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A Mini Review: Cholera Outbreak via Shellfish

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Abstract: Problem statement: Food borne illness occurs all over the world. *Vibrio cholerae* is the etiological agent of cholera which is spread by contaminated food, water or direct fecal contact with food handlers. There are also examples of sporadic outbreaks of illness attributed to raw products eaten unprocessed. Consequently, there was a widespread concern that food in international trade carries pathogenic microorganisms that could result in outbreaks of illness. **Approach:** A review was done on the role of shellfish and seafood in the transmission of cholera. Google, Pubmed and Scpus were used in preparation of this review. **Results:** This review clarified that shellfish is one of the main seafood sources for the transmission of cholera. In natural waters *Vibrio cholerae* can be presented in both free-living state or attached to copepods, zooplankter and algae. *Vibrio* cholerae can adhere strongly to the shellfish digestive tract and cannot be effectively removed by rinsing the shellfish or by depuration. Colonization or attachment of *Vibrio* cholerae to shellfish increased the resistance of these bacteria to heat, drying and low pH. **Conclusion:** Therefore, sea food in general and shellfish in particular provided suitable background for cholera outbreaks. Unfortunately, this mode of transmission was underestimated. Accordingly, proper cooking, storing and re-heating of foods before eating were considered as main safety measures for preventing food-borne transmission of cholera. It was recommended to reconsider this mode of transmission for cholera again as source of cholera epidemics.

Key words: Food borne illness, *vibrio cholerae*, copepods, sea food

INTRODUCTION

Cholera has been recognized as a killer disease since earliest time. Since 1817, six pandemics have swept over the world and the seventh one is in progress. The disease is caused by infection of the small intestine by *Vibrio cholerae* O1 and O139 and is characterized by massive acute diarrhea, vomiting and dehydration. Unfortunately, death may occurs in severe and untreated cases[1]. In cases of cholera which take a severe course, fluid loss of 500-1000 mL an hour can occur. If left untreated, this results in death in less than 24 h[2]. Cholera and typhoid fever impose both a private economic burden on patients and their families include treatment costs, lost productivity, suffering and risk of death. Besides that, financial burden on the public health systems in developing countries[3].

Vibrio cholerae is the etiological agent of cholera which is transmitted by contaminated food, raw sources of seafood or direct fecal contact with food handlers. *V. cholerae* live in both marine and freshwater habitats and in association with aquatic animals[4]. Shellfish is one of sources for its transmission. *Vibrio cholerae* is an autochthonous, which is frequently related with phyto-and zooplankton[5]. The marine vibrio, requiring salt for growth, enters into a dormant, viable but nonculturable stage when conditions are unfavorable for growth and reproduction[6]. The association of *Vibrio cholerae* with plankton, notably copepods, provides further proof for the environmental origin of cholera, as well as an explanation for the sporadic and erratic occurrence of cholera epidemics[7]. Several *Vibrio* species form part of the natural biota of fish and shellfish[7-9].

The *Vibrio* genus is characterized by a large number of species; some of these are human pathogens causing gastrointestinal and wound infections through the ingestion or manipulation of contaminated fishes and shellfish[10]. Vibrio species that play role in distribution of cholera outbreak around the world include *Vibrio cholerae* O1, non O1, O139 and non O139. *V. cholerae* O1 causes diarrhea disease that

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infect thousands of people yearly. Accordingly, *V. cholerae* O1 was frequently detected in estuary and coastal waters and its growth in filtered natural seawater under defined laboratory conditions has been reported[11-13]. Currently more than 200 serogroups of *V. cholerae* are recognized based on the somatic O antigen but only strains belonging to serogroups O1 and O139 are associated with epidemic and pandemic forms. The O1 serogroup is further classified into two biotypes, namely classical and El Tor[14]. Clinically, apart from the O1 and O139 serogroups, the non-O1, non-O139 serogroups continue to be of negligible significance since these strains are associated with illness in only a low percentage of patients hospitalized due to acute secretory diarrhea[15].

In the autumn of 1993, *V. cholerae* serogroup O139, was implicated in outbreaks of cholera in Bangladesh and India[16]. *V. cholerae* serogroup O139, causes characteristic severe cholera symptoms and has been implicated in a case of a travelers returning from India to the US. The O139 strain arose by horizontal gene transfer between a non-O1 and O1 strain[17].Other vibrios may be clinically significant also. These include *V. parahaemolyticus*, a halophilic (salt-loving) *Vibrio* associated with enteritis is acquired by ingestion of raw or improperly cooked seafoods[18]. Another halophilic vibrio, which ferments lactose and for this reason was called the L+*Vibrio*, has recently been identified as *V. vulnificus*[19].

The origin of cholera has been elusive, even though scientific evidence clearly shows it is a waterborne disease. On a global scale, cholera epidemics can now be related to climate and climatic events, such as El Nino, as well as the global distribution of the plankton host[20]. The objective of this review is to highlight that consumption of shellfish is one of the main sources for cholera outbreaks either shellfish was from marine or freshwater environments.

The free living or copepod attachment of *Vibrio cholerae* in marine and freshwater environment: *V. cholerae* can be present in both a free-living state[13] or attached to copepods, zooplankters and algae[21,22]. Salinity and temperature are reported to be important parameters controlling growth of *V. cholerae* in estuarine environments[23]. *V. cholerae* is not only able to survive, but also able to grow in freshwater samples[24]. Furthermore, a more frequent detection of *V. cholerae* O1 in estuary waters and a higher morbidity rate among people in Bangladesh was shown to be associated with increasing temperatures[11,25]. Beside salinity and temperature, nutrients are also important element to control growth of *V. cholerae* in aquatic systems[7]. In several studies, the bacterium was able to use a large fraction (12-62%) of the Assimilable Organic Carbon (AOC_{app}) available to the bacterial AOC-test community, indicating that *V. cholerae* has the ability to gain access to substrates present in freshwater even in competition with an autochthonous bacterial lake water consortium. *V. cholerae* is able to adapt to both copiotrophic and oligotrophic environments.

The role of marine shellfish in transmission of cholera disease: Microbiological contamination of foods continues to be a main concern in public health. Biological toxins are one type of significant contaminants that can cause various human diseases[26]. In Malaysia, the cockle (*Anadara granosa*) is popular as an ingredient of several types of local foods. They are cultured in coastal waters, which are normally not depurated after harvest and are kept and sold at local markets. It is well recognized that raw shellfish frequently carry pathogenic *Vibrio* spp., including *V. cholerae* and are frequently concerned in transmission of these bacteria[27]. In addition numbers of *Vibrio* spp., including *V. cholerae*, may increase during storage of shellfish[28]. Shrimp, especially the ones still in their shells and imported from Asian countries such as India and Indonesia, were significantly contaminated with *V. cholera*[29]. Mangroves are being increasingly exploited near ports by industry, being developed as a result of urban spread and used to generate wealth through shrimp farming. Shrimp farming is seen as a key activity linked to change in habitat use, degradation and loss of mangroves[30]. The genus *Vibrio* was chosen as a group of microorganisms to assess the effects of shrimp farming effluent for reasons such as they are native to marine and estuarine environments used by shrimp farms[31].

Mussels are filter feeding animals, therefore, they can accumulate pathogens contained in the water environment. In a recent study [under publishing], *V. vulnificus* was more numerous in market oysters than in oysters at harvest. This was expected because on-board refrigerator is rarely available on coast harvest vessels and *V. vulnificus* multiplies rapidly in un re-frigerated oysters that are exposed to ambient temperatures[32]. A case-control study showed that although case patients of cholera were neighbors to control subjects and subjected to the same environmental conditions, not all of them contracted the disease. This was explained that cholera patients ate cooked crabs or cooked/raw shrimp one week before

illness whilst control subjects did not. Moreover, case-patients who ate crabs were more likely than control subjects who ate crabs to have undercooked and mishandled the crabs after cooking[33]. A research done by the US Food and Drug Administration (FDA), *V. cholerae* non-O1 were isolated from 14% of freshly harvested oyster lots[28].

The role of freshwater shellfish in transmission of cholera disease: *Vibrio cholerae* also can survive well in freshwater environment. When environmental stresses and other factors have led to reduced diversity in the pond water, *Vibrio* community was found to be increased due to the lack of competition. Along with increased susceptibility to disease by tshrimp, then disease has become liable to strike the shrimp population more effectively[34]. *Vibrio* spp. have also been closely related with mass mortality of cultured fresh water prawn in Taiwan[35]. In a previous study[36], outbreaks of cholera disease were associated with increases in the proportion of potentially pathogenic species in the *Vibrio* population of the cultured pond water. Immunostimulation of shrimp was found beneficial for reducing infection rated with *Vibrio* suggesting that shrimp is highly liable to *V. cholera* infection[36]. Table 1 shows the Risk ratio estimates and 95% confidence intervals for cholera incidence in Bakerganj, Bangladesh.

According to this study[37], an increase of one $\log_{10}$ in copepod count was significantly related with increased cholera cases in three lakes and two ponds in three of the four surveillance areas, with a lag of 0-8 weeks. In fact, the evidence points to the zooplankton as the significant factor. In Bakerganj, considering lake and pond copepod data, the risk ratio was 1.36-1.73 and 1.48-2.30, respectively and the lag time was 8 and 4 weeks, respectively. In Bakerganj, water and air temperature, water depth, total rainfall, conductivity, dissolved oxygen, cholera toxin probe-positive count and copepod counts in the surface water sites were considerably associated with the number of cholera cases. These findings indicate that *V. cholerae*, unexpectedly, survive, effectively in freshwater environment giving a significant source for cholera outbreak.

Table 1: Risk ratio estimates and 95% confidence intervals for cholera incidence in Bakerganj, Bangladesh[37]

Site	Variable (lag, in weeks)	Estimate d risk ratio	95% confidence interval for risk ratio
River	Water temp (6)	3.46 (Δ = +5°C)	2.41, 4.97
	Rainfall (8)	1.73 (Δ = -50 mm)	1.45, 2.07
	Conductivity (2)	1.16 (Δ = +150 μS)	1.10, 1.23
	Water depth (8)	1.12 (Δ = -2 ft)	1.06, 1.18
Pond	Air temp (6)	1.85 (Δ = +5°C)	1.48, 2.30
	Copepods (4)	1.82 (Δ = +1 log)	1.48, 2.24
	Water depth (4)	1.59 (Δ = -2 ft)	1.24, 2.03
	Dissolved O_2 (6)	1.51 (Δ = +2 mg dL^{-1})	1.28, 1.77
	Conductivity (2)	1.48 (Δ = 150 μS)	1.10, 1.98
Lake 1	Conductivity (2)	1.85 (Δ = +150 μS)	1.64, 2.08
	Copepods (8)	1.53 (Δ = +1 log)	1.36, 1.73
Lake 2	Water temp (6)	3.31 (Δ = +5°C)	2.38, 4.59
	Probe (0)	3.09 (Δ = +1 log)	2.24, 4.25
	Conductivity (0)	2.35 (Δ = +150 μS)	1.60, 3.45
	Rainfall (8)	1.72 (Δ = 50 mm)	1.45, 2.06

Epidemics and local endemics spread of cholera due to consumption of shellfish: The first pandemic spread of cholera outside Asia was in 1817. Since then seven pandemics have been recorded. The fifth and sixth were caused by the classical biotype of O1 strains but the nature of the strains causing the first four pandemics is unknown. In contrast, in 1961 the seventh pandemic started in Indonesia and was due to the El Tor biotype[38]. Recently, in 1992 an epidemic clone of a non-O1 strain with serogroup O139 Bengal caused a large cholera outbreak in Bangladesh and neighboring countries[39]. Infections are usually acquired by ingesting raw of shellfish[40].

A case-control investigation, residents more than 5 years old who were hospitalized for treatment of acute, watery diarrhea and two matched controls for each interviewed regarding sources of water and food and eating, drinking and hygienic habits[41]. Interviewers inspected homes of case-patients and controls to document water treatment, food-handling and hygienic practices. It was found that fecal specimens and shellfish yielded the same strain of *Vibrio cholerae* O1. Another study, it was found that mild heat treatment, just sufficient to open the shells, may not bring the interior of the shellfish to a high enough temperature (140°F) to destroy *Vibrios*. however, even adequate cooking cannot prevent foodborne illness if cooked shellfish are subsequently recontaminated[42]. An example of improper handling, cooked shrimp were kept in boxes in which raw shrimp had been shipped in Louisiana in 1978 and then were held at warm temperatures for several hours before serving[43]. This resulted in one of the largest cholera outbreaks in the United States in over a century. Another case study, patients ate crabs were more likely than control subjects who ate crabs to have undercooked and mishandled the crabs after cooking[33].

Many cholera outbreaks in Thailand were associated with marine foods, *Vibrio cholerae* in mussels smuggled in/from Thailand[44]. There have also been recent outbreaks of oily diarrhea related with

consumption of "escolar" and other fish species containing indigestible wax esters[45]. *V. cholerae* O1 epidemics are uncommon in the USA. Ten outbreaks associated with consumption of seafood harvested from the Gulf Coast were reported in the US between 1973 and 1992[1]. Eight more outbreaks were reported after eating imported seafood, or in travelers returning from Latin America. Large outbreaks in airline passengers who ate contaminated seafood served on the aircraft have been reported[46]. In the South American epidemics in 1991, a marinated, raw fish dish was initially implicated epidemiologically as a source of transmission, while crabs have been implicated as the source of US Gulf coast-associated cholera cases[4]. Eight outbreaks were reported after consumption of imported seafood, or in travelers returning from Latin America.

Control measures for shellfish's food borne disease: *V. cholerae* O1 was recovered from a pooled sample of bivalve mollusks and from 68% of stool samples from case-patients. Thirty-six percent of the isolates from stool specimens were resistant to multiple antimicrobial agents. In future, specific prevention measures should be taken into account to prevent transmission of this pathogen in vehicles of choleras disease such as shellfish[47]. To reduce the risk of food-borne transmission of cholera, it is suggested that foods should be prepared, served and eaten in a hygienic environment, free from fecal contamination[48]. Proper cooking, storing and re-heating of foods before eating and hand-washing with safe water before eating and after defecation are main safety measures for preventing food-borne transmission of cholera[1].

The following are recommendations for improving surveillance of foodborne diseases- including those associated with fish and shellfish:

- At the national level, enhance the global capacity to respond to disease threats, with coordination through WHO, focusing in particular on threats in the developing world
- Encourage and, where necessary, reward physicians and local jurisdictions for contributing to national and regional databases
- Consolidate existing databases to generate one set of national data for each agent (e.g., notifiable diseases vs. lab isolations) that can be compared with those in other countries
- Have technical resources to interpret the data to look for meaningful trends that can point to appropriate control measures
- Encourage more focusing on active surveillance with population-based sentinel studies and use special case-control studies to identify risk factors for each type of food borne illness. This would better allow regulators to incorporate data into risk assessments, to consider intervention strategies for prevention and control and to assist in meaningful educational programs
- Set public health goals to reduce food borne disease for each country and monitor progress with surveillance data; it is obviously difficult to determine appropriate budget allocations without achievable goals
- Support research on innovative systems of surveillance for rapid detection, (e.g., syndromic surveillance), GIS and for specific pieces of information that are needed for targeted mitigation and control strategies
- Have adequate and committed long-term funding both from nations and donor organizations for the poorest developing countries. Investments should take the form of financial and technical assistance (medical, veterinary and entomological surveillance, as well as laboratory capacity, i.e., epidemiological, statistical and communication skills); and the development harmonized systems to ensure the rapid sharing of information across national boundaries

Develop and improve regional networks in different parts of the world to obtain quality population-based data on disease burden and trends in the developing world through global surveillance. The eventual aim is a global surveillance system into which member states contribute to and draw upon information needed to mitigate the risk in a system that not only integrates economically but shares risks globally as well[44].

To ensure inactivation of *V. Cholerae* in shellfish, it is important, to know the extent to which this pathogen under some condition, may tolerate heat treatment and pH levels that are usually sufficient to destroy *V. Cholerae* when it is present in other kinds of food[49]. Preventing seafood-associated cholera in the long term will depend on maintaining sewage-free harvest beds and improving sanitation in processing plants. In coastal areas where the organism persists in the environment, even in the absence of sewage contamination, education to discourage the consumption of raw or undercooked shellfish is also needed[4]. Besides that, monitoring of environmental conditions, such as water temperature and salinity, may help determine when shellfish harvesting areas should

be closed and re-opened to harvesting. Guidelines regulating the harvesting of oysters and clams rely on quantitative measurement of this pathogen levels in oyster or clam.

Mechanism of survival of *Vibrio cholerae* in shellfish: *Vibrio* spp. adheres strongly to the shellfish digestive tract and cannot be effectively removed by rinsing the shellfish or by depuration. A competition assay using a reference strain discovered that the average infectivity of the aggregate form is significantly higher than that of the corresponding planktonic cells. It was, therefore, recommended that the hyperinfectivity of *V. cholerae* shed in human stools might be due to the presence of the aggregate form of *V. cholerae*, which delivers a high infectious dose of pathogen to the human host due to better capacity to survive *in vivo* stresses[50]. The phenotype of *V. cholerae* dispersed into an aquatic environment is also of epidemiological significance, as different forms of the pathogen are likely to exhibit different survival properties which in turn decide the likelihood of transmission to the next human host.

V. cholerae form biofilms by attaching to surfaces of phytoplankton and zooplankton[51]. Phytoplanktons excrete a variety of organic compounds that can sustain the growth of associated (biofilm) or free-living (planktonic) *V. cholera*[52]. Similarly, attachment to chitinous surfaces of zooplankton and the subsequent degradation of chitin to *N*-acetyl glucosamine, which serves both as a carbon and nitrogen source, by several chitinases produced by *V. cholerae* can support growth of the pathogen in an aquatic environment[52]. Indeed, laboratory microcosm studies have shown that during co-culture, *V. cholerae* effectively attaches to surfaces of zooplankton and phytoplankton and this association increases the survival period of the organism[51]. Thus, attachment of *V. cholerae* to plankton and subsequent biofilm growth facilitates the environmental survival and growth of this pathogen. This property of the pathogen may, in turn, aid its transmission. In cholera-epidemic areas, there is a correlation between phytoplankton and zooplankton blooms and the timing of epidemics[50]. This suggests that the plankton in *V. cholerae*'s aquatic habitat acts as a reservoir for the organism and that the plankton's life cycle controls the abundance of *V. cholerae* populations and epidemic cycles[53].

Recent studies have shown that *V. cholera* found in the surface waters of cholera endemic areas exists in biofilm-like aggregates in which cells are in a so-called “conditionally viable” state. “Conditionally Viable Environmental Cells” (CVECs) are viable but “metabolically impeded” *V. cholera* cells. CVECs can regain metabolic activity under specific in vitro conditions, as wells by inoculation into legated rabbit ileal loops suggesting that these forms of *V. cholerae* might play a critical role in the transmission of the pathogen[54]. The source of CVECs is thought to be biofilm-like aggregate populations of *V. cholerae* present in human stools. Similarly, the active and passive detachment of *V. cholerae* from various surfaces it colonizes in aquatic habitats could also lead to formation of bacterial aggregates. *V. cholerae* can produce an amorphous exopolysaccharide which encourage biofilm development when colonizing on shellfish[54]. Cells in biofilms are noticeably more tolerant than planktonic cells to environmental conditions such as low pH or high temperature[49]. It was shown that colonizing cells of *V. cholerae* O1 have fairly modified metabolic functions or structures that confer an increased ability to withstand the effects of environmental stresses[55]. Zooplankton can also act as an important disease reservoir[56], they have been found to house the bacterium *Vibrio cholerae* by allowing the cholera *vibrios* to attach to their chitinous exoskeletons[57]. This symbiotic relationship greatly enhances the bacterium's ability to survive in an aquatic environment, as the exoskeleton provides the bacterium with an abundant source of carbon and nitrogen[58].

CONCLUSION

Vibrio cholerae is a natural component of the bacterial flora of both freshwater and marine environments. Seafood especially shellfish acts as a vehicle for the transmission of cholera disease. Several studies have demonstrated that once *V. cholerae* has attached to chitin particles or crustacean external surfaces, the microorganism is able to initiate a process of colonization. The food-borne cholera can be spread by eating raw or improperly cooked shellfish. Many parts in the world where the spread of cholera disease caused by ingesting raw shellfish such as Indonesia, Bangladesh, Thailand, Ecuador, South America and the United states. To understand disease emergence, it is important to investigate the disease agent as well as its interactions with its environmental reservoir, vector and other animal hosts. Therefore, it is recommended conducting further studies and research on the role of shellfish and sea food in the deadly outbreaks of cholera disease.

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