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Shellfish as reservoirs of bacterial pathogens

Harry Hariharan*, Victor Amadi

Department of Pathobiology, School of Veterinary Medicine, St. George's University, St. George's, Grenada, West Indies

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ABSTRACT

The objective of this article is to present an overview on bacterial pathogens associated with shellfish in Grenada and other countries including the authors' experience. Although there have been considerable published work on vibrios, there is a lack of information on *Salmonella* serovars associated with various shellfish. In Grenada, for instance the blue land crabs collected from their habitats were found to harbor several *Salmonella* serovars. Also, it is notable that only minimal research has been done on shellfish such as conchs and whelks, which are common in the Caribbean and West Indies. Information on anaerobic bacteria, particularly, non-spore forming bacteria associated with shellfish, in general, is also scanty. This review re-examines this globally important topic based on the recent findings as well as past observations. Strategies for reduction of bacteria in oysters are briefly mentioned because of the fact that oysters are consumed commonly without complete cooking.

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 guanhumii*), 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 cholerae* O1, *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

*Corresponding author: Harry Hariharan, Department of Pathobiology, School of Veterinary Medicine, St. George's University, St. George's, Grenada, West Indies.

Tel: +1-473-444-4175, ext. 3677

E-mail: hhariharan@sgu.edu

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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* (*C. sapidus*), for instance inhabit waters that contain roughly 10^6 bacteria/mL sea water and 10^9 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 bioconcentration 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 tissues[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*[27]. 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 tubiashii*[41]. Mass mortalities of aquacultured carpet shell clam (*Ruditapes decussatus*) larvae 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 damsela* subsp. *damsela*.

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 pelagius*, *Vibrio damsela* (*Photobacterium damsela*), *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, septicemia, 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 cephalothin (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 carbon dioxide[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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