Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 5(6) pp. 204-211, June, 2016 Issue.
Available online http://garj.org/garjas/home

Full Length Research Paper

Determination and monitoring of seafood Contamination with pathogenic and non-pathogenic bacteria at Majmaah province, Saudi Arabia

Hala Ali Abdel-Salam

Majmaah University, Saudi Arabia
Email: h.saleh@mu.edu.sa

Accepted 08 June, 2016

Seafood has traditionally being a popular part of the diet in many parts of the world and in some countries constituted the main supply of animal protein, lipids, carbohydrates, amino acids, fatty acids, vitamins and minerals. Seafood is part of a healthful diet, but seafood consumption is not risk-free. Seafood is responsible for an important proportion of food-borne illnesses and outbreaks in the world.A number of bacterial illnesses may arise from the consumption of seafood. Understanding the transfer of bacteria contaminants through the food web is critical to predict the exposure of humans to contaminants either through subsistence or commercial consumption of seafood and the possible health consequences of such exposure. In addition, such information is crucial in making accurate risk assessment for seafood safety purposes, a topic which is attracting much National and International attention. The present work deals with the isolation of pathogenic and non-pathogenic bacteria from two important commercially edible crustacean speciesin order to understand the health status andconsumer safety of these crustacean species as seafood. The results of the present study recorded that nine bacterial species were isolated from different tissues of both sexes of shrimp species (*Penaeussemisulcatus*) and crab species (*Portunuspelagicus*) as seafood products. In this research the occurrence of Gram negative bacteria was more frequent comparing with Gram positive ones. Furthermore, bacteria communities isolated from different organs of crustacean organisms collected from the study area showed a highly diverse and varied bacteria population associated with different organs, sex and species.

Keywords: Pathogenic, Nonpathogenic, bacteria, contamination, Seafood, shrimps, crabs

INTRODUCTION

Seafood products have attracted considerable attention as important sources in the human diet. Among seafood products crustacean organisms that considered as important of the aquatic fauna in the world. Edible crustacean consumption have been increasing worldwide during the past decades (Barrento et al., 2008; Wardiatno et al., 2012; Wardiatno and Mashar 2010; Abdel Salam 2013b; Abdel Salam and Hamdi 2015). The main reason for this increasing demand is thatedible crustaceans, such as crab, prawn, crayfish and lobsters are a good source of protein, lipids, carbohydrates, amino acids, fatty acids, vitamins and minerals (Rangappa et al., 2012; Sallam and

Temraz 2006; Oksuz et al., 2009; Ehigiator and Oterai 2012). Although edible crustaceans are part of healthful diet, but their as seafood consumption is not risk free; since seafood is considered as an important vehicle for marine toxins and chemical contamination. Furthermore, seafood is responsible for an important proportion of food born illness and outbreaks. Seafood associated infections are caused by variety of bacteria, viruses and parasites. This diverse group of pathogens results in a wide variety of clinical syndrome, each with its own epidemiology(Iwamoto et al., 2010). In general, illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity (Edema et al., 2005).

Edible crustaceans (crabs and shrimps) can be harvested from a range of aquatic environments – deep seas, shallow coastal waters and rivers. They are also grown in fish farms. Shrimps are caught in nets and refrigerated on board ship until they can be delivered to the processing plant where they are washed, sorted, and usually peeled before being cooked. Some raw shrimps are sold frozen. The shrimps die immediately after capture and they start to deteriorate quickly. They can be contaminated with bacteria from mud and the surrounding water, from ice and the boat. Because it takes several days from being caught to being processed, the microbes multiply (Wan Norhana et al., 2010). Most of these edible marine organisms are washed off, but more bacterial contamination can occur during peeling and other manufacturing steps. When frozen seafood products are consumed raw, there is the likelihood of endangering the health of the consumer especially when the micro-organism present includes pathogenic ones (Adebayo-Tayo et al., 2012a).

Bacteria may be found on the skin, chitinous shell, gills as well as the intestinal tracts of marine organisms (Adedeji et al., 2012). The microbiological flora in the intestines of seafood is quite different being psychotrophic in nature and to some extent believes to be a reflection of general contamination in the aquatic environment (Adebayo-Tayo et al., 2012a). So, raw and undercooked edible crustaceans as shrimps and crabs represent an important marine environment vector of infectious agents and marine biotoxines this is due to the ability of marine organisms to concentrate pathogens and toxins during the filter-feeding process (EUROPEAN COMMISSION (EC) 2001). During this process, they can accumulate and concentrate pathogenic microorganisms that are naturally present in harvest waters, such as vibrios (Iwamoto et al., 2010). *Vibrio* spp are the most important bacterialpathogens of prawn. Over 20 species are recognized, some of these are human pathogens (Amira Leila et al., 2013). *Vibrio* are widely distributed in marine and estuarine environments and in seafood throughout the world, which constitutes in many countries a real public health issue (Amira Leila et al., 2013). Furthermore, *Salmonella* have been shown to survive for over a month in the aqueous-sediment microcosm (Fish and Pettibone 1995), and also thermophilic *Campylobacter* have been isolated from sea water. Contamination of seafood by pathogens with a human reservoir can occur also when growing areas are contaminated with human sewage. Outbreaks of seafood-associated illness linked to polluted waters have been caused by calicivirus, hepatitis A virus, and *Salmonella enterica* serotype Typhi (Desenclos et al., 1991; Morse et al., 1986). Recent years have seen increased levels of production and consumption of seafood, leading to more frequent reporting of bacterial contamination. Therefore, the current study aimed to isolate and identify pathogenic, healthy- threatened bacteria strains and beneficial bacteria species from seafood crustaceans (shrimps and crabs). Furthermore, to stress on health implication of their presence in these edible seafood. The result would help creating awareness among the processors and consumers of the potential sources of contamination

MATERIAL AND METHODS

Collection of Samples

Two crustaceans species; Both sexes *of Penaeussemisulcatus* (shrimps), and *Portunuspelagicus* (crabs) were collected from local fishermen at Majmaah province, Saudi Arabia. To avoid further contamination, during transportation from the source to laboratory. Samples were carried by special sterile bags packed in insulated box with ice to maintain the temperature around 5 to 6 C Samples were placed inseparate sterile plastic bags to prevent spilling and cross contaminationand immediately transported to the laboratoryin a cooler with ice packs.After rinsing the samples insterile distilled water to remove any debris on their shell, samples were further prepared for bacterialisolation by cutting out, with sterile tools. 10 and25 g ofinterior flesh content (edible muscle, viscera and gills of both sexes of shrimp and crab species.

Bacteriological analysis and identification

For the isolation and identification of bacteria from edible muscles, viscera and gills of both sexes of shrimp and crab species collected and homogenized under aseptic condition. The homogenated each tissue sample was serially dilutedand spread over on nutrient agar medium. The plates were incubated at 37C for 24 to 48 hoursobserved the bacterial colonies.The bacterial colonies wereenumerated with the formula, Bacterialcount (TBC/g) = Number of colonies ×Dilution factor/ Volume of sample (g).Morphological identification of thebacteria present in all samples was carried out with the Gram stain,

Table 1: Bacteria identified from edible muscle, viscera and gill tissues of male and female shrimps and crabs

Tissues	***Bacteria*species**			
	P. semisulcatus		***P. pelagicus***	
	Males	**Females**	**Males**	**Females**
Edible muscles	*Shigella*spp	*Shigellaspp*	*Shigella*spp	*Shigella*spp
	Klebsiellaspp	-	*Klebsiellaspp*	-
	-	*Salmonella typhimurium*	*Salmonella typhimurium*	*Salmonella typhimurium*
	Aeromonasspp	*Aeromonasspp*	*Aeromonasspp*	*Aeromonasspp*
	-	*Staphylococcus aureus*	-	-
	Enterobacter spp	-	-	-
	*Serratia*spp	-	-	-
	-	*Citrobacter*	-	-
Viscera	*Shigella*spp	*Shigellaspp*	*Shigellaspp*	*Shigellaspp*
	*Aeromonas*spp	*Aeromonasspp*	*Aeromonasspp*	*Aeromonasspp*
	-	*Staphylococcus aureus*	*Staphylococcus aureus*	-
	-	*Enterobacter*	-	*Enterobacter*
	Salmonella typhimurium	*Salmonella typhimurium*	*Salmonella typhimurium*	*Salmonella typhimurium*
	Escherichia coli	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*
Gills	*Shigella*spp	*Shigellaspp*	*Shigellaspp*	*Shigellaspp*
	*Aeromonas*spp	*Aeromonasspp*	*Aeromonasspp*	*Aeromonasspp*
	Staphylococcus aureus	*Staphylococcus aureus*	*Staphylococcus aureus*	-
	Salmonella typhimurium	*Salmonella typhimurium*	*Salmonella typhimurium*	*Salmonella typhimurium*
	Escherichia coli	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*
	Enterobacter	-	-	*Enterobacter*

acid fast stain and spore staining (Holt et al., 1996), followed by biochemical tests.

Statistical analysis

A test of significance of observed differences inlevels of identified bacteria specieswas conducted using a onewayanalysis of variance (ANOVA). Meanswith the same letter for each parameter are not significantlydifferent, otherwise they do ($P < 0.05$). SPSS, for Windows (Version 15.0) was used for statistical analysis.

RESULTS

The bacteria enumerated from edible muscle, viscera and gill tissues of both sexes of shrimp and crab species are illustrated in Table 1. The recorded data detected that *Shigella*spp, *Aeromonas*spp, *Salmonella typhimurium,*

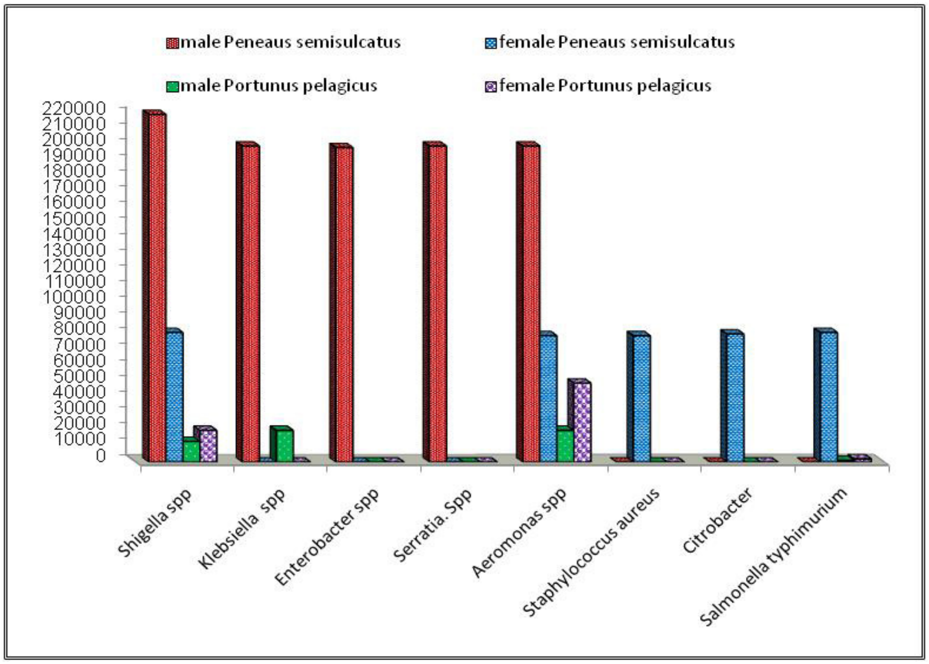

Figure 1. Bacteria species (TBC/g) identified from muscle tissues of both sexes of *P. semisulcatus* and *P. pelagicus.*

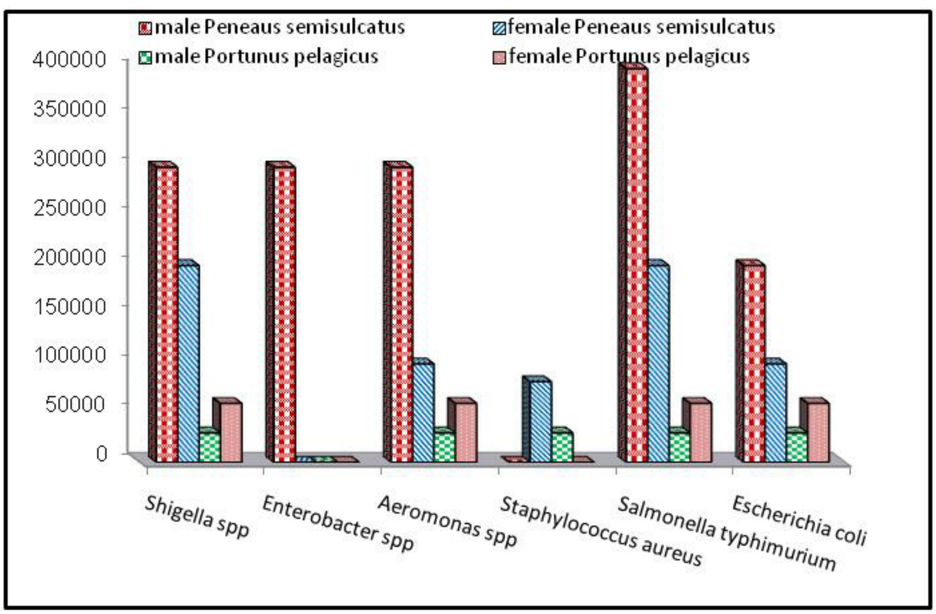

Figure 2. Bacteria species (TBC/gm) identified from viscera tissues of both sexes of *P. semisulcatus* and *P. pelagicus.*

Staphylococcus aureus and *Enterobacter*spp were the prominent bacteria that are isolated from all studied tissues of crustacean organisms. Whereas, the muscle tissue of male shrimps had *Klebsiella*spp and *Serratia*spp. Additionally, edible muscle portion of male crabs contained *Klebsiella*spp. Furthermore, *Citrobacter*spp was isolated from edible muscle tissue of female shrimps.While,*Escherichia coli*wasisolated only from viscera and gills of bothsexes ofshrimp species and crab species.

Regarding with the total bacteria count in studied tissues of male and female shrimps and crabs , the results in Figures 1- 3 indicated that muscle tissues of all studied samples had relatively few bacteria concentration compared with viscera and gill tissues. Furthermore, the results declared that both sexes of shrimp species contained higher level of all isolated bacteria comparable

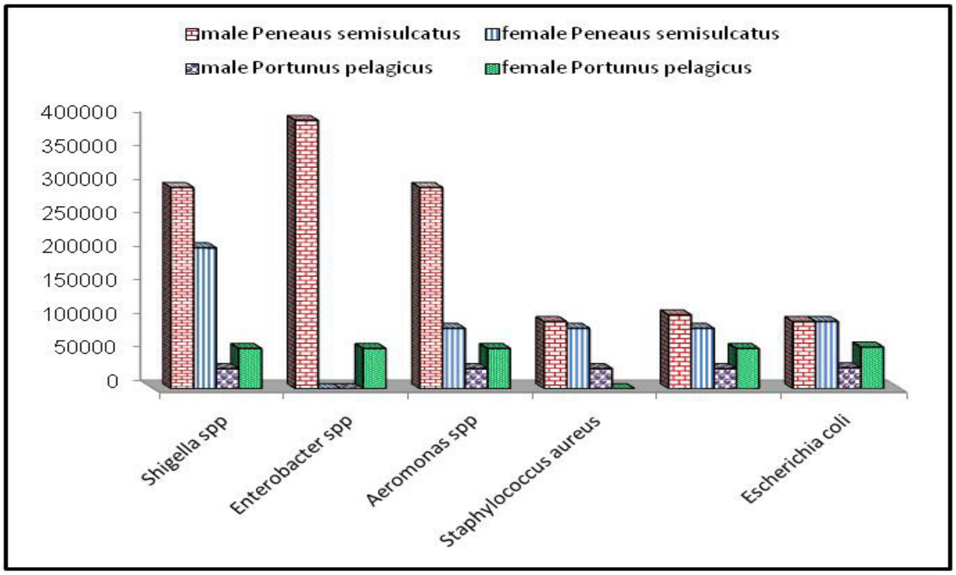

Figure 3. Bacteria species (TBC/gm) identified from gill tissues of both sexes of *P. semisulcatus* and *P. pelagicus.*

with crab species except beneficial bacteria species (*Escherichia coli*) in viscera tissues where significant decrease is recorded(P < 0.001).

As shown in Figure 1, the total bacteria countin edible muscle of male shrimps were higher than their females where significant increase was recorded (P < 0.001). Furthermore, it was observed that the concentration of bacteria in muscle tissues of male shrimps had this decreasing order: *Shigella*spp>*Aeromonas*spp>*Serratia* spp and *Klebsiell*spp>*Enterobacter* spp. While, in their females this descending arrangement was recorded: *Shigella*spp and *Salmonella typhimurium*>*Citrobacter*>*Aeromonas*spp>*Staphylococcus aureus*. In edible muscle tissues of male crab species, four pathogenic Gram negative bacteria species were isolated. Whereas, female crabs had threepathogenic Gram negative bacteria species. Also, it was detected that female crabs had higher bacteria count compared than their males and this increase is significant (P < 0.001). The concentration of bacteria found in muscles as per the order: *Klebsiell*spp and *Aeromonas*spp>*Shigella*spp>*Salmonella typhimurium* and *Aeromonas*spp>*Shigella*spp> *Salmonella typhimurium*in male and female crabs respectively.

As shown in Figure 2, six bacteria species were isolated from viscera of both sexes of shrimp and crab species. These species are represented by *Shigella*spp, *Enterobacter, Aeromonas*spp, *Staphylococcus aureus*, *Salmonella typhimurium* which were pathogenic bacteria and beneficial non-pathogenic bacteria species; *Escherichia coli*. The viscera of male and female shrimps had higher bacteria count of *Shigella*spp, *Aeromonas*spp and *Salmonella typhimurium* comparable with both sexes of crab species where significant increase was recorded. Whereas, *Escherichia coli* were fewer than in crab species, statistically, this decrease is significant (P < 0.001).Regarding the other bacteria species, it was observed that the viscera of male shrimps and female crabs had *Enterobacter* ($3x10^5$TBC/g and $6x10^4$ TBC/g respectively). While, *Staphylococcus aureus*; Gram positive bacteria was isolated only from female shrimps and male crabs (8.2 $x10^4$ TBC/g and $3x10^4$ TBC/g respectively). While, from gills *Shigella*spp, *Aeromonasspp, Salmonella typhimurium* and *Escherichia coli* were identified from both sexes of shrimp and crab species (Figure 3). Male shrimps had higher bacteria count comparable with their females. On the other hand, female crabs had greater bacteria count than their males. Furthermore, the present result showed that, Gram negative bacteria *Enterobacter*isolated from male shrimps and female crabs($4x10^5$ TBC/g and $6x10^4$ TBC/g respectively).While bacteria strain *Staphylococcus aureus*were enumerated from male and female shrimps ($10x10^4$ TBC/g and $9x10^4$ TBC/g respectively) as well as from male crabs ($3x10^4$ TBC/gm). But this species was not identified from female crabs.

DISCUSSION

A number of bacterial illnesses may arise from the consumption of seafood that has either beencontaminated at source or which becomes contaminated during the processing and retail chain. Such illnesses may arise from infection with the bacteria themselves or by the ingestion of toxins formed in the foodstuff prior to consumption

(Cappuccino and Sherman 1999). When frozen seafood products are consumedraw, there is the likelihood of endangering the healthof the consumer especially when the micro-organismof the consumer present includes pathogenic ones (Adebayo-Tayo et al., 2012a). Therefore, detection of pathogenic bacteria in edible crustaceans seafood is essential to ensure safe products for consumers and sustainable crustacean growing activities.

In the present study, studies on the bacteria communities isolated from different organs (edible muscle, viscera and gills) of both sexes of shrimp and crab species collected from Majmaah fisherman market showed a highly diverse and varied bacteria population associated with different organs, sex and species of crustaceans. Nine bacteria species were isolated from studied tissues of both sexes of shrimp (*P. semisulcatus*) and crab *(P. pelagicus).* From these bacteria , eightbacteria species were Gram negative bacteria (*Shigella*spp, *Aeromonasspp, Salmonella typhimurium, Enterobacter*, *Klebsiella*spp, *Serratia*spp, *Citrobacter* and *Escherichia coli*.Allof these bacteria strains are pathogenic bacteria except *Escherichia coli*.Furthermore, one Gram positive bacteria species was isolated from tissues of crustaceans species that was represented by pathogenic*Staphylococcus aureus*. in this research the occurrence of Gram negative bacteria was more frequent comparing with Gram positive ones. This finding is supported by (Lee and Rangdale 2008), who recorded five genera of Gram negative bacteria and 2 genera of Gram positive bacteria isolated from shrimps (*Penaeus monodon*). Also, in edible crab species; *Portunuspelagicus* and *Portunussanguinolentus* (Hermin and Muhammad 2015), showed that total of nine bacterial species was isolated and were predominant by gram-negative bacteria.

Additionally, the recorded results indicated that the total bacteria count are higher in viscera and gills of male and female of shrimp and crab species comparable with their edible muscles. This finding is in agreement with the results of (Hermin and Muhammad 2015). The elevation of bacteria concentrations in gills of crustacean organisms was attributed by (Hermin and Muhammad 2015), due to the micro-flora associated with gills islikely to have significant effect on crustacean organism as constant movement of water over gillsmight provide opportunity forcontamination with bacteria and colonization. In this respect, contamination of seafood with pathogenic bacteria at source (i.e. in the sea) primarily arises from two different origins. The first with bacteria that occur naturally in the marine environment when consumed in seafood in large enough numbers, will cause illness in humans (Hermin and Muhammad 2015). The second origin is environmental loading of fecal byproductsfrom humans and their associatedanimals is significant and can affect thequality of water and food resources incoastal ecosystems (Boopathi et al., 2013).Many bacterial species of enteric origin can be isolated from harbours which are located around sites of human habitation, including*, Staphylococcus aureus, Salmonella* spp, *Escherichia coli, Shigella* and *Klebsiella spp.* These bacterial species are *commonly* isolated from waters which contain fecal materials (Hermin and Muhammad 2015; Boopathi et al., 2013). In the present research, *Staphylococcus aureus, Salmonella typhimurium , Escherichia coli, Shigella,* and *Klebsiellaspp* are isolated from crustaceans species. Furthermore, the bacteria concentrations in all studied tissues of both sexes of shrimp species were higher than crab species. The elevation of bacteria count in shrimps might be attributed due to mode of feeding of shrimps. The filter-feeding process (EUROPEAN COMMISSION (EC) 2001). During this process, shrimps can accumulate and concentrate pathogenic bacteria that are naturally present in harvest waters. Pathogenic bacteria in seawater are most abundant in sediments (Boopathi et al., 2013), but are also seen in increased concentrations in the surface film, as compared with the water column (Martinez-Manzanarez et al., 1992). Therefore, untreated sewage can cause diseasein humans as a result of eatingcontaminated edible crustaceans especially bottom dwelling species.In addition, infected edible shrimps crabscan represent a significant public health problem for marine organisms themselves as well as for human health.

In the present study, it was observed that *Shigella*spp, *Salmonella typhimurium, Aeromonasspp, Enterobacter* and *Staphylococcus aureus*were most frequently isolated bacteria being present in almost all studied tissues of crustacean samples, followed by *Escherichia coli* that were isolated only from viscera and gills of both sexes of shrimps and crabs. While *Klebsiella*spp was isolated only from edible muscle of both male shrimps and crabs. Furthermore, *Serratia*spp and *Citrobacter* were isolated from edible muscle of male and female shrimps respectively.However, along with the nutrients and benefits derived from these species of shrimp and crab, consumption come the potential risks of eating this contaminated seafoodwith mentioned former bacteria. Because, the isolated pathogenic bacteria from the present study threaten human life such as *Shigella* species are Gram-negative bacilli. Four species have been identified, and clinical presentations vary by species. Clinical manifestations of *Shigella* infection range from watery, loose stools to more severe symptoms, including fever, abdominal pain, tenesmus, and bloody diarrhea. *Shigella* infection is highly communicable, because ingestion of as few as 10 viable organisms is sufficient for infection to occur (Plusquellec et al., 1991). Furthermore, contamination of shrimps and crabs with *Salmonella*which is considered as primary microbial pathogens responsible for the majority of food-borne illnesses (Wasley et al., 2008). According to the World Health Organization, annually there are about 17 million cases of acute gastroenteritis or diarrhea due to nontyphoidal salmonellosis with 3 million deaths (Wafaa et al., 2011). *Aeromonas* also, is considered one of the majorcauses of bacterial infections affecting aquatic organisms (World Health Organization (WHO) 2007). Moreover*, Aeromonas*sp. has been recognized as potential food borne pathogens for morethan 20 years (Hermin and Muhammad 2015). Regarding with *Enterobacter*spp, this gram negative bacilli has been associated with nosocomial outbreaks, and are considered opportunistic pathogens (Li and Cai 2011). Additionally, *Enterobacter* spp can cause numerous infections, including cerebral abscess, pneumonia, meningitis, septicemia, and wound, urinary tract and abdominal cavity/intestinal infections (Abbott 2007). The genus *Staphylococcus* contains a number of species which have been implicated as causative agents of disease in man and animals. *Staphylococcus aureus* is a major cause of food poisoning in man as well as of a range extra intestinal infections (Adedeji et al., 2012). *Escherichia coli* lives in the digestive tracts of human and animals. There are many types of *E. coli*, and most of them are harmless. But some can cause bloody diarrhea. Some strains of *E. coli* bacteriamay also cause severe anemia or kidney failure, which can lead to death (Farmer et al., 2007). Moreover, bacteria from the genus *Klebsiella* causes numerous infections in human (Adedeji et al., 2012). During this study it was observed that collected edible crustacean samples from studied area were more contaminated with pathogenic bacteria species. The fact is that, all frozen products such as shrimps and crabs are stored togetherthat is in turn may lead to cross contamination. Not onlythat but also the product is stored for relatively longperiod until sold and sometimes storage conditionbe ensured properly due to technological disruption (Myron et al., 1985; Clem and Garrett 1968). Based on the present study, it is concluded that the pathogenic bacteria are perhaps that mostimportant pathogens in shrimp and crabs ponds causing severe mortalities and financial losses. Furthermore, this contaminated seafood is responsible for an important proportion of food born illness and outbreaks. So, control strategies to prevent seafood-associated illnesses include monitoring harvest waters, identification, implementation of process controls, and consumer education

ACKNOWLEDGEMENT

Sincere gratitude is expressed to Deanship of Scientific Research, Majmaah University, Saudi Arabia for the financial support, fruitful cooperation and continuous help throughout this work.

REFERNCES

Abbott L (2007). Klebsiella, Enterbacter, Citrobecter, Serratia, Plesiomonnas. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller and M. L. Landry (Eds.), *Manual of Clinical Microbiology* (9 th ed., pp. 698-715). Washington, DC: ASM

Abdel Salam HA (2013b). Evaluation of nutritional quality of commercially cultured Indian white shrimp Penaeusindicus. Intern. J. Nutr. Food Science; 2(4): 160-166.

Abdel Salam HA, Hamdi SAH (2015). Evaluation of the edible muscles of four species of crustaceans from three regions of Egypt and Saudi Arabia .Glo. Adv. Res. J. Agric. Sci. (GARJAS) (ISSN: 2315-5094). 4(2) pp.105-112.

Adebayo-Tayo AC, Odu NN, Michael MU, Okonko IO (2012a). Multi-Drug Resistant (MDR) Organisms isolated from Sea-foods in Uyo, South-Southern Nigeria. Nature and Science; 10(3): 61-70.

Adedeji OB1, Okerentugba PO2 , Innocent-Adiele HC, Okonko IO2 (2012). BENEFITS, PUBLIC HEALTH HAZARDS AND RISKS ASSOCIATED WITH FISH CONSUMPTION New York Science Journal 2012;5(9) 229-237

Amira Leila Dib1, Amina Chahed 2, Rachid Elgroud 1, Rachid Kabouia 1, Nedjoua, Lakhdara 1, Omar Bouaziz 1, Miguel Espigares García (2013). Evaluation of the contamination of sea products by Vibrio and other bacteria in the eastern coast of Algeria Arch. Appl. Sci. Res., 2013, 5 (3):66-73

Barrento S, Marques A, Teixeira B, Vaz-Pires P, Carvalho ML, Nunes ML (2008). "Essential elements and contaminants in edible tissues of European and America lobsters," Food Chemistry. 111(4): 862–867

Boopathi M, Kasturi R, Chelladurai R, Kannan A, Anandan S (2013). Distribution of microbial population associated with crabs from Ennore sea coast Bay of Bengal north east coast of IndiaInt. J. Curr. Microbiol. App.Sci , 2(5): 290-305

Cappuccino FG, Sherman N (1999). Microbiology A Laboratory Manual; 4th Edition. Replica Press, Delhi

Clem JD, Garrett S (1968). Sanitation Guidelines for the Breaded-Shrimp Industry. U.S. Dept. of Interior, Fish and Wildlife Service, Bureau of Commercial Fisheries, 308.

Desenclos JC, Klontz KC, Wilder MH, Nainan OV, Margolis HS, RA, Gunn (1991). A multistate outbreak of hepatitis A caused by the consumption of raw oysters. Am. J. Public Health 81:1268-1272.

Dinakaran GK, Soundarapandian P, Varadharajan D (2013). Tail Rot Disease in Macrobrachiumidellaidella (Hilgendorf, 1898). J Drug Metab Toxicol:24-1-3

Edema MO, Omemu AM, Bankole MO (2005). Microbiological safety and quality of ready-to-eat foods in Nigeria. University of Agriculture, Abeokuta, pp: 26

Ehigiator FAR, Oterai EA (2012). Chemical composition and amino acid profile of a caridean prawns (Macrobrachiumvollenhoveanil) from Ovia river and periwinkle (Tympanonusfusctus) from Benin river", Edo tropical state , Nigeria. JRRAS 11 (1) :162-167.

EUROPEAN COMMISSION (EC) (2001). Journal of Applied Microbiology ISSN 1364-5072.Opinion of the ScientificCommittee on Veterinary Measures relating to Public Health on *Vibrio vulificus* and *Vibrio parahaemolyticus* raw and undercooked seafood). Adopted on 19-20 September 2001.

Farmer JJ, Boatwright KD, Janda JM (2007). Enterobacteriaceae: Introduction and identification. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry and M. A. Pfaller (Eds.), *Manual of Clinical microbiology* (9th ed., pp. 649-669). Washington, DC, USA: ASM press.

FISH JT, PETTIBONE GW (1995). influence of freshwater sediment on the survival of Escherichia coli and Salmonella sp. as measured by three methods of enumeration Lett. Appl. Microbiol, 1995, 20, 277–281.

Hermin PK, Muhammad Z (2015). Detection of bacteria and fungi associated with Penaeus monodon postlarvae mortality. Envir. Sci. 23 329 – 337

Holt JG, Krie NR, Sneath PHA, Stately JT, Williams ST (1996). Bergey s Manual of Determinative Bacteriology, 9th Edition, BaltimoreWilliams and Wilkins, p.787.

Iwamoto M, Ayers M, Swerdlow DL (2010): Epidemiology of Seafood-Associated Infections in the United States. Clin. Microbiol.23-399-401

Kim JH, Grant SB, McGee CD, Sanders BF, Largier JL (2004). Locating sources of surf one pollution: a mass budget analysis of fecalindicator bacteria at Huntington Beach, California. Environ. Sci. technol. 38: 2626-2636.

Lee RJ, Rangdale RE (2008). Bacterial pathogens in seafood.In Improving Seafood Products for the Consumer, Woodhead Publishing Series in Food Science,

Li Y, Cai SH (2011). Identification and pathogenicity of Aeromonassobria on tail-rot disease in juvenile tilapia *Oreochromisniloticus*. Curr. Microbiol. 62: 623-627.

Martinez-Manzanarez E, Morinigo MA, Castro D, Balebona MC, Sanchez JM, Borrego JJ (1992). Influences of the faecal pollution ofmarine sediments on the microbialcontent of shell fish. Mar. Pollu. Bull.24(7): 342-349.

Morse DL, Guzewich JJ, Hanrahan JP, Stricof R, Shayegani M, Deibel R, Grabau JC, Nowak NA, Herrmann JE, Cukor G (1986). Widespread outbreaks of clam- and oyster-associated gastroenteritis. Role of Norwalk virus. N. Engl. J. Med. 314:678-681

Myron ML, James PN, Helge K, Mary MB, James BK, Mary Lou, Clements Robert EB (1985). Encoding an Enteroadhesiveness Factor ans to Some Classic Serotypes of Enteropathogenic *Escherichia coli* is Dependent on a Plasmid Encoding an Enteroadhesiveness Factor. J Infect Dis. (1985) 152 (3): 550-559.

Oksuz A, Ozyilmaz A, Aktas M, Gercek G, Motte J (2009). A comparative study on proximate, mineral and fatty acid compositions of deep seawater rose shrimp (Parapenaeuslongirostris, Lucas 1846) and red shrimp (Plesionikamartia, A. Milne-Edwards, 1883). J. Anim. Vet. Adv.; 8: 183-189.

Plusquellec A, Beucher M, Le Lay C, Le Gal Y, Cleret JJ (1991). Quantitative and qualitative bacteriology of the marine water surface micro layer in a sewage.polluted area. Mar. Environ. Res. 31:227-239.

Rangappa A, Kumar RT, Jaganmohan P, Reddy SM (2012): Studies on the Proximal Composition of Freshwater Prawns Macrobrachiumrosenbergii and Macrobrachiummalcomsonii World. J. Fish Marine Sci.. 4 (2): 218-222.

Sallam WSTA, Temraz HR (2006). Gabar Biochemical compositions and heavy metals accumulation in some commercial crustaceans from the Mediterranean coast off Port Said, Egypt. J. Egypt. Ger. Soc. Zool. 51D:127.

Tauxe RV, Pavia AT (1998). Salmonellosis: nontyphoidal, p. 613-630. *In* A. S. Evans and P. S. Brachman (ed.), Bacterial infection of humans: epidemiology and control, 3rd ed. Plenum Publishing Corporation, New York, NY.

Wafaa MK, Bakr, Walaa A, Hazzah, Amani F Abaza (2011). Detection of *Salmonella* and *Vibrio* species in some seafood in Alexandria. J. Amer.Sci.;7 (9):663-668.

Wan Norhana MN, Susan E, Poole C, Hilton C, Deeth A, Gary A (2010). Dykes dPrevalence, persistence and control of Salmonella and Listeria in shrimp and shrimp products: A review (2010) Food Control 21 (2010) 343–361

Wardiatno Y, Mashar A (2010). Biological information on the mantis shrimp, Harpiosquillaraphidea (Fabricius(1798) Stomatopoda, Crustacea) in Indonesia with highlight of its reproductive a aspects. J. Tropical Biol. Conserv., 7: 65–71.

Wardiatno Y, Santoso J, Mashar A (2012). Biochemical composition in two populations of the mantis Shrimp Harpiosquillaraphidea (Fabricius 1798) Stomatopoda Crustacea. Maret, 17 (1) 49-58.

Wasley A, Grytdal S, Gallagher K (2008). Surveillance for acute viral hepatitis—United States, 2006. MMWR CDC Surveill. Summ. 57:1-24.

World Health Organization (WHO) (2007). Food safety issues associated with products from Aquaculture. Report of a Joint FAO/NACA/WHO study Group, WHO Technical reprint Series: 883 Geneva.