EJAR

Microbiological monitoring of some pathogenic bacteria in common commercial molluscan shellfish in Lake Timsah, Suez Canal, Egypt

Hanaa Mohamed Fadel[1] and Samya Hussein Mohammad[2]

[1]Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Suez Canal University, Egypt
[2]Department of Zoology, Faculty of Science, Suez Canal University, Egypt
**E-Mail: samya_hm@hotmail.com*

Received 7th May 2009, Accepted 30th July 2009

Abstract

Seafood is considered as an important source of protein and also it includes several important nutritive elements. Owing to its low fat content, it is considered the most favourable food for many people. On the other hand, eating raw or slightly cooked shellfish is one of the feeding habits that lead to infection with some diseases. This study was carried out to evaluate the prevalence of some pathogenic bacteria in the common street seafood. A total of 114 specimens of the gastropod *Thais carinifera* and 358 specimens of the bivalves; *Ruditapes decussata*, *Venerupis aurea* and *Venerupis white* were collected during the period from July to December, 2008. A diverse array of bacterial species, including several human pathogens, was isolated from these species. *Aeromonas hydrophila, Salmonella* spp., *Staphylococcus aureus* and *Escherichia coli* were isolated from soft tissue of the examined gastropod and bivalve samples at the rates of 34.88% and 28%, 20.9% and 26.3%, 86% and 57.9 % and 41.9 % and 57.9 %, respectively. They were also isolated from hand swabs collected from fishermen and shellfish sellers at the rates of 10%, 0%, 100% and 50%, respectively. The current research showed that bivalve species have higher bacterial counts than that of the gastropod. This study assessed that *Aeromonas hydrophila* was the most harmful bacteria infected bivalve species especially *Venerupis aurea*. It adversely affected their growth with size. The isolation of potentially pathogenic bacteria from the examined shellfish indicates a risk for health of people who consume or handle raw seafood.

Keywords: Shellfish, gastropod, bivalve, pathogenic bacteria, allometric growth, public health significance.

1. Introduction

Seafood constitutes an important food for humans particularly as a source of animal protein. The drill *Thais carinifera* is the most popular gastropod species harvested and consumed in Ismailia due to its cheap price and its big size. On the other hand, the bivalves *Ruditapes decussate*, *Venerupis aurea* and *Venerupis white* are by far the species most exploited and most eaten by the recreational harvesters in the area of study. Most harvesters believe that there is no particular health risk associated with molluscan shellfish consumption. In fact, bivalve molluscs filter large quantities of water and thereby concentrate a variety of aquatic contaminants pathogenic for man within their edible viscera (Lalitha and Surendran, 2005). Shellfish are identified as vehicles of *Aeromonas hydrophila, Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli* and many other pathogens (Rippey, 1994).

Aeromonas are found in soil, fresh, marine and brackish water (Herrera *et al*., 2006 and Jennifer *et al*., 2006). They are indigenous in sea water worldwide. They may be introduced into water courses by sewage contamination. This microorganism is isolated at high rates from seafood and aquaculture food (Thayumanavan *et al*., 2003). As a result, the US Food and Drug Administration has designated *Aeromonas* spp. as emerging food borne organism of increasing importance. Aeromonads have multiple opportunities for transmission to human through ingestion and contact with human, animal or handling fish and shellfish. (Hanninen *et al*., 1997). In immunocompetent persons, it causes gastroenteritis or localized wound infection. In immunocompromised patient, the organism may disseminate resulting in septicemia with multiple organ involvement (Lehane and Rawlin, 2000).

Coliforms are used as indicator of fecal pollution. Moreover, several strains of *Escherichia coli* are implicated in human illness. Enterohemorrhagic *E. coli/ Shiga* toxin producing *E. coli* (EHEC/STEC) serotypes cause infection ranging from self limiting diarrheal illness to bloody diarrhea with severe complications such as haemolytic uraemic syndrome (HUS) and nervous symptoms. Both syndromes contribute to fatalities in man (Yamashiro *et al.,* 1998 and Sayers *et al*., 2006). Enterotoxigenic *E. coli* (ETEC) is the most frequently isolated enteropathogen in the developing world of children 5 years old or younger; it is also the major cause of travelers' diarrhea (Schultsz *et al*., 2000 and Qadri *et al*., 2005). Enteropathogenic *E. coli* (EPEC) is a leading cause of severe diarrhea in infants

and young children in the developing world (Donnenberg, 2005). Enteroinvasive *E. coli* (EIEC) is a cause of diarrhea; it has been implicated in several food borne outbreaks (Gordillo *et al.*, 1992).

Bacteria in polluted marine environment include other species such as *Salmonella* spp. which reach water via the discharge of raw sewage and agricultural wastes. Animals and birds can also disseminate this microorganism into water. *Salmonella* infection can be severe with diarrhea, septicemia, bowel bleeding as seen with *S. typhi* and *S. paratyphi* infection. Other serotypes of *Salmonella* cause gastroenteritis with diarrhea, fever, abdominal pain and vomiting (Bean *et al.*, 1997 and Mearin *et al.*, 2005).

Staphylococcus aureus can be recovered from seafood (Le Loir *et al.*, 2003). This microorganism produces enterotoxin, subsequent ingestion of this enterotoxin leads to food borne intoxication characterized by vomiting, abdominal pain, diarrhea with subsequent fatalities in the extreme ages due to increased fluid and electrolyte loss.

This study was undertaken: to determine the prevalence of some pathogenic bacteria of public health importance in molluscan shellfish, to assess the hazards associated with consumption or handling molluscan shellfish, and on the other hand, to study the effect of bacterial infections on the growth of molluscan shellfish.

2. Materials and Methods

2.1. Sampling

During the period from July to December, 2008, a total of 114 specimens of the gastropod *Thais carinifera* and 358 specimens of the bivalves (151 of *Ruditapes decussata,* 100 of *Venerupis aurea* and 107 of *Venerupis white*) were collected alive from fishermen at Lake Timsah, Ismailia, Egypt. These samples were placed in sterile bags and immediately transported in an ice box to the laboratory. In addition, a total of 30 hand swabs were collected from fishermen and sellers who handle shellfish using sterile swabs (average hand surface area was 100±10 cm^2). Each swab was placed in a sterile tube containing buffered peptone water and transported in a refrigeration unit (APHA, 1992).

2.2. Relative growth

For the establishment of the weight/length relationships and biometric relationships, three bivalve shell axes were measured to the nearest 0.01 mm with a vernier caliper. These axes namely: length (maximum distance along the anterior-posterior axis), height (maximum distance on the dorsal-ventral, across the shell middle axis) and width (maximum distance on the lateral axis, between the two valves of the closed shell) (Gaspar *et al.*, 2002). The shell length (L) was measured from the apex to the base or anterior end of the shell in the drill *Thais carinifera*. Shell height (H) is the largest distance between any two points on the circumference of the shell. The shellfish were accurately weighed on a top-loading digital balance with a precision of 0.001g. In this way total weight of live animal (TW), weight of the flesh without shell (soft wt) and operculum weight (op wt) were recoded.
The allometric relationship between two characters can be expressed by the general equation:

$$Y = aX^b$$

This equation can also be expressed in its linearised logarithmic form:

$$\log Y = \log a + b \log X$$

Where:
Y- length (L-mm) (biometric relationship) or weight (W-g) (weight/length relationship)
X- height (H-mm) or width (wi- mm) (biometric relationship) or length (L- mm) (weight/length relationship)
a- intercept (initial growth coefficient)
b- slope (growth coefficient)

The allometry coefficient is expressed by the exponent *b* of the linear regression equations. In these equations, whenever both measurements are linear variables and are expressed in the same unit, when b=1, the biometric relationship describes an isometric growth. In relations between different types of variables and/or between different measuring units, when the exponent b=3, the weight/length relationship reflects an isometric growth.

The relationship parameters (*a* and *b*) were estimated by linear regression analysis on log-transformed data and the association degree between the variables was calculated by the determination coefficient (R^2).

2.3. Microbiological analysis

2.3.1. Preparation of samples (APHA, 1992)

Bacterial analysis was carried out within 2-4 hours of sample collection. The shellfish were washed under running drinking water, scrubbed free of dirt with sterile stiff brush and shucked with a sterile knife. Tissue and shell liquor samples (7-10 g) were weighed, then transferred to a sterile blender jar containing sterile buffered peptone water and homogenized for 90 seconds to make a 1:10 dilution, and immediately added to the appropriate enrichments or dilutions. The microbiological analysis was performed according to standard methods adopted from **(**USFDA, 2001**)**

2.3.2. Isolation and identification of Aeromonas hydrophila

A. hydrophila was enumerated by standard plate count on RS agar (Shotts and Rimler, 1973) supplemented with ampicillin 10 mg /liter. After incubation at 35°C, bright yellow colonies were

selected; confirmed with Gram stain, oxidase test, indole test, Voges Proskauer test, H_2S production, hemolysis, catalase, no growth in 1% NaCl, production of gas from glucose, D-mannitol, sucrose, D-sorbitol, lysine decarboxylase as recommended by (Martin-Carnahan and Joseph, 2005).

2.3.3. Isolation and identification of Staphylococcus aureus

One hundred microliters from the selected dilutions were plated on Baird Parker's agar medium (BPA) using a sterile smooth bent glass rod. After 48 hours of incubation at 37°C, the characteristic black colonies with peripheral clearance zone were counted and tested for Gram stain, catalase, DNase, mannitol fermentation and coagulase activity.

2.3.4. Isolation and identification of Salmonella species

Shellfish homogenates were pre-enriched on lactose broth, incubated overnight at 37°C followed by enrichment in Rappaport-Vassiliadis broth (RV) overnight at 42°C. A loopful of growth was streaked on Xylose Lysine Desoxycholate agar medium (XLD), incubated for 24 hours at 42°C. Both typical and atypical colonies were picked up, purified and further identified by Gram stain, urease test, TSI, indole, methyl red, Voges Proskauer test, utilization of citrate, lysine decarboxylase **(**USFDA, 2001**)**. Then, confirmed serologically at The Central Laboratory, Ministry of Health, Cairo.

2.3.5. Isolation and identification of Escherichia coli

Isolation and enumeration of *E. coli* was performed using spread plate method on Eosin Methylene blue (EMB), incubated for 24 hours in sealed but vented containers in a water bath at 44.5± 0.2°C. Green, purple coloured colonies with a metallic sheen were selected. Presence of *E. coli* was confirmed by indole, methyl red, Voges-Proskauer and citrate (IMViC), urease, TSI. Serotyping was carried out as aforementioned.

Other types of colonies were individually picked, purified and biochemically identified.

2.4. Genetic confirmation of *Salmonella* and *Aeromonas hydrophila* isolates by polymerase chain Reaction (PCR) technique

This test was done at The PCR Unit, Department of Infectious Diseases, Faculty of Veterinary Medicine, Suez Canal University.

Preparation of DNA template (Sambrook *et al.*, 1989)

Colonies on XLD and RS media suspected to be *Salmonella* and *Aeromonas hydrophila*, respectively were subjected to PCR. DNA extraction was carried out by boiling method; a loopful of bacterial colony was suspended in 100 µl of PBS. Centrifugation was occurred at 3000 rpm for 5 min., bacterial pellet was then dissolved in 100 µl of PBS and was subjected to heating in a boiling water bath at 100°C for 10 min., then centrifugation at 13000 rpm for 15min., and the supernatant was transferred into a new sterile tube. The DNA purity and concentration were measured by spectrophotometer (Davis *et al*., 1986).

Oligonucleotide primers

The oligonucleotide primer pairs corresponding to *inv A* gene of *Salmonella* (Rahn *et al.*, 1992) and lipase gene of *Aeromonas hydrophila* (Anguita *et al*., 1993) were synthesized by Biobasic Inc., Canada.

Polymerase chain reaction

DNA samples (100 ng per reaction) were amplified in a 25 µl reaction mixture consisting of 1.5 unit *Taq* polymerase (Sibenzyme, Russia), 1 X TAQ polymerase buffer, 200 µM of dNTPs mixture, 20 pmole of each primer and sterile distilled water up to 25 µl, amplification was performed in thermal cycler (Techne Progene, UK).

Table 1. Sequence of primers used for PCR assay.

Primer	Sequence	Gene	Nucleotide position
INVA-1 INVA-2	5'-ACA GTG CTC GTT TAC GAC CTG AAT-3' 5'-AGA CGA CTG GTA CTG ATC GAT AAT-3'	*Inv A* *Inv A*	104 – 127 324 - 347
Lipase-1 Lipase-2	5'-AACCTGGTTCCGCTCAAGCCGTTG-3' 5'-TTGCTCGCCTCGGCCCAGCAGCT-3'	Lipase Lipase	442-467 1181-1205

Parameters for *Salmonella* spp. amplification included an initial denaturation at 95°C for 5 min., followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 5 min.

For *Aeromonas hydrophila* amplification, a total of 40 PCR cycles were run under the following conditions: DNA denaturation at 94°C for 1 min., primer annealing at 62°C for 1 min. DNA extension at 72°C for 1.5 min., followed by a final extension at 72°C for 5 min.

Amplified products were separated by electrophoresis in a 1.7 % agarose gel (Biobasic), stained by ethidium bromide (0.5 µg / ml) in 1 X TAE buffer at constant voltage of 4 v/cm, and photographed with Sony digital camera. A 100 bp DNA marker (Axygen) was used as a DNA molecular size standard.

2.5. Statistical analysis

Minimum, maximum, mean and standard deviation of shell measurements and weights were estimated by subjecting the data to pivot table in the Excel package. The relationships between the shell length and either of the shell width, shell height, total weight and soft tissue weight were studied using the linear regression technique. The difference of bacterial counts between the examined gastropod and bivalve species was tested using t-test.

3. Results

During the present study, 114 specimens of *Thais carinifera* were collected. Their shell length and shell height ranged between 41.70 and 69.30 mm and 31.05 and 54.15 mm respectively. Means of their weights were 0.54, 45.37 and 11.22 g for operculum, total and soft weight, respectively. Other individual counts, body measurements and weights for the examined shellfish were recorded in table (2). *Thais carinifera* had the highest body measurements and weights followed by *Ruditapes decussata*, *Venerupis aurea* and *Venerupis white* in a decade manner. This gastropod also recorded higher infection rate with the most studied bacteria (*Aeromonas hydrophila, Pseudomonas* species, *Staphylococcus aureus*, *Citrobacter diversus, Enterobacter cloacae* and *Klebsiella oxytoca*) than the total examined bivalves (Figure 1).

The mean counts of *A. hydrophila* was higher in bivalve species ($3.61x10^2$) cfu ⁄g than *Thais carinifera* ($1.2x10^2$) cfu/g as shown in table (3). This difference was significant ($p<0.05$). Within the examined bivalve species, *Ruditapes decussata* recorded the highest prevalence rate (43.5%).

The overall rate of isolation of *Pseudomonas* spp. from molluscan shellfish was 15.6%. *Pseudomonas* spp. were isolated from the gastropod *Thais carinifera* at a higher rate (39.5%) than the total bivalve species (3.5%). Within the bivalve species, it could not be recovered from *R. decussata* and *V. white*.

Staphylococcus aureus showed the highest prevalence rate (86%) in *Thais carinifera* followed by *V. aurea, V. white* and *Ruditapes decussata* at the rates of (77.8%, 50%, and 47.8%), respectively. Overall, *S. aureus* was isolated from molluscan shellfish at the rate of 67.3%. The mean counts of *S. aureus* was higher in bivalve ($10x10^7$) cfu/g than gastropod ($2.4x10^7$) cfu/g. This difference was highly significant ($p<0.001$).

Salmonella spp. revealed a reverse trend (table 3). Its isolation rate was higher in bivalve (26.3%) than the gastropod *Thais carinifera* (20.9%). Totally, *Salmonella* spp. were isolated from molluscan shellfish at the rate of 24.5%. The predominant serotype was *S. kentucky*.

Similar to *Salmonella, E. coli* was isolated at a higher rate from bivalve species (57.9%) than the gastropod *Thais carinifera* (41.9%). The mean counts of *E. coli* was higher in bivalves ($3.1x10^2$) than the gastropod *Thais carinifera* ($2.65x10^2$). The overall isolation rate of *E. coli* in molluscan shellfish was 52.5%.

The serotypes recovered belonged to four pathogenic groups; the enteroinvasive *E. coli* (EIEC), the enterotoxigenic *E. coli* (ETEC), shiga toxin producing *E. coli ⁄* enterohemorrhagic *E. coli* (STEC ⁄ EHEC) and the enteropathogenic *E. coli* (EPEC). EIEC was represented by two serotypes; O124:H30 and O164:NM. The serotype O124:H30 represented 60% and 33.3% of the total *E. coli* isolates from *V. white* and the gastropod *Thais carinifera*, respectively. While, O164:NM was isolated only from *Ruditapes decussata.* ETEC was isolated only from *Thais carinifera*; represented by serotypes; O25:NM and O78:H11. The (STEC) was represented by two serotypes O26:H11 that was isolated from *Ruditapes decussata* and *Thais carinifera.* The serotype O111: H8 predominated in *V. aurea* (60% of its total *E. coli* isolates). EPEC was represented by the serotype O86: H34 which was detected in the bivalves *V. aurea* and *R. decussate* Table (4) illustrates that handling molluscan shellfish led to heavy contamination of hands by *Aeromonas hydrophila*, *E. coli* and *S. aureus*. *Aeromonas hydrophila*, *E. coli*, *Salmonella* spp.*,* and *S. aureus* were isolated from hand swabs collected from fishermen and shellfish sellers at the rates of 10%, 50%, 0% and 100%, respectively. The mean counts of *Aeromonas hydrophila*, *E. coli* and *S. aureus* were 3x10, $5x10^2$ and $2.3x10^6$ cfu/100 cm^2, respectively.

The analysis of tables (5) and (6) showed a consistent type of growth in height/length and width/length relationships, with the maintenance of a negative allometry in all shellfish species infected with the bacteria. With respect to weight/length relationships, table (5) showed a slight modification in the growth pattern of the total weight of *Thais carinifera*. It transited from a positive allometry (b=3.709) to a negative (b=2.829) allometric growth when infected with *Aeromonas hydrophila*. The soft weight/L relationship of this gastropod revealed the same pattern when infected with *Aeromonas hydrophila, Salmonella* spp. and *Escherichia coli*. For its operculum weight/L relationship, two modifications occurred: from a negative to isometric growth (when infected with *Aeromonas hydrophila*) and from a positive to a

negative growth when infected with *Staphylococcus aureus.* Table (6) shows that soft weight/L relationship of *Ruditapes decussata* transited from a negative allometry to positive (when infected with *Staphylococcus aureus*) and isometric allometry (when infected with *Salmonella* spp.). The only change from positive to negative allometry was recorded with the infection of *Aeromonas hydrophila*. Also the transition of soft weight /L relationship from a positive to a negative allometry was recorded in the infection with *Staphylococcus aureus* and *Escherichia coli* for the bivalves *Venerupis aurea* and *Venerupis white* respectively. The only transition from isometric to negative allometric growth was shown in *Venerupis white* when infected with *Staphylococcus aureus*. Among the studied shellfish, *Venerupis aurea* was harmly affected by *Aeromonas hydrophila* that negatively alter their growth (Figure2)

Presumptive *Salmonella* and *Aeromonas hydrophila* colonies were subjected to PCR test to detect (*inv A*) and (*lip*) genes specific for the genus Salmonella and *Aeromonas hydrophila*, respectively. Photograph 1(A) illustrates that the amplification of *Salmonella* samples produced a band (284 bp) of the gene (*inv A*) in three samples (lanes, 3, 4, and 5). Photograph 1(B) illustrates that the amplification of *Aeromonas hydrophila* samples produced a band (760 bp) of the lipase (*lip*) gene in three samples (lanes, 3, 4 and 5).

Table 2. Numbers, shell measurements (mm) and body weights (g) of the studied molluscan shellfish species.

Molluscan species	***Thais carinifera***		***Ruditapes decussata***		***Venerupis aurea***		***Venerupis white***	
Measurements and weights	**No.**	**min-max (mean ± SD)**	**No.**	**min-max (mean ± SD)**	**No.**	**min-max (mean ± SD)**	**No.**	**min-max (mean ± SD)**
shell length	114	41.70-69.3 (55.54 ± 6.27)	151	19.30-33.80 (26.13 ± 2.84)	100	14.90-31.20 (20.92 ± 4.39)	107	14.00-28.90 (20.10 ± 3.21)
shell height		31.05-54.15 (41.39 ± 5.08)		12.30-22.70 (17.88 ± 1.99)		9.70-19.40 (13.53 ± 2.69)		9.50-17.80 (12.70 ± 1.91)
shell width				8.70-15.50 (11.77 ± 1.25)		5.50-12.40 (8.44 ± 1.99)		5.60-11.20 (7.93 ± 1.39)
operculum weight		0.17-1.17 (0.54 ± 0.20)						
total weight		15.70-94.52 (45.37 ± 19.27)		1.46 -6.88 (3.39 ± 1.11)		0.58-4.19 (1.64 ± 0.97)		0.50-3.39 (1.31 ± 0.66)
soft tissue weight		5.21-21.77 (11.22 ± 3.61)		0.20-2.29 (1.07 ± 0.38)		0.13-1.16 (0.45± 0.28)		0.06-0.85 (0.35± 0.16)

No.: individual counts, min: minimum, max: maximum, SD: standard deviation

Table (3): Results of microbiological examination of molluscan shellfish.

Types of shellfish / Bacterial species	**Gastropod (*Thais carinifera*)**					**Bivalves**											**Grand total**	
						R.. decussata		***V. white***		***V. aurea***		**Total bivalve positive**		**Counts Cfu/g**				
	Positive		Counts Cfu/g			positive		positive		positive				min	max	Mean	positive	
	N	%	Min	Max	Mean	N	%	N	%	N	%	N	%				N	%
Aeromonas hydrophila	30	34.88	3.2 x10	3.16 $x10^2$	1.2 $x10^2$	30	43.5	6	12.5	12	22	48	28	2 $x10^2$	4.47 $x10^2$	3.61 $x10^2$	78	30.4
***Pseudomonas* spp.**	34	39.5	-	-	-	-	-	-	-	6	11.1	6	3.5	-	-	-	40	15.6
S. aureus	74	86	3 $x10^6$	7 $x10^7$	2.4 $x10^7$	33	47.8	24	50	42	77.8	99	57.9	1 $x10^6$	4 $x10^8$	10 $x10^7$	173	67.3
***Salmonella* spp.**	18	20.9	-	-	-	15	21.7	12	25	18	33.3	45	26.3	-	-	-	63	24.5
E. coli	36	41.9	10 x10	4.47 $x10^2$	2.65 $x10^2$	39	56.5	30	62.5	30	55.6	99	57.9	2.7 x10	4.36 $x10^2$	3.1 $x10^2$	135	52.5
Proteus vulgaris	52	60.5	-	-	-	45	65.2	24	50	36	66.7	105	61.4	-	-	-	157	61.1
Citrobacter diversus	24	27.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24	9.3
Enterobacter cloacae	18	20.9	-	-	-	12	17.4	-	-	6	11.1	18	1o.5	-	-	-	36	14
Klebsiella oxytoca	38	44.2	-	-	-	27	39.1	30	62.5	6	11.1	63	36.8	-	-	-	101	39.3

N: Number of positive samples.
Number of pooled samples: in *T. carinifera* =86, in *R.. decussate* =69, in *V. white* =48, in *V. aurea* =54

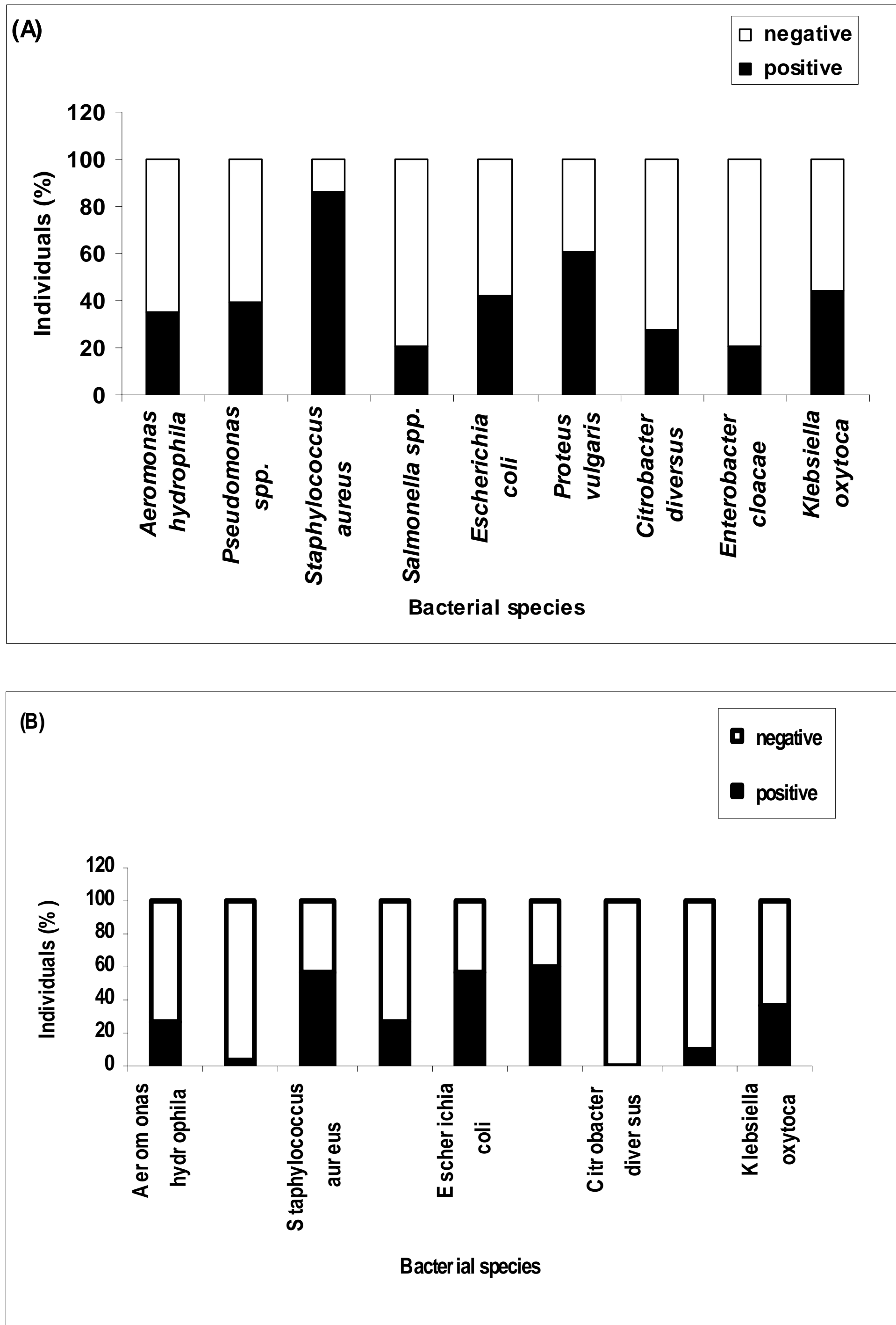


Figure 1: Prevalence of bacterial species in the gastropod (A) and all bivalve species (B).

Table 4. Prevalence of pathogenic bacteria in hand swabs collected from fishermen and molluscan shellfish sellers.

Number examined	**Bacteria recovered**										
	Aeromonas hydrophila			***S. aureus***			***Salmonella* spp.**		***E. coli***		
	Positive			Positive			Positive		Positive		
	N	%	Mean count Cfu/100 cm^2	N	%	Mean count Cfu/100 cm^2	N	%	N	%	Mean count Cfu/100cm^2
30	3	10	3x10	30	100	$2.3x10^6$	-	-	15	50	$5x10^2$

N: Number of positive samples

Table 5: Biometric and weight-length relationships of Thais carinifera infected with pathogenic bacteria

Bacterial species	**Infection**	**Biometric equation of**			
		L/H (R^2)	**TWL (R^2)**	**soft wt/L (R^2)**	**op wt/L (R^2)**
Aeromonas hydrophila	infected	$y = 0.5474x + 10.796$ (R^2=0.470)	$y = 0.0005x^{2.8285}$ (R^2= 0.70)	$y = 8E\text{-}05x^{2.9147}$ (R^2= 0.87)	$y = 3E\text{-}06x^{3.0015}$ (R^2= 0.54)
	uninfected	$y = 0.6252x + 6.0796$ (R^2=0.0.58)	$y = 1E\text{-}05x^{3.7087}$ (R^2= 0.77)	$y = 8E\text{-}05x^{3.037}$ (R^2= 0.80)	$y = 7E\text{-}06x^{2.7621}$ (R^2= 0.58)
***Salmonella* spp.**	infected	$y = 0.2765x + 24.89$ (R^2=0.37)	$y = 3E\text{-}05x^{3.}5641$ (R^2= 0.76)	$y = 0.0006x^{2.4204}$ (R^2= 0.85)	$y = 4E\text{-}06x^{2.2822}$ (R^2= 0.42)
	uninfected	$y = 0.6443x + 5.2911$ (R^2=0.58)	$y = 4E\text{-}05x^{3.4646}$ (R^2= 0.74)	$y = 3E\text{-}05x^{2.1389}$ (R^2= 0.82)	$y = 4E\text{-}06x^{2.9145}$ (R^2= 0.59)
Staphylococcus aureus	infected	$y = 0.6127x + 7.2045$ (R^2=0.59)	$y = 5E\text{-}05x^{3.4213}$ (R^2= 0.76)	$y = 3E\text{-}06x^{3.6742}$ (R^2= 0.86)	$y = 9E\text{-}06x^{2.6976}$ (R^2= 0.55)
	uninfected	$y = 0.6156x + 4.9115$ (R^2=0.43)	$y = 7E\text{-}07x^{4.4428}$ (R^2= 0.71)	$y = 3E\text{-}06x^{3.5742}$ (R^2= 0.63)	$y = 7E\text{-}09x^{4.4572}$ (R^2= 0.76)
Escherichia coli	infected	$y = 0.4465x + 15.099$ (R^2=0.40)	$y = 6E\text{-}05x^{3.3304}$ (R^2= 0.73)	$y = 0.0005x^{2.4736}$ (R^2= 0.74)	$y = 4E\text{-}05x^{2.3278}$ (R^2= 0.29)
	uninfected	$y = 0.5934x + 8.4366$ (R^2=0.54)	$y = 5E\text{-}05x^{3.4043}$ (R^2= 0.72)	$y = 2E\text{-}05x^{3.276}$ (R^2= 0.84)	$y = 4E\text{-}06x^{2.8921}$ (R^2= 0.64)

L: shell length, H: shell height, TW: total weight, soft wt: soft tissue weight, op wt: operculum weight, R^2: correlation coefficient

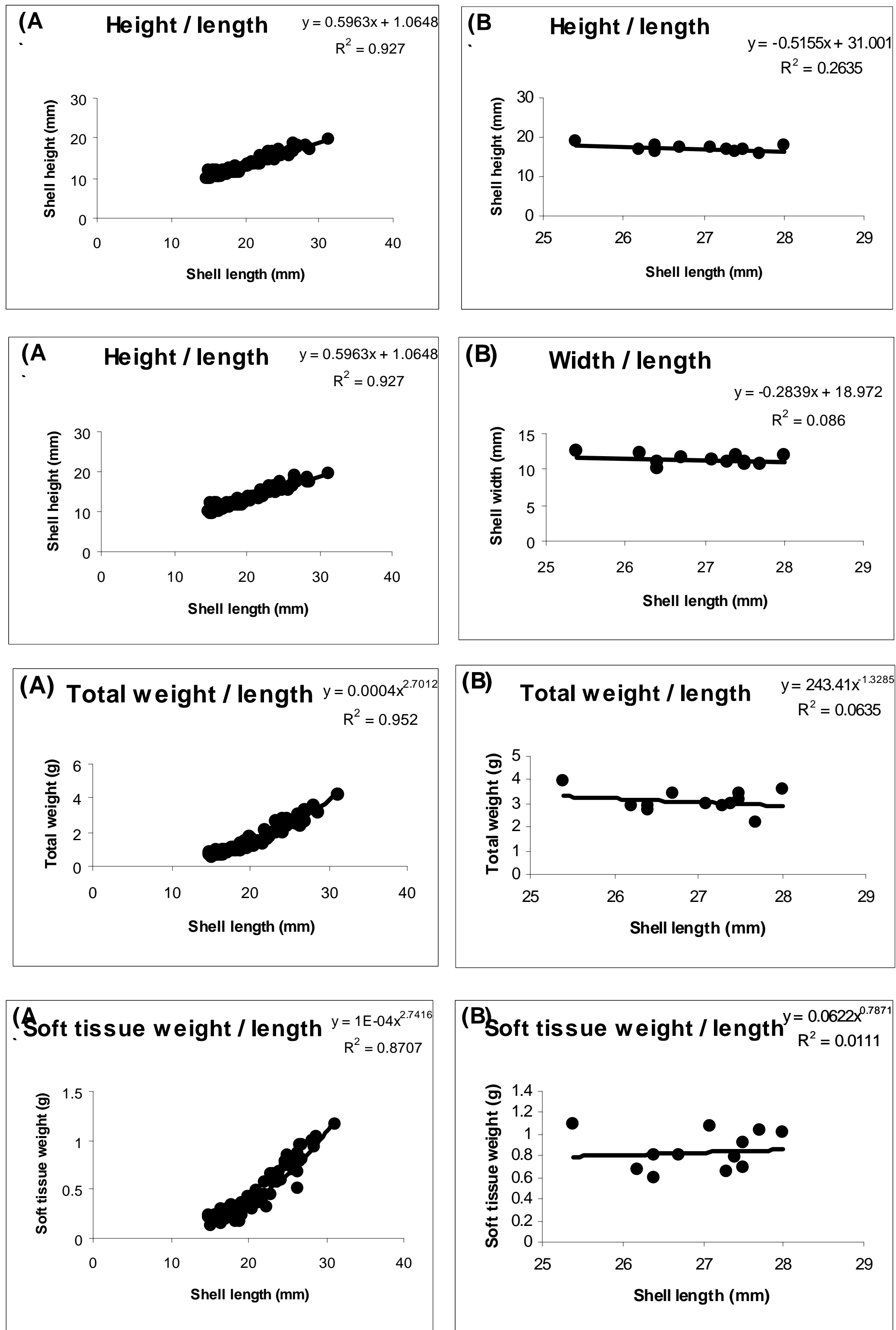


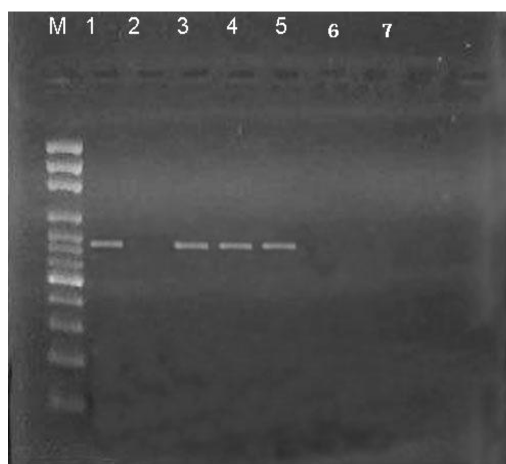
Figure 2: Relationships between shell length and either of some body measurements and weights of the uninfected (A) and infected (B) Venerupis aurea with *Aeromonas hydrophila*.

Table 6. Biometric and weight-length relationships of the studied bivalves infected with pathogenic bacteria.

Bivalve species	Bacterial species	Infection	Biometric equation of L/H	R^2	L/Wi	R^2	TW/L	R^2	soft wt/L	R^2
Ruditapes decussata	***Aeromonas hydrophila***	infected	$y = 0.6596x + 0.6356$	0.83	$y = 0.3419x + 2.7672$	0.71	$y = 0.0003x^{2.8685}$	0.87	$y = 0.0002x^{2.2852}$	0.77
		uninfected	$y = 0.6691x + 0.3687$	0.92	$y = 0.3840x + 1.7757$	0.56	$y = 0.0004x^{2.7714}$	0.91	$y = 4E\text{-}05x^{3.1489}$	0.57
	***Salmonella* spp.**	infected	$y = 0.6786x + 0.1285$	0.95	$y = 0.3488x + 2.5158$	0.80	$y = 0.0003x^{2.8755}$	0.97	$y = 6E\text{-}05x^{3.0044}$	0.92
		uninfected	$y = 0.6601x + 0.6122$	0.86	$y = 0.3539x + 2.5902$	0.60	$y = 0.0004x^{2.7736}$	0.86	$y = 0.0001x^{2.7484}$	0.67
	Staphylococcus aureus	infected	$y = 0.6663x + 0.5373$	0.85	$y = 0.3379x + 2.6414$	0.53	$y = 0.0003x^{2.8464}$	0.84	$y = 2E\text{-}05x^{3.2832}$	0.58
		uninfected	$y = 0.6357x + 1.1602$	0.85	$y = 0.3648x + 2.4153$	0.72	$y = 0.0005x^{2.6843}$	0.87	$y = 0.0002x^{2.7013}$	0.76
	Escherichia coli	infected	$y = 0.6720x + 0.2724$	0.86	$y = 0.3150x + 3.3796$	0.55	$y = 0.0002x^{2.9410}$	0.86	$y = 0.0001x^{2.7583}$	0.59
		uninfected	$y = 0.6635x + 0.5396$	0.90	$y = 0.3773x + 1.9857$	0.69	$y = 0.0004x^{2.7565}$	0.92	$y = 0.0001x^{2.8215}$	0.75
Venerupis aurea	***Aeromonas hydrophila***	infected	$y = -0.5155x + 31.001$	0.26	$y = -0.2839x + 18.972$	0.09	$y = 243.414x^{-1.3285}$	0.06	$y = 0.0622x^{0.7871}$	0.01
		uninfected	$y = 0.5961x + 1.0653$	0.93	$y = 0.4017x - 0.0266$	0.90	$y = 0.0004x^{2.7012}$	0.95	$y = 1E\text{-}04x^{2.7416}$	0.87
	***Salmonella* spp.**	infected	$y = 0.6371x -0.0064$	0.94	$y = 0.4317x - 0.6345$	0.91	$y = 0.0002x^{2.9047}$	0.97	$y = 2E\text{-}05x^{3.2044}$	0.92
		uninfected	$y = 0.5630x + 1.8531$	0.93	$y = 0.4109x - 0.1395$	0.90	$y = 0.0005x^{2.7565}$	0.95	$y = 0.0002x^{2.5391}$	0.91
	Staphylococcus aureus	infected	$y = 0.5656x + 1.8014$	0.93	$y = 0.4124x - 0.1637$	0.91	$y = 0.0005x^{2.6534}$	0.96	$y = 0.0002x^{2.5795}$	0.93
		uninfected	$y = 0.6929x -1.0212$	0.93	$y = 0.4447x - 0.8781$	0.86	$y = 0.0002x^{2.9744}$	0.93	$y = 2E\text{-}05x^{3.2665}$	0.84
	Escherichia coli	infected	$y = 0.5549x + 2.029$	0.92	$y = 0.4094x - 0.172$	0.88	$y = 0.0006x^{2.5583}$	0.95	$y = 0.0002x^{2.511}$	0.91
		uninfected	$y = 0.6176x + 0.6333$	0.91	$y = 0.4412x - 0.703$	0.69	$y = 0.0003x^{2.8462}$	0.94	$y = 8E\text{-}05x^{2.8009}$	0.84
Venerupis white	***Aeromonas hydrophila***	infected	$y = 0.3889x + 5.9386$	0.50	$y = 0.0996x + 7.9574$	0.10	$y = 0.11654x^{0.9749}$	0.21	$y = 0.0024x^{1.7508}$	0.57
		uninfected	$y = 0.5844x + 0.9467$	0.87	$y = 0.3917x + 0.0243$	0.76	$y = 0.0003x^{2.735}$	0.86	$y = 0.0001x^{2.5761}$	0.67
	***Salmonella* spp.**	infected	$y = 0.4582x + 3.1011$	0.78	$y = 0.3056x + 1.5142$	0.64	$y = 0.0008x^{2.4256}$	0.81	$y = 1E\text{-}04x^{2.6522}$	0.49
		uninfected	$y = 0.5597x + 1.626$	0.91	$y = 0.3939x + 0.1123$	0.82	$y = 0.0004x^{2.7244}$	0.90	$y = 0.0003x^{2.3352}$	0.85
	Staphylococcus aureus	infected	$y = 5678x + 1.603$	0.94	$y = 0.4002x + 0.1210$	0.86	$y = 0.0003x^{2.7887}$	0.95	$y = 0.0003x^{2.3647}$	0.86
		uninfected	$y = 4927x + 2.4891$	0.82	$y = 0.3773x + 1.9857$	0.69	$y = 0.0006x^{2.5075}$	0.82	$y = 3E\text{-}05x^{3.0694}$	0.65
	Escherichia coli	infected	$y = 0.5725x + 1.3379$	0.91	$y = 0.3185x + 1.2829$	0.72	$y = 0.0003x^{2.7809}$	0.92	$y = 0.0003x^{2.381}$	0.81
		uninfected	$y = 0.4894x + 2.548$	0.84	$y = 0.319x + 12357$	0.76	$y = 0.0006x^{2.4954}$	0.83	$y = 3E\text{-}05x^{3.1171}$	0.64

L: shell length, H: shell height, Wi: shell width, TW: total weightotal weight, soft wt: soft tissue weight, R^2: correlation coefficient

Photograph 1(A)
Agarose gel electrophoresis pattern of *Salmonella inv A* gene, 284 bp specific PCR product amplified with the forward and reverse primers.
M: DNA molecular weight ladder 100 bp.
Lane1: control positive (*Salmonella typhimurium*).
Lane 2: control negative (*E. coli* DNA).
Lanes 3, 4, and 5: positive samples.
Lanes 6, 7, 8 and 9: negative samples.



Photograph 1(B)
Agarose gel electrophoresis pattern of *Aeromonas hydrophila lip* gene, 760 bp specific PCR product amplified with the forward and reverse primers.
M: DNA molecular weight ladder 100 bp.
Lane 1: control positive (*Aeromonas hydrophila*).
Lane 2: control negative (*E. coli* DNA).
Lanes 3, 4, and 5: positive samples.
Lanes 6 and 7: negative samples.

4. Discussion

Sea food is responsible for a significant proportion of food borne diseases worldwide (Rippey, 1994). Pathogenic bacteria can be divided into two groups: the indigenous and non-indigenous bacteria. The indigenous bacteria (*Aeromonas hydrophila* and *Plesiomonas shigelloides*) are widely distributed in the aquatic environment in various parts of the world (Jennifer *et al*., 2006 and Herrera *et al*., 2006). The non-indigenous bacteria (*Salmonella* spp., *E. coli*, *Staphylococcus aureus* and others) can contaminate sea food by direct fecal contamination in growing area, by infected food handlers or storage in unhygienic conditions. This research focused on types of shellfish that are generally processed on streets (or what is called street food). The pathogens of concern for this research are *A. hydrophila, Salmonella* spp., *E. coli* and *Staphylococcus aureus.*

Aeromonas hydrophila was isolated from the studied molluscan shellfish at the rate of 30.4%. The present result was in accordance with Lalitha and Surendran (2005) who reported that *Aeromonas* comprise a high percentage of the total flora of black clam in India. *Aeromonas hydrophila* has been implicated in cases of gastroenteritis following the consumption of contaminated shellfish. In 1986, 472 cases of gastroenteritis were associated with frozen raw oysters which had been stored at -72˚C for 18 months, highlighting the survival properties of *A. hydrophila* under extreme condition (Rippey, 1994). *Aeromonas* species are generally considered to cause infection ranging from gastroenteritis, localized wound infection to septicemia and life threatening disease (Lehane and Rawlin, 2000).

The total rate of *Pseudomonas* spp. isolation from molluscan shellfish was 15.6%. Pseudomonas infections are caused by several types of the gram-negative bacteria of the genus Pseudomonas, especially *Pseudomonas aeruginosa*. They usually affect immunocompromised patients; the infection ranges from mild external ones (affecting the ear or hair follicles) to serious internal infections (Koneman *et al*., 1997).

Staphylococcus aureus is present in nasal passage, throats, on the hair and skin of probably 50% or more of healthy individuals and can be found in air, dust, water and human and animal wastes. In the present study, the isolation rate of *Staphylococcus aureus* from molluscan shellfish was 67.3%. This result was higher than those of Carlos Abeyta (1983) who reported that the incidence of *Staphylococcus aureus* in molluscs was 26.3 % and the count was 3.6-240 cfu/g. Green wood *et al*. (1985) isolated *Staphylococcus aureus* from 21% of shell fish samples. Mousa (1986) reported that the counts ranged from $26x10^3$ to $30x10^4$ cfu/100g. Ayulo *et al.* (1994) isolated *Staphylococcus aureus* from 60% of samples of shellfish meat. On the other hand, the result of the present study was lower than Mousa (1986) and Takwa (1994) who found *Staphylococcus aureus* in 100% and 98% of the examined molluscs, respectively. However, the mean counts of *S. aureus* ($2x10^3$ cfu/g, Takwa, 1994) was lower than our results. The high counts of *S. aureus* have a public health importance. This microorganism can multiply in food to reach 10^5 or 10^6 cfu/g. This is

followed by production of thermostable toxin responsible for vomition, diarrhea and dehydration in consumers. *S. aureus* was implicated in cases of food poisoning caused by consumption of contaminated shellfish (Le Loir *et al*., 2003).

The overall isolation rate of *Salmonella* spp. from the studied shellfish was 24.5%. This result was much higher than those of D' Aoust *et al.* (1980), D' Aoust *et al.* (1990) Takwa (1994), Wilson and Moore (1996) and Martinez-Urtaza *et al.* (2003). They isolated *Salmonella* spp. from molluscan shellfish at the rates of 3%, 6.6%, 2%, 8% and 1.8%, respectively. On the contrary, Carlos Abeyta (1983), Green wood *et al.* (1985), Abd El-Massih (1989) and Mona *et al*. (2003) couldn't detect *Salmonella* in the examined molluscan shellfish samples at all. On the other hand, the current result was lower than that of Fraiser and Koburger (1984). They isolated *Salmonella* spp. from clams at the rate of 45%. *Salmonella* strains are well known to cause gastroenteritis in man worldwide. Several outbreaks have been traced to the consumption of contaminated shellfish (Cantoni *et al*., 1985 and Rippey, 1994).

Human gastrointestinal illness caused by pathogenic *E. coli* has been recognized for several decades. Fecal pollution is the most common cause of shellfish contamination by *E. coli*. Our study revealed out that *E. coli* was isolated from the examined bivalves at the rate of 57.9%. This result was much higher than those reported by Green wood *et al.* (1985), Mousa (1986) Abd El-Massih (1989) and Takwa (1994) at rates of 14%, 30%, 28%, and 30%, respectively. Comparing our result (in Lake Timsah) with a previous study (in El Max, Abd El-Massih, 1989) it was found that the prevalence rate of *E. coli* in Lake Timsah (52.5%) was lower than that in El Max (100%). On the other hand, *E. coli* counts in the present study were higher than that recorded by Mona *et al*. (2003) ($1.5x10^2$and 1.7x10 cfu/g) for Gandofli & Om-El-Khloul samples, respectively. Isolation of *E. coli* in high rates and counts indicates fecal pollution. The recovered serotypes belonged to pathogenic groups which cause diarrheal illness, urinary tract infection, meningitis, pneumonia, osteomyelitis and wound sepsis.

Other members of *Enterobacteriaceae* (*Proteus vulgaris*, *Citrobacter diversus*, *Enterobacter cloacae*, and *Klebsiella oxytoca*) were isolated at high rates from the examined molluscan shellfish. These bacteria are often referred to as opportunistic pathogens. They can cause a variety of extra intestinal infections (Koneman *et al.*, 1997).

Hand swabs collected from occupational workers revealed the presence of several pathogenic bacteria. This highlights the possible role of molluscan shellfish in transmitting some pathogenic bacteria.

Generally, the current study revealed higher bacterial counts in the examined bivalve species than in the gastropod *Thais carinifera*. It may be concluded that infection with bacteria may depend on feeding habit. Since these bivalves are filter feeding, they magnify public health problems associated with water contamination because they accumulate microbial pathogens within their edible viscera many fold over the densities found in overlying water (Correa *et al*., 2006). Accordingly, they are infected with higher number of bacteria than the gastropod *Thais carinifera*, which is carnivorous (Broom, 1982). Fortunately, this physiological trend can be used to combat infection in bivalves by putting them in clean estuarine water, which leads to a decrease in the shellfish bacterial load.

In this study, we amplified 284 bp of *inv A* gene responsible for invasion of *Salmonella* into host's intestinal cells and 760 bp of *lip* gene specific for *Aeromonas hydrophila* using PCR protocol. Three of the seven presumptive *Salmonella* spp. colonies and three of the five presumptive *Aeromonas hydrophila* colonies gave positive results. Colonies examined by PCR were further identified biochemically and serologically. The biochemical tests classified all these isolates as *Salmonella* species and *Aeromonas hydrophila*. Concerning *Salmonella*, results from PCR and serotyping gave identical results for both negative and positive samples. This was in accordance with the results obtained by (Chiu and Ou, 1996). The PCR procedure described shows promise as rapid and specific technique to detect pathogens. Microbiological methods including isolation, biochemical and serology tests are time-consuming and laborious process. They take 15 days for diagnosis of salmonellosis and Aeromonas infection. When PCR technique was applied on suspect colonies, the PCR run takes 28 hours. The whole cost of PCR assay is much reduced by extraction of DNA by boiling. The technique can be modified for rapid diagnosis of *Salmonella* and *Aeromonas hydrophila* directly from food samples and enrichment broth, which is a very helpful tool for diagnosis of infection in case of outbreaks.

Apparently, the negative allometry in the H/L relationship is frequent in the gastropod *Thais carinifera*, as the same type of growth was found by Radwan *et al*. (2009). In the current study, this relationship was also negative and was not affected with bacterial infection. On the other hand, soft weight/L relationship was the most affected relation in which infection transited it from the positive to the negative allometry. Among the pathogenic bacteria, *Aeromonas hydrophila* was the most dangerous species that altered all weight/length relationships of this gastropod. So, it can be hypothesized that infection with these pathogenic bacteria can alter growth in weight (especially soft weight). This was more evident in the bivalve species that had the same pattern with bacterial infection. Concerning *Aeromonas* spp., it is evident that *Venerupis aurea* was harmfully affected than other bivalve species. Its soft wt/L relationship indicated a non significant positive growth (b=0.79 and R^2=0.01) reflecting its influence by this species. Moreover, direct negative growth of its shell height (b=-0.52 and R^2=0.26), shell width (b=-0.28 and R^2=0.09) and total weight (b=-1.33 and R^2=0.06) with

size were evidently appeared. Hence, it can be proved that infection of molluscan shellfish with these pathogenic bacteria not only alter their growth but also vary from species to another.

Finally, although clams and gastropods are sources of iron, B12, phosphorous and Zinc (Thierry *et al*., 1999) they are considered sources of pathogenic bacteria if they are eaten raw.

From this study, it can be concluded that the isolation of potentially pathogenic bacteria from the examined shellfish indicates a risk for health of people who consume or handle raw seafood. Therefore, it is recommended to take the following measures to control shellfish borne diseases: 1) Appropriate sewage and waste water disposal. 2) Adoption of basic hygienic measures and hand washing after contact with shellfish. 3) Decrease risk of contact by use of gloves and covering wounds. 4) Applying depuration technique in the aqua fisheries before selling to consumers.

References

Abd El-Massih, S.: 1989, 'Occurrence of some food poisoning agents in Alexandria', MV.Sc. Thesis, Fac Vet. Med., Alex. Univ.

Cantoni, C.D., Abubert, S. and Soncini, G.: 1985, 'Food poisoning outbreak caused by *Salmonella*', *Archivio Veterinario Italiano*, 36: 74-75.

Carlos-Abeyta, J.R.: 1983, 'Bacteriological quality of fresh seafood products from Seatle retail markets', *J. Food Protection*, 46:901-909.

Chiu, C-H and Ou, J.T.: 1996, 'Rapid identification of *Salmonella* serovars in feces by specific detection of virulence genes, *Inv A* and *Spvc*, by an enrichment broth culture-Multiplex PCR Combination Assay', *J. Clin. Microbiol.*, 34:2619-2622.

Correa, A.A, Toso, J., Albarnaz, J.D., Simoes, C.M.O. and Barardi, C.R.M.: 2006, 'Detection of *Salmonella typhimurium* in oysters by PCR and molecular hybridization', *J. Food Quality*, 29: 458-469.

D'Aoust, J.Y., Gelinas, R. and Maishment, C.:1980, ' Presence of indicator organisms and recovery of *Salmonella* in fish and shellfish', *J. Food Protection*, 43:679-682S.

D' Aoust, J.Y., Sewell, A. and Jean, A.: 1990, 'limited sensitivity of short (6 h) selective enrichment for detection of food borne *Salmonella*', *J. Food Protection*, 53:562-565.

Davis, L., Dibner, M. and Battey, J.:1986, Basic Method in Molecular Biology. Elsevier Science Publishing Co. Inc. 52 Vanderbilt Avenue, New York, 10017.

Donnenberg, M.S.: 2005, '*Enterobacteriaceae*. In: Mandell, G.L., Bennett, J.E. and Dolin, R: Mandell, Douglas, and Benett's Principles and Practice of Infectious Diseases'. Ed. 6. Philadelphia: Elsevier Churchill Livingstone, 2:2567-86.

Anguita, J., Rodriguez Aparicio, L. B. and Naharro, G.: 1993, 'Purification, gene cloning, amino acid sequence analysis, and expression of an extracellular lipase from an *Aeromonas hydrophila* human isolate', *Appl. Environ. Microbiol.*, 59:2411–2417.

APHA, American Public Health Association: 1992, 'Compendium of methods for the microbiological examination of food', 3rd ed., APHA, Washington, DC.

Ayulo, A.M., Machado, R.A. and Scussel, V.M.: 1994, 'Enterotoxigenic *Escherichia coli* and *Staphylococcus aureus* in fish and seafood from the southern region of Brazil', *Int. J. Food Microbiol.*, 24:171-8.

Bean, N.H., Goulding, J.S., Daniels, M.T. and Angulo, F.J.: 1997, 'Surveillance for food borne disease outbreaks-United States, 1988-1992: Review', *J. Food Prot*ection, 60: 1265-1286.

Broom, M.J.: 1982, 'Size selection, consumption rates and growth of the gastropods *Natica maculosa* (Lamark) and *Thais carinifera* (Lamark) preying on the bivalve *Anadara granosa* (L.)', *J. experimental Marine Biology and Ecology*, 56:213-233.

Fraiser, M.B. and Koburger, J.A.: 1984, 'Incidence of Salmonellae in clams, oysters, crabs and mullet', *J. Food Protection*, 47:343-345.

Gaspar, M. B., Chícharo, L.M., Vasconcelos, P., Garcia, A., Santos, A.R. and Monteiro, C.C.: 2002, 'Depth segregation phenomenon in *Donax trunculus* (Bivalvia: Donacidae) populations of the Algarve coast (southern Portugal)', *Sci. Mar.*, 66:111-121.

Gordillo, M.E., Reeve, G.R. and Pappas, J.: 1992, 'Molecular characterization of strains of enteroinvasive *E. coli* O 143, including isolates from a large outbreak in Houston Texas', *J. Clin. Microbiol.*, 30:889-893.

Greenwood, M.H., Coetzee, E.F.C., Ford, B.M., Gill, P., Hooper, W.L., Mathews, S.C.W. *et al*.: 1985, 'The bacteriological quality of selected retail ready to eat food products. III cooked crustaceae and mollusks', *Env. Health*, 93:236-239.

Hanninen, M. L., Oivanen, P. and Hirvela-Koski, V.: 1997, '*Aeromonas* species in fish, fish-eggs, shrimp and freshwater', *International Journal of Food Microbiology*, 34:17-26.

Herrera, F.C., Santos, J.A., Otero, A. and García-López, M.-L.: 2006, 'Occurrence of foodborne pathogenic bacteria in retail prepackaged portions of marine fish in Spain', *J. Appl. Microbiol.*, 100:527-536.

Jennifer, R. H., Zak, J.C. and Jeter, R.M.: 2006, 'Antimicrobial Susceptibilities of *Aeromonas* spp. isolated from environmental sources', *Appl. Env. Microbiol.*, 72:7036-7042.

Koneman, W.E., Allen, S.D., Janda, W.M., Schreckenberger, P.C. and Winn, W.: 1997, 'Colour Atlas and text book of Diagnostic

Microbiology'. Fifth ed. Lippincott Raven publishers.

Lalitha, K.V. and Surendran, P.K.: 2005, 'Bacterial profile of black clam (*Villorita cyprinoides var. cochinensis*) and clam harvesting waters from Vembanad Lake in Kerala (India)', *Fishery Technology*, 42:183-190.

Lehane, L. and Rawlin, G.T.: 2000, 'Topically acquired bacterial zoonoses from fish: a review', *Med. J. Aust*. 173, 256-259.

Le Loir, Y., Baron, F. and Gautier, M.: 2003 '*Staphylococcus aureus* and food poisoning', Genet. Mol. Res. 2: 63-76.

Martin-Carnahan, A. and Joseph, S. W.: 2005, 'Aeromonadaceae', In Brenner, D. J., Krieg, N. R., Staley, J. T. and Garrity, G. M. Eds. The Proteobacteria, Part B, Bergey's Manual of Systematic Bacteriology, second edition, Volume 2, Springer-Verlag, New York, NY.

Martinez-Urtaza, J., Saco, M., Hernandez-Cordova, G., Lozano, A., Garcia Martin, O. and Espinosa, J.: 2003, 'Identification of *Salmonella serovars* isolated form live molluscan shellfish and their significance in the marine environment', *J. Food Protection*, 66:226-232.

Mearin, F., Pérez-Oliveras, M., Perelló, A., Vinyet, J., Ibañez, A., Coderch, J. and Perona, M.: 2005, 'Dyspepsia and irritable bowel syndrome after a Salmonella gastroenteritis outbreak: one-year follow-up cohort study', *Gastroenterology*, 129:98-104.

Mona, S.M., Azza, H., Hoda, A.A. and Zeinab, I.S.: 2003, 'Studies on some hazards associated with shellfish', *J. Egypt. Vet. Med. Assoc*., 63:213-223.

Mousa, M.M.I.: 1986, 'Microbiology of some fish and shellfish in local markets and its relation to public health. Ph.D. Thesis, Fac. Vet. Med., Alex. Univ.

Qadri, F., Svennerholm, A.M., Frauque, A.S.: 2005, 'Enterotoxigenic *E.coli* in developing countries: Epidemiology, microbiology, clinical features, treatment, and prevention', *Clin. Microbiol. Rev*., 3:465-83.

Radwan, N. A., Mohammad, S. H., Mohammed, S. Z., and Yaseen, A. E.: 2009, 'Biometric studies on *Thais carinifera* in Lake Timsah, Suez Canal', *CATRINA*, 4:31-37.

Rahn, K., De Grandis, R.C., Clarke, S.A., Mcewen, J.E., Galan, C., Ginocchio, R., Curtiss, I. and Gyles, C.L.: 1992, 'Amplification of an *inv A* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella', Molecular and Cellular Probes*, 6: 271-279.

Rippey, S.R.: 1994, 'Infectious diseases associated with molluscan shellfish consumption', *Clin. Microbiol. Rev*., 7: 419-425.

Sambrook, J., Fritsch, E.T. and Maniates, T.: 1989, 'Molecular Cloning, A Laboratory Manual'. Second edition. Cold Spring Harbor Laboratory Press, USA.

Sayers, G., Mccarthy, T., O'connell, M., O'leary, M., O'brien, D., Cafferkey, M. and Mcnamara, E.: 2006, 'Haemolytic uraemic syndrome associated with interfamilial spread of *E. coli* O26:H11', *Epidemiology and Infection*, 134: 724-728.

Schultsz, C. , Van Den Ende, J., Cobelens, F., Vervoort, T., Van Gompel, A., Wetsteyn, J. C. F. M. and Dankert, J.: 2000, 'Diarrheagenic *Escherichia Coli* and acute and Persistent diarrhea in returned travelers', *J. Clin. Microbiol*., 38: 3550-3554.

Shotts, E.B. and Rimler, R.: 1973, 'Medium for the isolation of *Aeromonas hydrophila*', *Appl. Microbiol*., 26:550-553.

Takwa, I. H.: 1994, 'Studies on the microbiological quality of molluscs in Suez Canal Area', M.VSc. Thesis. Hygiene and Control of meat, fish and their by product, Fac. of Vet. Med., Suez Canal University.

Thayumanavan, T., Vivekanandhan, G., Savithamani, K., Subashkumar, R. and Lakshmanaperumalsamy, P.: 2003, 'Incidence of haemolysin-positive and drug-resistant *Aeromonas hydrophila* in freshly caught finfish and prawn collected from major commercial fishes of coastal South India', *FEMS Immunology and Medical Microbiology*, 36: 41-45.

Thierry, T., Cartier, J. and Gagnon, F.: 1999, 'Analysis of the chemical and microbiological risks associated with consumption of recreationally harvested shellfish from the Baie Comeau Area of Prime Concern', Public Health Branch, North Shore Regional Health and Social Services Board, 150 p.

USFDA: 2001, 'United States Food and Drug Administration for detection, enumeration and identification to species level of individual organisms, Bacteriological Analytical Manual', 8th edition.

Wilson, I.G. and Moore, J.E.: 1996, 'Presence of *Salmonella spp*. and *Campylobacter* spp. in shellfish', *Epidemiology and Infection*, 116:147-153.

Yamashiro, T., Nakasone, N., Higa, N., wanaga, M., Insisiengmay, S., Phounane, T., Munnalath, K, Sithivong N., Sisavath, L., Phanthauamath, B., Chomlasak, K., Sisulath, P., and Vongsanith P.: 1998, 'Etiological study of diarrheal patients in Vientiane, Lao People's Democratic Republic', *J. Clin. Microbiol.,* 36: 2195-2199.

الرصد الميكروبي لبعض أنواع البكتريا الممرضة فى الرخويات الصدفيه الشائعة فى بحيرة التمساح، قناة السويس، مصر

[1]هناء محمد فاضل و[2]سامية حسين محمد

[1]قسم صحة الحيوان و الأمراض المشتركة كلية الطب البيطرى جامعة قناة السويس.

[2]قسم علم الحيوان كلية العلوم جامعة قناة السويس

المأكولات البحرية تعتبر مصدر هام من مصادر البروتين للإنسان كما أنها تحتوى على العديد من العناصر الغذائية الهامة. ونظرا لقلة محتواها من الدهون فإنها تعتبر الغذاء المفضل لكثير من الناس. ومن ناحية أخرى من العادات الغذائية الشائعة لبعض الأشخاص أكل المحار النىء أو الغير مطهى جيدامما قد يؤدى الى إصابتهم ببعض الأمراض .وقد تم اجراء هذه الدراسة لتقييم مدى تواجد بعض البكتريا الممرضة والتى تؤثر على صحة الإنسان فى بعض الرخويات المحارية التى يقبل الناس على تناولها خاصة فى الشارع نظرا لرخص سعرها. حيث تم تجميع عدد 114 عينة من بطنية القدم *Thais carinifera* و 358 عينة من ذوات المصراعين *Ruditapes decussata* ، *Venerupis aurea* و*Venerupis white* بمنطقة الدراسة خلال الفترة من يوليو الى ديسمبر2008. وقد أظهرت الدراسة أن نسبة الاصابه بـ *Aeromonas hydrophila*، *Salmonella spp* ، *Staphylococcus aureus* و *Escherichia coli* فى النسيج الرخوى لبطنية القدم و ذوات المصراعين قد بلغ 34.88% و 28% ، 20.9% و 26.3% ، 86% و 57.9% و 41.9% و 57.9% على التوالى . كما بلغت نسبة العزل من مسحات الأيدى للبائعين و صائدى المحاروالقواقع 10% ، 0% ، 100 % و 50 % على التوالى . وبالنسبة لأعداد *Aeromonas hydrophila* ، *Staphylococcus aureus* و *Escherichia coli* فقد كانت أعلى فى ذوات المصراعين عنها فى بطنية القدم. كما أكدت الدراسه الحالية على أن تأثير *A. hydrophila* كان فى غاية الضرر على ذوات المصراعين خاصة فى *V. aurea* حيث أثر بالسلب على النمو. وأظهرت الدراسة أن تواجد العديد من البكتريا الممرضة ذات الأهمية على صحة الإنسان فى القواقع و المحاريات يشكل خطورة على الأشخاص الذين يتداولون أو يتناولون هذه الكائنات البحرية النيئة أو الغير مطهية جيدا.