Detection of some pathogenic bacteria from chikal(Mantis shrimp) with special reference to enterotoxin producing strains

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Abstract
The present study tried to detect some pathogenic bacteria and their toxins in chikal. The study included 100 samples of fresh caught collected randomly from seafood markets and street vendors in different districts of Port-Said city. The samples were divided to 2 groups 50 each. Group (A) complete with shells and heads, group (B) peeled without shells and heads. All samples were examined for presence of some pathogenic bacteria as *E.coli*, *Staphylococcus aureus* and *Aeromonas hydrophila* and their ability to produce toxin. The pathogenic strains of *E.coli* and its toxins not detected, in both groups while *A.hydrophila* detected in 36 and 28% of samples in group (A) and (B) respectively. *Staphylococci* species detected in 24% of group(A) and14% in group (B) .The coagulates positive staphylococcus aureus strains detected in one, sample from 12 isolate as 8.33 % in group(A)and 0% in group (B) and their toxins detected in 8.3% of all sample. The ability *A.hydrophila* isolates to produce toxin by using infant mouse assay was carried out.

Key words:
Mantis shrimp, Staphylococcus aureus, Aeromonas hydrophila, E.coli, enterotoxins, Bacteria producing toxins, Public health.

Introduction

In port-said city a very popular seafood named Chikal **(Mantis shrimp)**. It resembling small lobsters, to which they are closely related similar to the much expensive shrimp and lobster, but much less in price. **Mantis shrimp** are crustaceans they are usually aquatic animal with hard skin (exoskeleton) over segmented body However it is also known that seafood can be a source of foodborne toxin infections, which emphasizes the need of a thorough control of its bacteriological characteristics **(Wallace *et al.,* 1999; Croci ana Suffredini, 2003).**

According to **(Thampuran *et al;* 2005).** *E. coli* is commonly associated with seafood contamination in the tropics, where it is encountered in high numbers. The occurrence of this bacterium in food is directly related to fecal contamination. On what seafood is regarded, the occurrence of *E. coli* is related to water contamination and/or unhygienic conditions during the handling process **(Huss, 1993).** *E. coli* is often nonpathogenic, although different strains may cause diseases in gastrointestinal, urinary, or central nervous systems **(Nataro and Kaper, 1998)**. On the other hand, the occurrence of *E. coli* in shell fish has been accounted in different parts of the world **(Baer *et al.,* 1976; Hood *et al.,* 1983 and Tusevlijake *et al.,* 2012). Gourmelon *et al.,* (2006)** suggested that shell- fish collected in coastal environments can serve as a vehicle for Shiga toxin-producing *E. coli* transmission.

Staphylococcus aureus is a bacterium that causes staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms. *S. aureus* produces staphylococcal enterotoxin (SE) and is responsible for almost

all staphylococcal food poisoning **(Montville and Matthews 2008; FDA 2012).** *S. aureus* isolates pathogenic for humans usually coagulate human and rabbit plasma.

The specific toxins include the enterotoxin causing food poisoning **(Shena and Sanjeev 2007).**

Aeromonas hydrophila is living in aquatic environments and the gastrointestinal tract of healthy fish **(Daskalove 2006; Edberg *et al.,* 2007 and Jandaand Abbot 2010).** It can be found in freshwater, seawater, and also chlorinated-drinking water. This bacterium has been considered as a food-borne pathogen since it is found in many food products, for example sea food, shrimp cocktail, ground meat and raw vegetables, and also in fish and shellfish, **Abeyta, *et al.,* 1990; Isonhood and Drake, 2002).**

Recently it was also isolated from freshwater crayfish **(Pikulet *et al.,* 2009)** and they have been shown to cause food borne outbreaks of gastrointestinal disease **(Zeng Shan *et al.,* 1988).** Its virulence related to many factors, such as enterotoxins **(Janda and Abbott, 1998).**

However, there is substantial evidence that fish and seafood are high on the list of foods associated with outbreaks of food borne diseases **(Huss, 2003).** Therefore, this study was carried out to determine the prevalence of *A. hydrophila*, pathogenic strains of *E, coli* and *S. aureus* in (Chikal) commercially sold in Port-Said city as well as the ability to produce enterotoxin. Also the public health significance of the isolated such bacteria was discussed.

Materials and Methods

1- Collection of samples.

This study was carried out on 100 sample of (Chikal) collected randomly from fish markets, and street vendors in different districts of Port-Said city at different times. All of the samples were collected in sterile plastic bags and transported to the laboratory on ice – box and divided to two groups (A and B) for bacteriological examination.

a- Group (A) 50 sample of complete chikal with head and shells

b- Group (B) 50 sample of chikal peeled without head and shells (peeling head and shells was done using sterile forceps and scissor.

2- Preparation of the samples.

The technique recommended by **APHA (1992)** was used for samples preparation. 25 grams of samples were aseptically added to 225 ml of Buffered peptone water and homogenized in a stomacher for 2 min (1:10) Ten fold serial dilutions were prepared using sterile 0.1% peptone water. 0.1 ml aliquot was aseptically transferred from each mixture and spread over the surface of duplicate plates of specific media to every type of organism. Aeromonas agar to which Aeromonas selective supplement was added. Eosin Methylene Blue agarfor *Escherichia coli*) and Baird Parker egg yolk supplement agar for (*Staph. aureus*) the plates were incubated at 37^{o}c for 24-48 hr aerobically.

3- Bacteriological examination.

a-Isolation of *E.coli* was displayed according to **APHA (1992)**.

b-Isolation and identification *S.aureus* was carried out according to **ISO (1999).**

c-Isolation and identification of *A.hydrophila* was done according **to Umadatt(1997)**

4- Detection of toxin produced by isolates.

Enterotoxin assay.

The ability to produce enterotoxin was assayed by the infant mouse test according to the technique described by **(Pai and Mors, 1978)** and **(Stavric *et al.,* 1992).**

1-Preparation of culture filtrate.

Isolated strains were inoculated into 25 ml of specific media, incubated at room temperature at 22-26°C, (as well as *A.hydrophila* isolates were tested as mentioned above) on a rotatory shaker (200 r.p.m) for 48 hours, centrifuged at 12000 for 10 minutes. The supernatant was filtered through Millipore membrane filter (Pore size 0.22µm), stored at - 20 □C, till used. A part of the sterile medium was used as a negative control.

2- Infant mouse assay.

A volume of 0.1 ml of culture filtrate was injected through the abdominal wall into milk-filled stomach of each of 2 mice (for each serotype) which were 2 to 4 days old. Another 2 infant mice were injected by 0.1 ml saline and were used as negative control. After 4 hours, the mice were killed and the entire intestine was removed. The intestine and the remaining body were weighted to calculate the ratio of (intestine weight) / (remaining body weight). A ratio greater than 0.083 was recorded as positive test for enterotoxin.

Result

Table 1. Prevalence of isolated pathogens in the examined chikal (A) and (B).

	E.coli		*A.hydrophila*		*S.aures*	
	No.	%	No.	%	No.	%
Group (A) 50	0	0	18	36	12	24
Group (B) 50	0	0	14	28	7	14
Total (100)	0	0	32	32	19	19

Group A = unpeeled samples (with shells and head).
Group B= peeled (without shells and head).

Table 2. Prevalence of *Staphylococcus aureus* isolated from the examined samples and toxins producing strains

	Type of samples					
	Group A n=50		Group B n=50		Total n=100	
	No.	%	No.	%	No.	%
Total *Saphylococcus aureus*	12	24	7	14	19	19
Coagulase positives *Saphylococcus aureus*	1	8.33	0	0	1	5.26
Coagulase negative *Saphylococcus aureus*	11	91.66	7	100	18	94.7
Enterotoxinss producing strain	1	8.33	0	0	1	5.26

Group A= unpeeled samples (with shells and head).
Group B= peeled (without shells and head)

Table 3. Detection of enterotoxin producing *Aromonas. hydrophila* strains isolated from the examined samples using infant mouse assay.

	Aeromonas hydrophila	Positive of enterotoxigenic *A. hydrophila*	
	No.	No.	%
Group A No. 50	18	4	22.1
Group B n=50	14	1	7.1
Total No=100	32	5	29.5

Group Group A= unpeeled samples (with shells and head).
Group B= peeled (without shells and head)

Discussion

E.coli is enteric bacteria causing gastroenteritis and it is cause with other bacteria as staphylococcus spp. indices of hazardous condition during handling and processing of food such organisms should not be present on fresh caught sea foods **(Chattopadhyay, 2000).**

This study investigated that *E.coli* pathogenic strains (O 157) not be detected and this agree with **(Kumar *et al.,* 2003 and Manna & Manna, 2008)** when they couldn't detect E.coli O 157 in shell fish and they said only few samples were contaminated with non O157 serotype .Our results also agree with the previous studies met with **(Lovel & Barakate, 1969)** when used MPN technique and detected E.coli in 100% of commercial processed shellfish but its suppressed in raw flesh samples. However different finding display by **(Mansour *et al.,* 1994)** who found 2 samples out of 44(5%) of shell fish were positive to O 157 . In Brazil **(Ayulo *et al.,* 1994**) isolate only one strain of *E.coli* from shellfish and it is non toxin producing while **(Mohamed, 2003)** recorded that *E.coli* was isolated as in 0.6% in giant shrimp shell and was 4.8% in head less shell. All of these differences may be attributed to different techniques and types of media and or, sources of sampling, handling of samples and different environment. Table2 show that *staphylococcus aurous* isolated as 24 and14% from unpeeled and peeled chikal respectively Only one strain of coagulase positive staphylococcus aureus was detected in whole chikal (8.33%), these finding agree with **(Ayulo *et al.,* 1994)** when detected *S.aureus* from 20% of the samples and 9 samples from 109 producing toxins as 8.25% also our result revealed toxins in 1 sample from 12 positives staph by 8.33% and coagulase positive in 8.3% in(group A) and it was 0% in group B . Our results disagree with **(Mohamed, 2003)** who isolated Staph in 1.3% in raw peeled shrimp and the coagulas positive staph were 5% in raw and peeled tail off. **David *et al.,* (1979)**, recorded higher incidence of coagulas positive *S.aureus* strains as (79 %) of processed shill fish and attributed it to contamination during processing. our result proved that, A.hydrophila was detected in 36% ,and 28% of unpeeled and peeled chikal respectively similar finding were observed by **(Ye *et al.,* 2013)** who examined 60 different fish and find A.hydrophila in 20 by 33.3% also **(Ali& Hossien, 2010)** found A.hydrophila in 35% of shell fish and 28% of fin fish higher incidence was reported by **(Khalil and Saad, 2013)** who detected A.hydrophila in 40.38% of fresh water shellfish. These results may disagree with that investigated by **(Mic and Jsyvokiene, 2011)** as they recorded Aeromonas species constitute up to 70% of shellfish eggs. but lower incidence was reported by **(Yosra, 2004)** who isolated A.hydrophila as 1.98% from fresh water shell -fish.

From the present study, it was concloded that Chikal which is considered popular seafood in Port-Said it may cause some bacterial hazard and may be harmful causing bacterial gastroenteritis and toxicity. There is a quantity differences between the peeled and unpeeled crustacean shellfish because of the hard exoskeleton which act as a first line of defense against microorganisms. So that to increase shelf life of seafood and to prevent the infections by these bacteria , 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 of the source of contamination from the harvested sites.

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الكشف عن مدى تواجد بعض البكتريا الممرضة من (الشيكال) مع الاشارة الى المنتجة منها للسموم المعوية

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الملخص العربي

أجريت هذه الدراسة علي نوع من الجمبرى معروف في محافظة بورسعيد باسم (شيكال) بهدف الكشف عن مدي تواجد بعض البكتريا الممرضة وكذلك تواجد بعض السموم البكتيرية لهذه الميكروبات وتأثيرها علي الصحة العامة. تم تجميع 100عينة من اسواق محافظة بورسعيد واخضاعها للفحص البكتيري ثم عزل وتصنيف بعض الميكروبات الممرضة من عضلات العينات موضع الدراسة. تم تقسيم العدد الكلي الي مجموعتين 50عينة لكل مجموعة المجموعة(أ) كامل العينة بالرأس 'مجموعة (ب) عينات تم تقشيرها ونزع رأسها. اسفرت الدراسة عن عدم تواجد للعترات الممرضة من ميكروب الأشيرشيا كولاي او سمومها فقد كانت نسبتها 0% في العينات بينما كانت نسبة تواجد الأيروموناس هيدروفيلا بنسبة 36% في المجموعة (أ) و 28% في المجموعة(ب) واثبتت الدراسة وجود ميكروب المكور العنقودي الذهبي في 24% من العينات في المجموعة (ا) و 14% في المجموعة (ب) والموجبة منها لتخثر البلازما بنسبة % 8.3 في كل من المجموعتين مع وجود سموم المكور العنقودي الذهبي في العينات موضع البحث بذات النسبة.