Shellfish as reservoirs of bacterial pathogens

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1. Introduction

Shellfish comprises a variety of animals including bivalves – oysters, clams, quahogs, scallops, mussels, and others including crustaceans – lobsters, crabs, and shrimps. Shellfish naturally found in Grenada, an island nation in the Caribbean, include: blue land crabs (Cardisoma guanhumi), clams (Mercenaria mercenaria), oysters (Isognomon isognomon), queen conchs, also called “lambi” (Strombus gigas), and whelks (West Indian top shell) (Cittarium pica), all of which are used for human consumption in this country.

Bacteria are ubiquitous in the marine environment. Shellfish can be a source of commensal bacteria that can be pathogenic to shellfish, as well as those pathogenic to humans. Raw and partially cooked molluscan shellfish (clams, oysters, and mussels) have a long history as vectors of infectious agents [1]. According to Potasman et al. [2], the earliest reports of shellfish-transmitted diseases were documented in the late 19th and early 20th century. Apart from typhoid fever, the most notable examples include Vibrio species, which account for 20% of all outbreaks of shellfish-associated human disease. Other bacteria are Salmonella species, Shigella species, Plesiomonas shigelloides, and Listeria species [2].

Several Vibrio species native to both marine and estuarine environments, have been identified as the causative agents of shellfish-associated human illnesses. These include Vibrio parahaemolyticus (V. parahaemolyticus), Vibrio vulnificus (V. vulnificus), Vibrio cholerae non-O1, Vibrio cholerae, Vibrio fluvialis (V. fluvialis), Vibrio hollisae, Vibrio mimicus, Vibrio alginolyticus (V. alginolyticus), and unidentified or miscellaneous Vibrio species. Among these, all except V. vulnificus, are associated with gastroenteritis of varying severity [1]. Oyster-associated V. vulnificus septicemia and death were first reported in 1975 [3].

Another bacterium of public health concern is Salmonella. Compared to vibrios, there have been much less studies on Salmonella in various shellfish. Setti et al. [4] studied the dynamics of Salmonella contamination in the coastal areas of Agadir, a populous region of Southern Morocco where shellfish production is important to the local economy. Salmonella enterica (S. enterica) serovar Kentucky was found almost exclusively in mussels, whereas Salmonella blockley was found in mussels and sediment samples. Identification of Salmonella to serovar level is required.
to understand the risks involved, because outcome of clinical salmonellosis in humans can vary depending on the serovar[5].

In addition, shellfish may serve as reservoirs of bacteria resistant to antimicrobial drugs. Multi-drug resistant *S. enterica* serovar Newport from oysters in the United States presents a significant health risk to consumers of raw oysters[6]. There is emerging evidence that antimicrobial-resistant *Salmonella* cause more severe infections[7]. Non-pathogenic bacteria present in shellfish also could transfer drug resistance genes to human pathogens, resulting in ineffectiveness of antimicrobial therapy. It is a well documented mechanism, common in bacteria belonging to Enterobacteriaceae.

2. Crabs and associated bacteria

Bacteria are often present in the gut contents of normal crabs. Presence or absence of bacteria in the gut lumen may depend on the crustacean species involved and their habitats[8]. The blue crab *Callinectes sapidus* (or sea crab), for instance inhabit waters that contain roughly 10^6 bacteria/mL sea water and 10^5 bacteria/mL fluid volume in marine sediments, and as such are constantly exposed to infectious agents[9].

Early publications indicate that hemolymph of healthy crabs may contain a variety of bacteria, including potential human pathogens. The soft shell crab, *C. sapidus* industry is a major fishery in the Eastern United States[10]. The predominant organisms consist of *Vibrio* spp. in these animals. Healthy *C. sapidus* have been found to carry *Vibrio* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Bacillus* spp., *Flavobacterium* spp., and coliforms[11]. Bacterial diseases of crabs sparked some debate in the 1970s[12]. The notion that blue crabs have sterile hemolymph was disproved. In a study on 290 normal, freshly collected blue crabs, the hemolymph samples of only 18% were found to be sterile[13]. In a more recent study on the same species of crabs from Galveston Bay, Texas, only 12% of 140 crabs had sterile hemolymph. *Vibrio cholerae*-like organisms, *V. vulnificus*, and *V. parahaemolyticus* were routinely isolated from culture positive animals. These organisms may be found in hemolymph and digestive tracts[14]. Hemolymph from specimens of the blue crab *C. sapidus* collected in 1994 from several Florida bays, including Pensacola Bay were positive for bacteria belonging to the following genera: *Aeromonas*, *Acinetobacter*, *Alcaligenes*, *Clavibacter*, *Enterobacter*, *Haemophilus*, *Klebsiella*, *Pasteurella*, *Proteus*, *Pseudomonas*, *Salmonella*, *Serratia*, *Shewanella*, *Shigella*, *Vibrio*, *Xanthomonas*, and *Yersinia*. Potential human pathogens from these isolates included *Aeromonas* hydrophila, *Salmonella* of undetermined serotypes, *Vibrio cholerae*, *V. parahaemolyticus*, and *V. vulnificus*[12].

In Grenada, Peterson[15] and Amadi et al.[16] reported isolation of *V. parahaemolyticus*, *V. fluvialis*, and *V. alginolyticus* from the hepatopancreas of blue land crabs (*Cardisoma guanhumi*) collected from natural habitats. Bacteremia, caused by vibrios has been reported in the blue crab, *C. sapidus*, *Callinectes bocourti*, and in the rock crab, *Cancer irroratus*[10]. Low dissolved oxygen and high carbon dioxide concentrations in the environment can affect bacterial clearance from the blue crab *C. sapidus*[17]. A recent prior exposure to *Vibrio campbellii* in these animals can enhance the ability of blue crabs to render bacteria non-culturable[18]. The harmful effects of hypoxia have also been found in the Dungeness crab, *Cancer magister*[19]. A majority of juvenile edible crabs, *Cancer pagurus* harbor cultivable bacteria in the hemolymph, but most of them have only small numbers of bacteria, which does not appear harmful to the host. Again, vibrios, including *Vibrio pectenicida*-like isolates may be present[20].

Potential contamination of shellfish with fecal matter, and biocloncentration in these animals are known to be human health hazards. Microcosm studies showed that certain bacterial populations, e.g., *Vibrio cholerae*, can be bioaccumulated in crab gill tissue[21].

Crabs, such as Dungeness crab (*Cancer magister*), king crab (*Paralithodes camtschatica*), rock crab (*Cancer irroratus*), and Tanner crab (*Chionoecetes opilio*), have been found to carry a variety of bacteria, both Gram-negative, and Gram-positive. The Gill was the most common reservoirs of these organisms, and it included food-borne pathogens such as *Vibrio* spp., and *Yersinia enterocolitica*. Other Gram-negative bacteria included *Acinetobacter* spp., *Aeromonas* spp., *Alcaligenes* sp., *Klebsiella* spp., *Citrobacter* spp., and *Pseudomonas* spp.[21]. *Vibrio* spp. are the organisms most commonly associated with infections in commercially stressed crabs.

It was established that uninjured healthy blue crab *C. sapidus* does not have sterile hemolymph, but instead harbors low-level bacterial loads[22].

Edible crab, *Cancer pagurus* can be infected naturally with a number of bacteria, predominantly from the genus *Vibrio*. They can not only cause infection of the crab shells, but also septicemic infection or death from extracellular bacterial products, terminating with death[23].

*Vibrio harveyi* (*V. harveyi*) can cause mortalities in the swimming crab (*Portunus trituberculatus*)[24].

*Vibrio cholerae* can colonize the gut of blue crab, *C. sapidus*. These animals, therefore, can be involved in the epidemiology and transmission of cholera in the aquatic environment[25].

Interestingly, the blue crab, *C. sapidus* has been found to be a reservoir of the small, comma-shaped predatory bacteria, *Bdellovibrio* spp.[26]. Recently, *Bdellovibrio bacteriovorus* has been shown to reduce colonization of *Salmonella enteritidis* in young chicks. This bacterium preys upon and kills Gram-negative bacteria, including zoonotic pathogens such as *Salmonella*. More studies on this predatory bacterium on shellfish pathogens will be rewarding.

*Vibrio* isolates from blue land crabs in Grenada included *V. parahaemolyticus*, *V. fluvialis*, and *V. alginolyticus*. *V. parahaemolyticus* has been reported as a public health problem in the commercial preparation of crab meat[28,29]. The most common clinical syndrome of exposure to *V. parahaemolyticus* is gastroenteritis[30].

Blue land crabs, *Cardisoma guanhumi*, in Grenada, are reservoirs of *Salmonella* serovars potentially pathogenic to humans[31]. Seventeen percent of 65 crabs were positive for *S. enterica* serovars Saintpaul, Montevideo, or Newport in Grenada. *Salmonella* Saintpaul has been linked increasingly to human disease outbreaks in several countries in Europe and other parts of the world, including Australia and the United States[31]. As a cause of human illness, S. Montevideo ranks as the fourth most common serovar in the Caribbean[32]. This serovar has also been found in sea lions and seals[33].

Marketed crabs *Ucides cordatus* in Brazil have been found to harbor *Salmonella* serovars Senftenberg and Poona. They also harbored *V. alginolyticus* and *V. parahaemolyticus*[34].

Crab meat was found to be a vehicle for *Escherichia coli* (*E. coli*) O157 outbreak in humans in England in 2011[35].

Zoonotic Gram-positive bacteria can also be associated with crabs. In a study on blue crab, *C. sapidus*, 19.5% of raw crabs collected
in US were positive for presumptive _Listeria_ spp. and 4.5% were confirmed to be _Listeria monocytogenes_[36], _Clostridium botulinum_, another food-borne, toxigenic pathogen of concern, can be present in fresh crab meat of blue crabs[37].

3. Bacteria associated with clams, oysters, mussels, conchs, and whelks

Bacteria associated with these marine animals in Grenada, were studied by Rodriguez et al.[38].

3.1. Clams

The bacterial isolates from clams ( _Mercenaria mercenaria_ ) in Grenada included _V. alginolyticus, V. parahaemolyticus, Aeromonas salmonicida_, and _Pantoea_ spp. _V. parahaemolyticus_ was isolated from softshell clams ( _Mya arenaria_ ) in the United States decades ago[39]. vibrios can also cause disease in clams. These include brown ring disease, with high mortalities in Manila clam _Ruditapes philippinarum_ due to _Vibrio tapetis_[40], and bacterial necrosis and systemic disease in larvae of hard clam or quahog ( _Mercenaria mercenaria_ ) caused by _Vibrio tubiiashii_[41]. Mass mortalities of aquacultured carpet shell clam ( _Ruditapes decussatus_ ) larvee associated with infections due to _V. alginolyticus_ and _V. splendidus_ have also been reported[42].

3.2. Oysters and mussels

Grenada isolates from oysters ( _Isognomon isognomon_ ) comprised of _V. alginolyticus, V. fluvialis, Stenotrophomonas maltophilia, Shewanella putrefaciens, E. coli, Moraxella lacunata, and Pantoea_ spp.[38]. According to Buller[41], _Vibrio_ species are commonly associated with oysters, and present in soft tissue and haemolymph. Normal flora also includes _Shewanella colwelliana_. Diseases causing bacteria in oysters include _Vibrio splendidus, Aeromonas_ spp., _Listonella anguillarum, and Photobacterium damselae_ subsp. _damselae_.

In a study by Hariharan et al.[43], on blue mussels ( _Mytilus edulis_ ), and eastern oysters ( _Crassostrea virginica_ ) collected from shellfish growing areas on Prince Edward Island, Canada, among 51 _Vibrio_ isolates, the most common species were _Vibrio anguillarum_ from oysters, and _V. alginolyticus_ and _V. splendidus_ from mussels. _V. parahaemolyticus_ and _V. vulnificus_, each were isolated from nearly 7% of oysters. The other species of vibrios from both mussels and oysters from Prince Edward Island included _Vibrio pelagiis, Vibrio damsela_ ( _Photobacterium damselae_), _Vibrio mediterranei, Vibrio tubiashii_, and _Vibrio ordalii_.

Concerning _V. vulnificus_, in the past, many cases of severe human infections from this species used to occur from eating raw oysters[44]. In a more recent study on blue mussels ( _Mytilus edulis_ ) from shellfish growing areas from the German Wadden Sea, _V. alginolyticus_ was the most frequently detected species, followed by _V. parahaemolyticus_. In addition, _V. vulnificus_ was present in 3.5% of the samples. It was also confirmed that _E. coli_ was not a reliable indicator for contamination with _Vibrio_ spp.[45]. Environmental parameters such as salinity and temperature can regulate the dynamics of _V. parahaemolyticus_ within the oyster[46]. _V. vulnificus_ has been isolated from oysters, mussels, clams, and crabs[47,48]. This organism has been subtyped into three biotypes, of which biotype 1 is pathogenic to crustaceans and humans. It can cause gastroenteritis, septiceama, and wound infections in humans[47]. Effective antibiotics for _V. vulnificus_ infections include tetracycline, ceftazidime, and imipenem[48].

Oysters can also harbor Gram-negative bacteria other than vibrios, including _Acinetobacter, Flavobacterium, and Pseudomonas_, and Gram-positive organisms, including _Staphylococcus_ spp., mainly _Staphylococcus capitis, and Bacillus_ spp., mainly _Bacillus pumilus_[43]. _Vibrio and Pseudomonas_ have been reported as dominating the bacterial flora of marine shellfish from unpolluted regions, whereas _Lactobacillus and Aeromonas_ are more common in oysters from regions where fecal contamination is a problem[49]. The anaerobes isolated from oysters by Hariharan et al.[43] included _Clostridium perfringens, Clostridium difficile, Clostridium sporogenes, Clostridium bifermentans_, and non-spore forming bacteria: _Bacteroides buccae, and Fusobacterium mortiferum_. The significance of non-spore forming anaerobes in oysters is not understood. _Clostridium perfringens_ may be considered as an indicator of fecal pollution[50].

In the south coast of Brazil, untreated oysters harbored _V. vulnificus, Aeromonas and Salmonella_; and water samples had _V. parahaemolyticus, V. vulnificus, Aeromonas, and Salmonella_ as well[51].

There have been limited studies on serovars of _Salmonella_ and their drug resistance. Research in Spain showed that mussels and oysters presented a higher incidence of _Salmonella_, compared to clams and cockles. The most frequent serovar was _S. Senftenberg_, followed by serovars Typhimurium and _Agona_[52,53]. In these studies in Spain, levels of fecal coliforms did not correlate with the occurrence of _Salmonella_.

_S. enterica_ serovar Newport has been shown to be present in 7.4% of oysters harvested from 36 US bays. Again, no relationship was found between the isolation of fecal coliforms and _Salmonella_ from oysters. Overall, the isolates were of a single pulsed-field gel electrophoresis type. For the most part, the isolates were resistant to ampicillin, and tetracycline, and susceptible to ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole[54,55].

In the United States, 43% of 28 oyster samples from eight Tucson restaurants contained _S. enterica_ serovar Newport[6]. The strain was resistant to seven antimicrobials tested. This particular strain of _Salmonella_ had a 200 times greater concentration than an _E. coli_ strain after 10 days of storage[56]. Persistence of _Salmonella_ serotype _Senftenberg_ for several years in the marine environment may have occurred in the coastal areas of Spain due to contamination from mussel processing facilities[53]. Once infected, _Salmonella_ can survive 100 times better than _E. coli_ in oysters. Unidentified virulence factors may play a role in _Salmonella_’s interaction with oysters[56].

Older literatures indicate that oysters can harbor _Clostridium botulinum_, a well known toxigenic organism of concern[57,58], _Clostridium botulinum_ types C, D, and E were found in marine sediment at the oyster sampling site. Types C and D are primarily associated with animal botulism[59].

3.3. Conchs

Conch ( _Strombus gigas_ ) samples from Grenada on culture yielded _V. fluvialis, Stenotrophomonas maltophilia, Enterobacter sakazakii_, and _Klebsiella pneumoniae_[38]. Little is known regarding
the microorganisms associated with the queen conch (Strombus gigas). A study of bacteria associated with queen conch, using both cultural and molecular methods in the Caribbean area of Colombia[60] showed presence of Vibrio spp., Pseudomonas spp., Pseudoalteromonas spp., Halomonas spp., Psychrobacter spp., and Cobetia spp. Many bacteria, including those which may not be culturable, can be involved in health and pathogen defense mechanisms of marine animals. Recently, Carrascal and co-workers[61] studied the bacterial diversity associated with wild and captive conchs from the Rosario Islands in Colombia using both culture-dependent methods and culture-independent techniques, particularly, denaturing gradient gel electrophoresis and sequence analysis of the 16S rRNA gene. The bacterial groups were identified at the phylum level: Proteobacteria (89%), Firmicutes (9%), and Actinobacteria (2%). The bacteria detected in conchs belonged to Burkholderia spp., Pseudoalteromonas spp., and Acinetobacter spp. These authors concluded that both culture-based methods and molecular approach are important in detection of diverse bacterial flora.

3.4. Whelks

Whelks (Cittarum pica), commonly known as West Indian top shell collected in Grenada were culture-positive for V. alginolyticus, Aeromonas hydrophila, Shewanella putrefaciens, Pseudomonas fluorescens, E. coli, and Chryseobacterium meningosepticum[38]. Interestingly, an outbreak of human salmonellosis due to S. enterica serovar Goldcoast occurred following consumption of whelks occurred recently in England[62].

4. Miscellaneous human infections arising from shellfish

Pathogenic Photobacterium damsela and Shewanella spp. could pose a health threat to humans through the ingestion of contaminated seafood, or by cuts or abrasions acquired in the marine environment. These organisms have been cultured from oysters and seawater[63]. Photobacterium damsela subsp. damsela has been isolated from marine sources in Grenada. It is of zoonotic importance as well[64]. Hundenborn et al.[65] reported an unusual case of severe wound infection caused by Photobacterium damsela and V. harveyi together, although V. harveyi was formerly considered to be non-pathogenic to humans.

Koh et al.[66] reported Streptococcus iniae cellulitis in an immunocompetent person resulting from a puncture wound caused by a crab pincer. Streptococcus iniae can cause septicemia, endocarditis, arthritis, meningitis, osteomyelitis, and pneumonia in humans[48].

5. Drug resistance of Gram-negative bacteria associated with shellfish

Crabs used for human consumption may harbor Gram-negative bacteria resistant antimicrobial drugs used in human medicine. In a recent study on blue land crabs in Grenada, isolates of Klebsiella pneumoniae, Citrobacter freundii, Enterobacter cloacae, and E. coli, showing resistance to several antibiotics used in human medicine were found. The drugs included amoxicillin-clavulanic acid, ampicillin, cephalotin, chloramphenicol, ciprofloxacin, tetracycline, and trimethoprim-sulfamethoxazole[16]. Being members of Enterobacteriaceae, these organisms could transfer their resistance genes to human pathogens via conjugation.

In the study in Grenada by Rodriguez et al.[38] on bacterial isolates from shellfish, comprising clams, oysters, queen conchs, and whelks, drug resistance among Gram-negative isolates was most frequent to ampicillin (60%), followed by cephalexin (40%), streptomycin (29%), amoxicillin-clavulanic acid (20%), tetracycline (17%), and chloramphenicol (8.6%). Resistance was none or less than 3% for enrofloxacin, ciprofloxacin, and trimethoprim-sulfamethoxazole.

6. Strategies to reduce zoonotic bacteria in oysters

Among shellfish, oysters are commonly consumed raw or undercooked. For this reason, methods on reduction of zoonotic pathogens, particularly, vibrios have focused on oysters. Extended depuration at 15 °C for 96 h has been found very effective in reducing V. parahaemolyticus and V. vulnificus in oysters. Higher and lower temperatures were not as effective as 15 °C[67]. High salinity relay could be an effective postharvest processing method to reduce V. vulnificus levels in oysters[68]. In this process, oysters from moderate salinities with high levels of V. vulnificus are exposed to high salinities resulting in decreases of 2 to 3 log densities of V. vulnificus. Other postharvest processing methods include use of high hydrostatic pressure[69], use of supercritical carbondioxide[70], and inactivation of zoonotic bacteria, including Salmonella and Vibrio spp. by ionizing radiation[71]. One study described X-ray inactivation of V. parahaemolyticus and inherent microflora in oysters to less than detectable limit[72]. Most recently, it has been shown that bacteriophage application by bath immersion can reduce considerably or even inhibit multi-drug resistant V. parahaemolyticus in oysters[73].

7. Conclusions

Blue land crabs in their natural habitats in Grenada are reservoirs of Salmonella serovars implicated in human disease. Oysters from various parts of the world can harbor Salmonella as well as vibrios of zoonotic significance. In addition, oysters may carry several species of clostridia and non-spore forming anaerobes found by one of the authors of this review in Prince Edward Island, Canada. On a global basis, microflora of conchs and whelks has not been studied adequately. In summary, shellfish of various kinds can be significant sources of bacterial infections in humans. Control strategies, including reduction at sources, and monitoring of antimicrobial resistance need continued attention.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Rippey SR. Infectious diseases associated with molluscan shellfish
consumption. Clin Microbiol Rev 1994; 7: 419-25.

[2] Potasman I, Paz A, Odeh M. Infectious outbreaks associated with bivalve shellfish consumption: a worldwide perspective. Clin Infect Dis 2002; 35(8): 921-8.

[3] Blake PA, Merson MH, Weaver RE, Hollis DG, Heublein PC. Disease caused by a marine Vibrio. Clinical characteristics and epidemiology. N Engl J Med 1979; 300(1): 1-5.

[4] Setti I, Rodriguez-Castro A, Pata MP, Cadarso-Suarez C, Yacoubi B, Bensmael L, et al. Characteristics and dynamics of Salmonella contamination along the coast of Agadir, Morocco. Appl Environ Microbiol 2009; 75(24): 7700-9.

[5] Johnson PT. Histology of the Blue Crab, Callinectes sapidus, a model for the Decapoda. New York: Praeger Publishers; 1980.

[6] Johnson NG, Burnett LE, Burnett KG. Properties of bacteria that trigger hemocytopenia in the Atlantic blue crab, Callinectes sapidus. Biol Bull 2011; 221(2): 164-75.

[7] Wang W. Bacterial diseases of crabs: a review. J Invertebr Pathol 2011; 106(1): 18-26.

[8] Huq A, Huq SA, Grimes DJ, O’Brien M, Chu KH, Capuzzo JM, et al. Colonization of the gut of the blue crab (Callinectes sapidus) by Vibrio cholerae. Appl Environ Microbiol 1986; 52(3): 586-8.

[9] Taylor J, Oshin F, O’Connor K, Oliver I. Crab Vibrio campbellii and Salmonella. Emerg Infect Dis 2006; 12(3): 381-8.

[10] Peterson R, Hariharan H, Matthew V, Chappell S, Davies R, Parker R, et al. Prevalence, serovars, and antimicrobial susceptibility of Salmonella isolated from blue land crabs (Cardisoma guanhumi) in Grenada, West Indies. J Food Prot 2013; 76(7): 1270-3.

[11] Galanis E, Lo Fo Wong DM, Patrick ME, Binsztein N, Cieslik A, et al. Prevalence, characterization and sources of potential human pathogens. Clin Microbiol Rev 2007; 20(1): 39-48.

[12] Phillips FA, Peeler JT. Bacteriological survey of the blue crab industry. Appl Microbiol 1972; 24: 958-66.

[13] Nair GB, Ramanathry T, Bhattacahrya SK, Duta B, Takeda Y, Sack DA. Global dissemination of Vibrio parahaemolyticus serotype O3K6 and its serovariants. Clin Microbiol Rev 2007; 20(1): 39-48.

[14] Holman JD, Burnett KG, Burnett LE. Effects of hypercapnic hypoxia on the clearance of Vibrio campbellii in the Atlantic blue crab, Callinectes sapidus Rathbun. Biol Bull 2004; 206(3): 188-96.

[15] Macey BM, Rathburn CK, Thibodeaux LK, Burnett LE, Burnett KG. Clearance of Vibrio campbellii injected into the hemolymph of Callinectes sapidus, the Atlantic blue crab: the effects of prior exposure to bacteria and environmental hypoxia. Fish Shellfish Immunol 2008; 25(6): 718-30.

[16] Scholnick DA, Haynes VN. Influence of hypoxia on bacteremia in the Dungeness crab, Cancer magister. Biol Bull 2012; 222(1): 56-62.

[17] Smith AL, Whitten MM, Hirschle L, Pope EC, Wootton EC, Vogan CL, et al. Bacterial septicaemia in prerecruit edible crabs, Cancer pagurus L. J Fish Dis 2014; 37(8): 729-37.

[18] Faghri MA, Pennington CL, Cronholm LS, Atlas RM. Bacteria associated with crabs from cold waters with emphasis on the occurrence of potential human pathogens. Appl Environ Microbiol 1984; 47(5): 1054-61.

[19] Welsh PC, Sizemore RK. Incidence of bacteremia in stressed and unstressed populations of the blue crab, Callinectes sapidus. Appl Environ Microbiol 1985; 50(2): 420-5.

[20] Vogan CL, Costa-Ramos C, Rowley AF. Shell disease syndrome in the edible crab, Cancer pagurus – isolation, characterization and pathogenicity of chitinolytic bacteria. Microbiology 2002; 148: 743-54.

[21] Ishimaru K, Muroga K. Taxonomical re-examination of two pathogenic Vibrio species isolated from milkfish and swimming crab. Fish Pathol 1997; 32: 59-64.

[22] Varma JK, Molbak K, Barrett TJ, Beebe JL, Jones TF, Ingram AL, Cieslak PR, Vugia DJ, Tobin-D’Angelo M, Hurd S, et al. Salmonellosis outcomes differ substantially by serotype. Appl Environ Microbiol 2002; 68(7): 263-70.

[23] Stoddard RA, DeLong RL, Byme BA, Jang S, Gulland FM. Prevalence, serovars, and antimicrobial susceptibility of Salmonella enterica serovar Typhimurium isolated from blue crabs (Callinectes sapidus) collected from Galveston Bay, Texas. Appl Environ Microbiol 1982; 43: 1092-7.

[24] Macey BM, Rathburn CK, Thibodeaux LK, Burnett LE, Burnett KG. Clearance of Vibrio campbellii injected into the hemolymph of C.
Ristori CA, Iaria ST, Gelli DS, Rivera IN. Pathogenic bacteria associated with Vibrio parahaemolyticus from Cape Cod soft-shell clams (Mya arenaria). Appl Microbiol 1975; 29: 635-40.

Allam B, Paillard C, Ford SE. Pathogenicity of Vibrio tapetis, the etiological agent of brown ring disease in clams. Dis Aquat Organ 2002; 48: 221-31.

Butler NB. Bacteria from fish and other aquatic animals: a practical identification manual. Cambridge: CABI Publishing; 2004.

Gómez-León J, Villamil L, Lemos ML, Novoa B, Figueras A. Isolation of Vibrio alginolyticus and Vibrio splendidus from aquacultured carpet shell clam (Ruditapes decussatus) larvae associated with mass mortalities. Appl Environ Microbiol 2005; 71: 98-104.

Haritharan H, Giles GS, Heaney SB, Arenault G, McNair N, Rainnie DJ. Bacteriological studies on mussels and oysters from six river systems in Prince Edward Island, Canada. J Shellfish Res 1995; 14: 527-32.

Centers of Disease Control and Prevention (CDC). Earle PM, Crisley FD. Isolation and characterization of the etiological agent of brown ring disease in clams. Rev Sci Tech 1989; 8: 297-300.

Madden RH, Buller H, McDowell DW. Madden RH, Buller H, McDowell DW.ימהם�סשתל רתוי קומונ瞭解ן, אודיסים לוס אנגליש, 296. 45: 621-4.

Hariharan H, Mitchell WR. Type C botulism: the agent, host spectrum and environmental parameters influence on the dynamics of total and pathogenic Vibrio parahaemolyticus densities in Crassostrea virginica harvested from Mexico’s Gulf coast. Mar Pollut Bull 2015; 91: 317-29.

O’Neill KR, Jones SH, Grimes DJ. Seasonal incidence of Vibrio vulnificus in the Great Bay estuary of New Hampshire and Maine. Appl Environ Microbiol 1992; 58: 3257-62.

Haenen OL, Evans JJ, Berthe F. Bacterial infections from aquatic species: potential for and prevention of contact zoonoses. Rev Sci Tech 2013; 32: 497-507.

Samata T. Microflora of healthy animals. In: Austin B, Austin DA, editors. Methods for the microbiological examination of fish and shellfish. Chichester: Ellis Horwood Ltd.; 1989.

Madden RH, Buller H, McDowell DW. Clostridium perfringens as an indicator of hygienic quality of deparated shellfish. J Food Prot 1986; 49: 33-6.

Ristori CA, Iaria ST, Gelli DS, Rivera IN. Pathogenic bacteria associated with oysters (Crassostrea virginica) and estuarine water along the south coast of Brazil. Int J Environ Health Res 2007; 17: 259-69.

Martinez-Urtaza J, Saco M, Hernandez-Cordova G, Lozano A, Garcia-Martin O, Espinosa J. Identification of Salmonella serovars isolated from live molluscan shellfish and their significance in the marine environment. J Food Prot 2003; 66(2): 226-32.

Martinez-Urtaza J, Liebana E. Identification of clonal distribution and persistence of Salmonella Senftenberg in the marine environment and identification of potential sources of contamination. FEMS Microbiol Ecol 2005; 52: 255-63.

Brands DA, Inman AE, Gerba CP, Mare CJ, Billington SJ, Saif LA, et al. Prevalence of Salmonella spp. in oysters in the United States. Appl Environ Microbiol 2005; 71: 893-7.

Brands DA, Billington SJ, Levine JF, Joens LA. Genotypes and antibiotic resistance of Salmonella Newport isolates from U.S. market oysters. Foodborne Pathog Dis 2005; 2: 111-4.

Morrison CM, Armstrong AE, Evans S, Mild RM, Langdon CJ, Joens LA. Survival of Salmonella Newport in oysters. Int J Food Microbiol 2011; 148: 93-8.

Presnell MW, Miessier CJ, Hill WF Jr. Clostridium botulinum in marine sediments and in the oyster (Crassostrea virginica) from Mobile Bay. Appl Microbiol 1967; 15: 668-9.

Sayler GS, Nelson JD Jr. Justice A, Colwell RR. Incidence of Salmonella spp., Clostridium botulinum, and Vibrio parahaemolyticus in an estuary. Appl Environ Microbiol 1976; 31: 723-30.

Haritharan H, Mitchell WR. Type C botulism: the agent, host spectrum and environment. Vet Bull 1977; 47: 95-103.

Acosta EA, Gomez E, Romero Tabarez M, Cadavid Restrepo GE, Moreno Herrera CX. Molecular identification of the bacterial populations associated with queen conch (Strombus gigas) from Colombian Caribbean. Acta Biolo Colomb 2009; 14: 83-96.

Carrascal OMP, Elorsa DD, Herrera CXM. Assessment of the bacterial community diversity associated with the queen conch Strombus gigas (Linnaeus, 1758) from the Caribbean coast of Colombia using denaturing gradient gel electrophoresis and culturing. Aquac Res 2014; 45: 773-86.

Inns T, Beasley G, Lane C, Hoppes V, Peters T, Pathak K, et al. Outbreak of Salmonella enterica Goldcoast infection associated with shellfish consumption, England, June to October 2013. Euro Surveill 2013; 18(49). pii: 20654.

Richards GP, Watson MA, Crane EJ 3rd, Burt IG, Bushek D. Shewanella and Photobacterium spp. in oysters and seawater from the Delaware Bay. Appl Environ Microbiol 2008; 74: 3323-7.

Herbers M, Haritharan H, Matthew V, Oliveira S, Rovira A, Sharma RN, et al. Photobacterium damsela in bighte scad, Selar crumenopthalmus (Bloch), from Grenada, West Indies. J Fish Dis 2014; 37: 675-7.

Hundenborn J, Thurig S, Kommreller M, Haag H, Nolte O. Severe wound infection with Photobacterium damsela spp. damsela and Vibrio harveyi, following a laceration injury in marine environment: a case report ad review of the literature. Case Rep Med 2013; 2013: 610632.

Koh TH, Sing LH, Yuen SM, Thomas CK, Tan PL, Tan SH, et al. Streptococcus cellulis after preparation of fresh seafood. Zoonoses Public Health 2009; 56: 206-8.

Chae MJ, Cheney D, Su YC. Temperature effects on the depuration of Vibrio parahaemolyticus and Vibrio vulnificus from the American oyster (Crassostrea virginica). J Food Sci 2009; 74: M62-6.

Audemard C, Kator HI, Rhodes MW, Gallivan T, Erskine AJ, Leggett AT, et al. High salinity relay as a postharvest processing strategy to reduce Vibrio vulnificus levels in Chesapeake Bay oysters (Crassostrea virginica). J Food Prot 2011; 74(11): 1902-7.

Papiwong N, Wallace RK, Arias CR. Bacterial loads and microbial composition in high pressure treated oysters during storage. Int J Food Microbiol 2009; 131: 145-50.

Meujo DA, Kevin DA, Peng J, Bowling JJ, Liu J, Hamann MT. Reducing oyster-associated bacteria levels using supercritical fluid CO2 as an agent of warm pasteurization. Int J Food Microbiol 2010; 138: 63-70.

Jakabi M, Gelli DS, Torre JC, Rodas MA, Franco BD, Destro MT, et al. Inactivation by ionizing radiation of Salmonella enteritidis, Salmonella infantis, and Vibrio parahaemolyticus in oysters (Crassostrea brasiliana). J Food Prot 2003; 66: 1025-9.

Mahmoud BS, Burrage DD. Inactivation of Vibrio parahaemolyticus in pure culture, whole live and half shell oysters (Crassostrea virginica) by X-ray. Lett Appl Microbiol 2009; 48: 572-8.

Jung JW, Kim HJ, Yun SK, Choi JY, Park SC. Eating oysters without risk of vibriosis: application of a bacteriophage against Vibrio parahaemolyticus in oysters. Int J Food Microbiol 2014; 188: 31-5.