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REVIEW ARTICLE

Food as a Vehicle of Transmission of Cholera

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ABSTRACT

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 diarrhoea, vomiting, and dehydration: death occurs in severe, untreated cases. Cholera is a highly contagious disease, and is transmitted primarily by ingestion of faecally-contaminated water by susceptible persons. Besides water, foods have also been recognized as an important vehicle for transmission of cholera. Foods are likely to be faecally contaminated during preparation, particularly by infected food handlers in an unhygienic environment. The physico-chemical characteristics of foods that support survival and growth of *V. cholerae* O1 and O139 include high-moisture content, neutral or an alkaline pH, low temperature, high-organic content, and absence of other competing bacteria. Seafoods, including fish, shellfish, crabs, oysters and clams, have all been incriminated in cholera outbreaks in many countries, including the United States and Australia. Contaminated rice, millet gruel, and vegetables have also been implicated in several outbreaks. Other foods, including fruits (except sour fruits), poultry, meat, and dairy products, have the potential of transmitting cholera. To reduce the risk of food-borne transmission of cholera, it is recommended that foods should be prepared, served, and eaten in an hygienic environment, free from faecal contamination. Proper cooking, storing, and re-heating of foods before eating, and hand-washing with safe water before eating and after defaecation are important safety measures for preventing food-borne transmission of cholera.

Key words: Cholera; *Vibrio cholerae*; Food; Disease transmission; Review literature

INTRODUCTION

Although cholera had been prevalent in many parts of the world for centuries, it is only during the last 50 years that we have learnt important aspects of the disease, including its pathogenic mechanism, treatment, and prevention. Robert Koch, during work in Alexandria and Calcutta, isolated *Vibrio cholerae*, and, in 1883, was the first to conclusively show that it was the cause of cholera. In 1953, SN De, a bacteriologist in Calcutta, discovered the crude cholera toxin, responsible for stimulating fluid secretion from the small intestine (1). Since then, rapid advances in research have illuminated the pathogenic mechanisms of fluid secretion and its regulation, the modalities of treatment, immune mechanisms, and have stimulated vaccine development.

V. cholerae O1, the causative organism of cholera, is a Gram-negative bacterium which infects and colonizes

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the small intestine, stimulating massive outpouring of fluid and electrolytes and leading to severe watery diarrhoea, dehydration, vomiting, electrolyte abnormalities, and metabolic acidosis. Although the majority of infections with *V. cholerae* lead to inapparant infection or mild disease, death occurs in 50 to 70% of the severe cases if they are not adequately rehydrated (2), either by intravenous infusion or, more simply, by oral rehydration solutions (ORS) (3). Antimicrobial agents, including tetracycline, are effective in reducing the volume of fluid loss and the duration of diarrhoeal illness (4). Although cholera occurs in individuals of all ages, children aged 2 to 9 years have the highest incidence of the disease in endemic areas, such as Bangladesh (5). Short-lived, protective immunity develops in adults in the endemic areas due to repeated exposures to infection, but long-term protection is rare.

Cholera is a disease of great epidemic potential. It is endemic along the Ganges valley, particularly in eastern India and Bangladesh. Since 1817, six cholera pandemics have swept over the world, exacting a heavy toll of human lives. The seventh pandemic started in Sulawesi, Indonesia in the early 1960s, and has now spread to most parts of the world. This last pandemic was the first caused by the El Tor biotype of *V. cholerae*.

Epidemiological evidence indicates that cholera is primarily a water-borne disease (6). Its faecal-oral transmission usually occurs by the ingestion of faecally contaminated water by susceptible individuals. Besides drinking water, food has also been recognized to be an important vehicle of transmission of cholera. In developing countries, where both poverty and poor sanitation are common, faecal contamination of domestic and commercial food is likely to occur, and in many outbreaks the infection has been traced to consumption of faecally-contaminated foods. In the developed countries, food-borne outbreaks of cholera have on many occasions occurred due to consumption of contaminated seafoods.

FOOD AS A MEDIUM FOR SURVIVAL OF V. CHOLERAE

The survival and growth of *V. cholerae* in foods depend on the physico-chemical properties of the particular foodstuff that has been contaminated. Food characteristics, which enhance the growth of *V. cholerae*, are low temperature, high-organic content, neutral or alkaline pH, high-moisture content, and absence of other competing micro-organisms in the food (7-10). *V. cholerae* are very sensitive to heat, and are rapidly killed when exposed to temperature of 100 ^{o}C. Drying and exposure to sunlight is also an effective means of killing *V. cholerae* (8,11). Domestic freezing is usually ineffective in sterilizing foods, and the organisms can survive for a long period in a frozen state.

Fruits and vegetables: In many countries, the practice of fertilizing gardens with untreated night soil and the habit of consuming uncooked vegetables have often resulted in cholera outbreaks. Vegetables may be contaminated during washing with polluted water. This can also occur when contaminated water is injected into fruits, such as water melons, to preserve their weight and taste (12). The pH of a specific fruit is an important factor that influences contamination by *V. cholerae.* Sour fruits, such as lemons and oranges, with lower pH (below 4.5) do not support the growth of *V. cholerae*, and, thus, do not pose risk of cholera transmission. Fruit pulp and concentrate preserved in cans are also less likely to be contaminated if they have an acidic pH. Spices, including raw onions and garlic, can support the survival of *V. cholerae* for 2 to 3 days at ambient temperature (8,13).

Seafoods: The importance of fish and shellfish as a vehicle of transmission of cholera has been recognized by early observers. Fishes are likely to be contaminated by *V. cholerae* when the surrounding water is contaminated by sewage or other environmental sources of *V. cholerae* O1. It has been shown that *V. cholerae* can survive in seawater in association with zooplankton (copepods). Zooplankton secrete a self-protective coat of chitin that can be dissolved by chitinase, an enzyme produced by *V. cholerae* O1. Seafoods, including molluscs, crustaceans, crabs, and oysters, feed on plankton and can become infected with *V. cholerae* (14). Once infected, particularly clams and oysters can harbour *V. cholerae* for weeks, even if refrigerated (7). In crabs, the organisms can rapidly multiply at ambient temperature, and boiling for less than 10 minutes or steaming for less than 30 minutes does not completely kill *V. cholerae* (7).

Dairy products: *V. cholerae* O1 can survive for more than two weeks in different dairy products, including milk, milk products, soft deserts, and cakes. Addition of sugar and eggs enhances bacterial survival. Although *V. cholerae* is killed by pasteurization of milk, the organisms can persist in raw milk as long as four weeks, even if refrigerated (13).

Poultry and meat: Contamination of meat of animal origin occurs exogenously during processing, cooking, storage, or consumption. It has been shown that *V. cholerae* can live and grow on cooked chicken, an increase in numbers of *V. cholerae* from 10^3 to 10^6 within 16 hours has been demonstrated (15). An early observation by Seligmann indicates that consumption of improperly cooked horsemeat was incriminated in a

small outbreak of cholera in Berlin in 1918 (16). The meat had been prepared by an infected butcher who succumbed to cholera the next day.

There are many other types of food that may be contaminated with *V. cholerae*. *V. cholerae* can survive on cooked rice, potatoes, eggs, and pasta for up to 5 days, and can also survive in spices, including pepper and cinnamon, for up to several days (8).

LABORATORY CHARACTERIZATION OF V. CHOLERAE O1

The mere presence of *V. cholerae* in food does not implicate food as the vehicle of cholera transmission. The organism must be tested in the laboratory to identify its biological characteristics with regard to pathogenicity, toxin production, antigenic type, and genetic structure. Application of modern molecular techniques to detect *V. cholerae* in food samples should be considered. Molecular probes of natural DNA fragments (17,18) and synthetic oligonucleotides have been developed to detect *V. cholerae* toxin genes (19). Polymerase chain reaction (PCR), a very sensitive tool, can detect trace amounts of DNA fragments, even from dead or lysed organisms. This assay may be very useful in investigating food-borne cholera outbreaks. Epidemiologic and medical implications should be considered to establish the cause-and-effect relation between the suspected source of infection and the outbreak of the disease. In this regard, the laboratory plays an important role with respect to characterization of the organism. When a specific food is suspected for contamination, a sufficient amount (25 g) of the food should be sampled, and specific laboratory procedures should be employed for the isolation and characterization of *V. cholerae*. Specific guidelines for laboratory examination of contaminated foodstuff, developed by the U.S. Food and Drug Administration, Department of Public Health, may be useful for proper identification of the suspected pathogens.

EPIDEMIOLOGY OF FOOD-BORNE TRANSMISSION OF CHOLERA

The epidemiological evidence reviewed by Politzer (20) suggests drinking water as the primary vehicle of transmission of cholera. However, early observations as well as those made during the last 30 years indicate that many outbreaks of cholera have been traced to consumption of contaminated foods throughout the world (21,22). The role of food as a vehicle of cholera transmission depends on several factors, including the likelihood of contamination of a specific food item, and the gastric acid-neutralizing capacity of a particular food.

Table 1. Food-borne outbreaks of cholera mostly in countrie other than USA*

Country	Year	Infected food	References
Philippines	1961	Raw shrimps	26
Malaysia	1963	Seafood/shellfish	36
Israel	1970	Raw vegetables	44
Bahrain/Sydney	1972	Hors d'oeuvres	46
Italy	1973	Mussels	37
Portugal	1974	Raw cockles/bottled water	39/59
Guam	1974	Salted fish	60
Gilbert Island	1977	Raw shellfish/salted fish	61/62
Louisiana, USA	1980	Inadequately steamed crabs	63
Sardinia	1980	Shellfish	35
Singapore	1982	Seafood	33
Gulf of Mexico	1983	Water-contaminated rice	42
Micronesia	1984	Food	64

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Seafood and shellfish: Seafoods, particularly fish and shellfish, have been incriminated in many cholera outbreaks since the nineteenth century (Table 1). Fish becomes infected with *V. cholerae* either due to sewage contamination of water or by ingestion of aquatic vegetation and zooplankton infested with *V. cholerae* (23). In a food survey in Taiwan, 1,088 vibrios, including *V. cholera*e and other species, were isolated from seafoods and aquacultured foods (24). In many countries, fish is eaten raw or undercooked (25,26). Outbreaks of cholera due to consumption of raw fish have been reported from Japan as early as 1886 (27) and from the Philippines in 1908 (28). Fish may serve as an important vehicle of transmission of cholera in the endemic areas

of Asia, where it is a major food item and is likely to be contaminated by *V. cholerae* due to both poor environmental sanitation and poverty prevailing in this region. Pandit and Hora (29) observed that, in India, the transmission of endemic cholera is maintained through infection of *hilsa* fish**,** which breeds abundantly in the Hoogly river that runs through Calcutta.

During the course of the seventh cholera pandemic, contaminated seafoods have been identified as the source of infection in several outbreaks. Seafoods and seafood products most frequently incriminated are raw shrimps, crabs, oysters, clams, shellfish, and mussels. These foods have been identified as a source of repeated outbreaks in the Unites States and elsewhere (30-32). In 1978, Singapore experienced another cholera outbreak, which was traced to consumption of prawns and squid, which were likely to be contaminated by infected food handlers (34). Again in Singapore, a food-borne cholera outbreak occurred in 1982 among 37 construction workers after they ate contaminated seafoods at a local cafeteria, where two food handlers were found to be infected with *V. cholerae* O1 (33). In 1979, an outbreak of cholera occurred in Sardinia; the source of infection was traced to eating of bivalves from which *V. choleare* O1 were isolated (35). In 1974 in the Pacific island of Guam, eating of small, salted fish was thought to be the vehicle of transmission of cholera (60).

The importance of shellfish as a vehicle of cholera transmission has been conclusively shown by a statistically significant association of cholera with shellfish consumption in Italy in 1973 (37,38). One year later, a severe cholera epidemic occurred in Portugal with 2,467 reported cases and 48 deaths (39). *V. cholerae* O1 was isolated from 42% of shellfish tested, and consumption of raw or poorly cooked cockles were significantly associated with cholera cases.

Reports of cholera outbreaks from the U.S. Gulf Coast during the last two decades provide epidemiological evidence of a definitive role of fish in the transmission of cholera (Table 2). In the United States, the first case of cholera after 1911 occurred in a shrimp fisherman in Texas in 1973 (40). The isolate was identified as *V. cholerae* O1, biotype El Tor. In spite of extensive investigations, the source of infection remained unidentified. Later, in 1978, 11 persons were attacked with *V. cholerae* El Tor in Louisiana after eating cooked crabs in different sites of the coastal marshes. In 1984 in Maryland, one person who had consumed infected crabs collected along the Texas coast developed cholera (41). In Florida (25) and Georgia (26), two isolated cases of cholera occurred in 1978 due to consumption of contaminated raw oysters. In the 1986 Louisiana cholera outbreak, 18 persons were attacked in 12 different clusters; all were infected by eating crabs and shrimps collected from different sites along the Louisiana coast (32). In the fall of 1991, a single cholera case was identified in an oil rig barge in Texas, which was followed by 13 secondary cases of cholera and one asymptomatic infection (42). The source of infection in the index case was traced to consumption of infected seafoods from local water. The secondary cases were infected by consuming rice prepared with water contaminated by the faeces of the index case through cross-connection between a sewer drain and the drinking water supply. Since 1973, a total of 65 cholera cases have been associated with the Gulf Coast reservoir in the United States. In all cases, the *V. cholerae* O1 isolated were of El Tor biotype and Inaba serotype; all strains produced cholera toxin and haemolysin on blood agar,

Table 2. Cholera cases in the Unites States associated with consumption of seafoods from Gulf Coast waters (1973-1992)*

State	Year	No. of cases	Implicated food	References
Texas	1973	1	Raw oysters	40
Louisiana	1978	11	Cooked crabs	63
Texas	1981	2	Fish/shrimps	65
Texas	1981	16	Cooked rice	53
Maryland	1984	1	Crabs	41
Florida	1986	1	Raw oysters	25
Louisiana	1986	18	Crabs/shrimps	63
Georgia	1986	18	Raw oysters	26
Louisiana	1987	2	Crabs	31
Colorado	1988	1	Raw oysters	66
Total	1973-1992	71		

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and possessed a characteristic bacteriophage, VcA3 (19). Genetically, all strains had the same restriction digestion pattern and ribotype pattern. All these data support the hypothesis that *V. cholerae* O1 strains have been persisting as a free-living organism along the Gulf Coast water for the last 20 years, independent of exogenous introduction during the pandemics.

Other foods. The African continent had been free of cholera for 70 years, but in 1971 cholera reappeared in Africa, and 30 of the 46 countries started reporting cholera. During a cholera outbreak in Mali in 1984, a case-control study showed that eating leftover millet gruel by villagers in an arid region was associated with cholera (43). In this community, millet is a major food item, which is prepared once a day and stored at room temperature for many hours and is often consumed without proper cleanliness by people in groups. In another outbreak in Guinea, consumption of leftover rice with peanut sauce has been incriminated as the vehicle of transmission of cholera. In contrast, leftover rice eaten with tomato sauce, having an acidic pH, unfavourable for *V. cholerae*, was not associated with cholera cases (43). There were also two reports of cholera outbreaks due to contamination of rice during preparation of a funeral feast by women who had cleaned the bodies of patients who died of cholera.

The importance of contaminated vegetables as a vehicle of cholera transmission is indicated by an outbreak in Jerusalem (44). In August 1970, an epidemic of cholera broke out in Jerusalem with 256 cases, the initial cases appearing simultaneously in three different places. However, the later cases appeared sporadically without specific primary cases; and the cases were limited to the Jerusalem region, despite considerable movement of people. Careful investigation of the epidemic could not establish a source of infection for the initial cases. However, the later cases were shown to be infected by secondary spread of *V. cholerae* through consumption of vegetables contaminated by faeces from the initial cases. In Jerusalem, vegetables are supplied from the surrounding villages, where sewage and night soil are used as fertilizer.

In the United States, an outbreak of cholera occurred in Maryland in 1991, in which four persons were infected, three of them having diarrhoea (45). Consumption of Thai-style rice topped with coconut milk was the source of infection. Laboratory examination of the same brand of coconut milk revealed its contamination by *V. choleare*, which differed in molecular characteristics from those strains causing the seventh pandemic in Asian and Latin American countries, and in the Gulf Coast of the U.S.A. These findings suggest that the coconut milk was contaminated during its preparation in the country of origin.

Table 3. U.S. cases of cholera in persons returning from Latin America or eating food from Latin America.*

State	Year	Nos. of cases	Suspected food	Country of food origin	References
Georgia	1991	1	Cooked crab meat	Peru	67
New Jersey	1991	8	Cooked crab/salad	Ecuador	68
Florida	1991	1	Raw oysters	Ecuador	68
New York	1991	4	Cooked crab/salad	Ecuador	69
New Jersey	1991	1	Crabs	Ecuador	CDC, Unpub.
California	1992	2	Seafood at stand	El Salvador	CDC, Unpub.
California	1992	76	Shrimp/fish in salad	Peru	53
California	1992	1	Raw seafood	El Salvador	CDC, Unpub.
Total	1991-1992	94			

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An outbreak of cholera was reported in 47 of the 331 passengers of an airline flight en route from London to Sydney in 1972. The source of the infection was traced to consumption of contaminated hors d'oeuvre served on the aircraft and obtained from a caterer in Bahrain, where an epidemic of cholera was going on (47).

Recent cholera outbreaks in Latin America: For the first time in the 20th century, cholera appeared in Latin America in 1991. Peru was the first country to report cholera during the epidemic (48). The disease spread quickly to other countries. By August 26, 1992, 640,000 cholera cases and 5,600 deaths were reported by 19 countries (48-50); these numbers were more than those reported for the entire world during the former five years (51,52). In the same wave of the epidemic, cholera

entered into the United States with travellers returning from Latin America (Table 3). Food has been incriminated as the vehicle of transmission of cholera from Latin America to the United States in several instances.

An index case of cholera was identified in New York in 1991. This case was followed by three secondary cases of symptomatic infection with *V. cholerae* O1 (69). The index case had travelled to Ecuador and brought with him boiled crabs which were found to be contaminated by the *V. cholerae* strain prevailing in Latin America, as detected by sensitive assays, including PCR and DNA ribotyping (42). The molecular characteristics of this strain of *V. cholerae* O1 were completely different from those isolated from the coastal areas of Texas and Louisiana. In another instance in 1991, 76 airline passengers returning from Argentina to Los Angeles were infected with *V. cholerae* O1 by eating contaminated shrimp and fish salads prepared in Lima, Peru (53). Although no secondary spread occurred, 37 passengers became ill, and of them one died. A total of 41 imported cases of cholera have been documented in the United States during 1961-1990. Most of these cases were associated with travel to Latin American countries or with eating of seafoods brought from there.

These observations indicate that there is a possibility of cholera transmission through eating contaminated food in another country. However, the risk of transmission of cholera through imported commercial foods seems to be small. There have been no cases of cholera in the United States as a result of importing commercial foodstuffs from Latin American countries.

FOOD-BORNE CHOLERA IN ENDEMIC REGIONS

In cholera endemic regions of Asia, including Bangladesh, contamination of food may be an important factor in the transmission of cholera. Case-control studies have shown that, in Bangladesh, the rate of contamination of household water with *V. cholerae* O1 is significantly higher in water used for cooking than in water used for drinking (54). It is likely that water may serve as a source of secondary contamination of food during its preparation. A household survey carried out in Bangladesh indicates that only 0.13% of the food samples cultured were contaminated with *V. cholerae* O1. This indicates that the risk of food-borne transmission of cholera during the non-epidemic season in the endemic areas may be small. Although fish and shellfish have been shown to be an important vehicle of cholera transmission in non-endemic areas (U.S. Gulf Coast, Africa, Latin America), in the endemic areas of India and Bangladesh, fish-borne transmission seems to be rare. In these communities, most people eat fresh water fish rather than salt water fish and other marine species, such as shellfish, clams, and oysters. Nevertheless, *V. cholerae* O1 has been isolated from aquatic flora and fauna in this region (55). It has also been found that blue-green algae can act as a reservoir of *V. cholerare* O1 in the aquatic environment of Bangladesh (55). These algae are eaten by fish. Since fish is usually well-cooked before eating and never consumed raw, fish-borne infections must be rare. However, cross contamination of foods through handling of infected fish remains a possible risk of transmission.

In endemic areas, transmission of cholera through contaminated foods served by street vendors and restaurants should be considered. In Dhaka, the capital city of Bangladesh, there were two outbreaks of cholera in 1974 and 1975 (56). The results of a case-control study indicated that the attack rates of cholera were significantly associated with eating in restaurants. Moreover, the free food distribution centres established in the city to feed the famine-affected people also played a significant role in the transmission of the disease. In the Hong Kong outbreak of cholera in 1990, the source of infection has been traced to consumption of a special rice dish called “moonsalus” (57).

CONCLUSIONS AND RECOMMENDATIONS

Cholera is a disease of great public health importance. The mainstay of treatment of cholera is the replacement of fluid and electrolytes lost in the stools. Rehydration can be easily achieved either by intravenous infusions or more simply by oral rehydration solutions. Treatment with an effective antimicrobial agent reduces fluid loss and the duration of diarrhoea, but this form of treatment is not considered a substitute for rehydration therapy. Since untreated diarrhoeal stools from cholera patients are the primary source of environmental contamination, including water sources and foods, proper treatment of cholera cases and safe disposal of faeces would reduce secondary spread and faecal contamination of the environment. Transmission of cholera occurs by the faecal-oral route, and water has been recognized as the primary vehicle of cholera transmission. Thus, to interrupt the transmission cycle, effective public health measures should be undertaken to prevent faecal contamination of drinking water supplies, as well as to establish sanitary disposal and sewage treatment systems. This is not an easy task in most situations, and, thus, calls for a considerable investment and commitment by the government and the community leaders.

Beside water, foods constitute an important vehicle for transmission of cholera in the environment. Consumption of *V. cholerae*-contaminated foods, particularly seafoods and fish, has been implicated in a large number of cholera outbreaks throughout the world. Seafoods can be infected if the surrounding water is faecally contaminated or during its processing by the infected food handlers. After cooking, food can be infected during storage and consumption in an unclean environment.

To prevent food-borne transmission of cholera, the foods must be safe and free from *V. cholerae* before consumption. The World Health Organization recommends the following food safety measures to prevent the spread of cholera (58):

- avoid raw food (exception: undamaged fruits and vegetables from which the peel can be removed are safe if hygienically handled);
- cook food until it is hot throughout;
- eat food while it is still hot, or reheat food thoroughly before eating;
- wash and thoroughly dry all cooking and serving utensils after use;
- handle and prepare food in a way that reduces the risk of contamination (e.g. cooked food and eating utensils should be kept separate from uncooked foods and potentially contaminated utensils); and
- wash hands thoroughly with soap (or ash) after defaecating, or after contact with faecal matter, and before preparing or eating food, or feeding children.

With regard to the risk of cholera transmission through food trade, the WHO recommends: "… although there is a theoretical risk of cholera transmission associated with international food trade, the weight of evidence suggests that this risk is small and can normally be dealt with by means other than an embargo on importation."

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