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

(With 4 Tables)

By

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

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في دراسة لتحديد مدي وجود أصناف بكتيريا الفيبريو المحبة للملوحة في بعض المأكولات البحرية تم فحص مائة وخمسون عينة صالحة ظاهريا من ام الخلول (*Donax trunculus*) ، (*anatinus*) ، بلكويز (*Tartufo di mare*) سمك بلطي (*Oreochromis niloticus*) ، سمك سردين (*Sardinella gibbosa*) و حبار (*Sepia spp.*) بواقع ٣٠ عينة من كل نوع والتي تم جمعها عشوائيا في الفترة من شهر مايو الي اغسطس ٢٠٠٦ من أسواق مدينة بورسعيد بهدف عد وعزل وتصنيف بكتريا الفيبريو المحبة للملوحة بالاضافة لتحديد العترات التي لها القدرة علي احداث اصابة مرضية من غير التي ليست لها هذه القدرة. وظهرت 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  $٣١٠ \times ٨.٣$  ،  $٣١٠ \times ٣.٥$  ،  $٣١٠ \times ١.٤$  ،  $٣١٠ \times ٣.٨$  ، و  $٣١٠ \times ١.٨$  خلية/ جرام في كل من ام الخلول (*Donax trunculus anatinus*) ، بلكويز (*Tartufo di mare*) سمك بلطي (*Oreochromis niloticus*) سمك سردين (*Sardinella gibbosa*) و حبار (*Sepia spp.*) علي التوالي. وتم عد وتصنيف عترات بكتريا الفيبريو المحبة للملوحة المعزولة فوجد أن عدد العترات كان ٤١ ، ٥٢ ، ٦٩ ، ٥٩ و ٥٠ عترة في كل من ام الخلول (*Donax trunculus anatinus*) ، بلكويز (*Tartufo di mare*) سمك بلطي (*Oreochromis niloticus*) ، سمك سردين (*Sardine Sardinella gibbosa*) و حبار (*Sepia spp.*) علي التوالي بينما كانت الانواع المعزولة من جميع العينات فيبريو الجينوليتكس، فيبريو باراهيموليتكس ، فيبريو فالنفيكس، فيبريو فورنوزاي، فيبريو فليوفاليس، فيبريو ميميكس و فيبريو ميتشنيكوفاي. و بدراسة العترات لتحديد مدي قدرتها علي احداث الاصابة فتم اختبار العترات لظاهرة كاناجاوا (Kanagawa) وكانت النسبة الايجابية في العينات موضع الدراسة ١٣.٠٤ % (٩) ، ٩.٦٢ % (٥) ، ٤.٨٨ % (٢) ، ٨.٤٧ % (٥) و ٤.٠٠ % (٢) علي التوالي وتم

مناقشة العلاقة بين عدد العترات المعزولة من جانب وايجابية العترات لظاهرة كاناجاوا (Kanagawa) في العينات 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  $8.3 \times 10^3$ ,  $3.5 \times 10^3$ ,  $1.4 \times 10^3$ ,  $3.8 \times 10^3$  and  $1.8 \times 10^3$  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 fasciitis 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  $35^{\circ}\text{C}$  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  $35^{\circ}\text{C}$  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 X 10 <sup>2</sup>	2.1 X 10 <sup>2</sup>	1.5 X 10 <sup>2</sup>	2.4 X 10 <sup>2</sup>	2.4 X 10 <sup>2</sup>
		Max.	4.0 X 10 <sup>4</sup>	2.4 X 10 <sup>4</sup>	4.6 X 10 <sup>3</sup>	2.4 X 10 <sup>4</sup>	4.6 X 10 <sup>3</sup>
		Mean	8.3 X 10 <sup>3</sup>	3.5 X 10 <sup>3</sup>	1.4 X 10 <sup>3</sup>	3.8 X 10 <sup>3</sup>	1.8 X 10 <sup>3</sup>
		S.E.	1.8 X 10 <sup>3</sup>	1.2 X 10 <sup>3</sup>	2.5 X 10 <sup>2</sup>	1.0 X 10 <sup>3</sup>	3.0 X 10 <sup>2</sup>
		S.D.	1.0 X 10 <sup>4</sup>	5.6 X 10 <sup>3</sup>	1.1 X 10 <sup>3</sup>	5.0 X 10 <sup>3</sup>	1.4 X 10 <sup>3</sup>

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 <sup>2</sup> - <10 <sup>3</sup>	4	13.33	7	23.33	5	16.67	3	10.00	5	16.67
10 <sup>3</sup> - <10 <sup>4</sup>	15	50.00	12	40.00	13	43.33	17	56.67	16	53.33
≥10 <sup>4</sup>	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 <i>Vibrio</i> 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	271	
		%	100.00	100.00	100.00	100.00	100.00	
Isolates type	<i>Vibrio alginolyticus</i>	No.	18	10	8	19	8	63
		%	26.09	19.23	19.51	32.20	16.00	23.25
	<i>Vibrio parahaemolyticus</i>	No.	13	6	5	11	11	46
		%	18.84	11.54	12.20	18.64	22.00	16.97
	<i>Vibrio vulnificus</i>	No.	5	1	1	3	1	11
		%	7.25	1.92	2.44	5.08	2.00	4.06
	<i>Vibrio furnissii</i>	No.	4	5	3	6	10	28
		%	5.80	9.62	7.32	10.17	20.00	10.33
	<i>Vibrio fluvialis</i>	No.	5	9	7	7	8	36
		%	7.25	17.31	17.07	11.86	16.00	13.28
	<i>Vibrio mimicus</i>	No.	11	13	10	9	7	50
		%	15.94	25.00	24.39	15.25	14.00	18.45
	<i>Vibrio metschnikovii</i>	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 <i>Vibrio</i> 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	
<i>Vibrio alginolyticus</i>	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	
<i>Vibrio parahaemolyticus</i>	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
<i>Vibrio vulnificus</i>	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
<i>Vibrio furnissii</i>	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
<i>Vibrio fluvialis</i>	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
<i>Vibrio mimicus</i>	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
<i>Vibrio metschnikovii</i>	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

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  $8.3 \times 10^3$ ,  $3.5 \times 10^3$ ,  $1.4 \times 10^3$ ,  $3.8 \times 10^3$  and  $1.8 \times 10^3$  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



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).

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

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