver, J. and Kaper, J. (1997): Vibrio species. In M. P. Doyle, L. R. Beuchat and T. J. Montville, eds. Food Microbiology: Fundamentals and Frontiers, p 228-264. Washington, D. C., ASM Press.

Overman, T.; Kessler, J. and Seabolt, J. (1985): Comparison of API20E, API Rapid E and API Rapid NFT for identification of members of the family *Vibrionaceae*. J. Clin. Microbiol. 22:778-781.

Parisi, A.; Normanno, G.; Addante, N.; Dambrosio, A.; Montagna, C.; Quaglia, N.; Celano, G. and Chiocco, D. (2004): Market survey of *Vibrio* spp. and other microorganisms in Italian shellfish. J. Food Prot. 67: 2284-2287.

Sakazaki, R.; Pivnick, H.; Jarvis, G.; Goddard, M.; Asakawa, Y.; Barrow, G.; Beuchat, L.; Colwell, R.; Gleeson, T.; Gray, R.; Nakanishi, H.; Sakai, S.; Stavric, S.; Takizawa, K.; Tamura, K.; Twedt, R.; Vanderzant, C. and West, P. (1986): ICMSF methods studies. XVI. Comparison of salt polymyxin broth and glucose salt teepol broth for enumerating *Vibrio parahaemolyticus* in naturally contaminated samples. J. Food Prot. 49: 773-780.

Schintu, M.; Meloni, P.; Marras, M.; Sau, M. and Contu, A. (1994): La ricerca di *vibrioni alofili* come metodologia complementare nella valutazione dei mollusschi eduli lamellibranchi. Ig. Mod. 101: 53-60.

Scoglio, M.; Dipietro, A.; Picerno, I.; delia, S.; Mauro, A.; Lagana, P. (2001): Virulence factors in *Vibrios* and *Aeromonads* isolated from seafoods. New Microbial, 24 (3): 273-280

Sherif Mona, M.; Housen Azza, A.; Aideia Hoda, A.; Soliman Zeinab, I. (2003): Studies on some hazards associated with shellfish. J. Egypt. Vet. Med. Assoc. 63 (2): 213-223

Smith, G. and Merkel, J. (1981): Collagenolytic activity of *Vibrio vulnificus*: potential contribution to its invasiveness. Infect. Immunol. 35: 1155-1156 Soliman Zienab I., Shalaby Amny M. and EL-Tabeey Azza A. (2002): Prevalence of *Vibrio* species in some kinds of shellfish sold in Port-Said markets. J. Egypt. Vet. Med. Assoc. 62(5): 7-13

Stavric, S. and Buchanan, B. (1995): The isolation and enumeration and *Vibrio vulnificus* from fish and seafoods. Research Division, Bureau of Microbial Hazardous, Food Directorate, Health Canada, Ottawa On, K1A 0L2.

Takeda, Y. (1983): Thermostable direct hemolysin of *Vibrio parahaemolyticus*. Pharmac. Ther. 19: 123-146.

Venkateswaran, K.; Kurusu, T.; Satake, M., and Shinoda, S. (1996): Comparison of a fluorogenic Assay with a conventional method for rapid detection of *Vibrio parahaemolyticus* in seafoods. Appl. Environ. Microbiol. 62: 3516-3520.

Ward, D.; Bernard, D.; Collette, R.; Kraemer, D.; Hart, K.; Price, R.; and Otwell, S. (1997): Hazards found in seafoods, Appendix III. In HACCP: Hazard Analysis and Critical Control Point Training Curriculum, 2nd ed., pp. 173-188. UNC-SG-96-02. North Carolina Sea Grant, Raleigh. NC.

Wong, H.; Chen, M.; Liu, S. and Liu, D. (1999): Incidence of highly genetically diversified *Vibrio parahaemolyticus* in seafood imported from Asian countries. Int. J. Food Microbiol. 52(3): 181-188.

Yeung, P. and Boor, K. (2004): Epidemiology pathogenesis and prevention of foodborne *Vibrio parahaemolyticus* infections. Foodborne Pathog. Dis. 1 (2): 74-88

Zanetti, S.; Deriu, A.; Duprè, I.; Sanguinetti, M.; Fadda, G. and Sechi, L. (1999): Differentiation of *Vibrio alginolyticus* strains isolated from Sadinian water by ribotyping and a new rapid PCR fingerprint method. Appl. Environ. Microbiol. 65: 1871-1875.