A Review on Emerging Pathogenic Bacteria in Marine Environment

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
Bachelor of Science in Microbiology

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Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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Abstract:

Marine pathogenic bacteria possess significant public health risks due to bathing in coastal water and consumption of seafood. Different man-made and environmental factors help these pathogenic bacteria grow and survive in the adverse condition of the ocean. The genetic mutation also influences the survival of pathogenic bacteria in the marine environment. The increasing rate of marine pathogen outbreaks has become a public health concern over the world. So, scientists must be aware of these groups of pathogenic bacteria while diagnosing a patient or detecting a new pathogen. Recently, researchers are using different developed detection methods like culture-based methods, RT-PCR, PFGE, etc., to detect these marine pathogenic bacteria. This paper overviews the occurrence of emerging marine pathogenic bacteria, their sources, route of transmission, and the disease that they cause.

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Chapter 1: Introduction

Worldwide human are greatly connected with ocean. However, man-made environmental changes, such as global warming, climate change, pollution etc can lead to ocean conditions that greatly harming the health of humans, marine species, and ecosystems. Contamination of lakes, rivers, and coastal waters raises significant public health issues. Among the leading sources of chemical and biological contamination of these waters and associated beaches are sewer systems, septic tanks, storm water runoff, industrial wastes, wastewater injection wells, cesspits, animal wastes, commercial and private boat wastes, and human recreation[1]. In 1997, 649 beach closings or advisories were caused by sewage spills and overflows [2]. In Florida alone, approximately 500 million gallons of sewage were released along the coast each year during the late 1980s[3].Thus one of the primary concerns in public health is the risk that humans using the marine environment for recreational activities will encounter microbial pathogens. The risk to human health due to recreational exposure— swimming where sewage contaminates marine waters, for example—has been documented [4][5]. Studies conducted along beaches in Hong Kong reported that swimmers were at higher risk of gastrointestinal and respiratory illness and eye, ear, and skin infections than no swimmers, not surprisingly, these risks were greatest at beaches known to be polluted [6]. Cheung and colleagues estimated that in 1990, approximately 58,000 episodes of illness in Hong Kong were the result of swimming at a single beach contaminated by sewage[6]. In Great Britain, Alexander and colleagues (1992) concluded that children who swam in contaminated marine waters were more likely than those who did not to develop symptoms of illness[7]. Apart from that, seafood poisoning, particularly secondary to microbial contamination, accounts for a large and growing proportion of all food poisoning incidents. In the United States, fish, shellfish, and other marine organisms are responsible for at least one of six food poisoning outbreaks with a known etiology, and for 15% of the deaths associated with these particular outbreaks [8][9]. From 1971 to 1990, seafood was the single most important vehicle in food poisoning outbreaks in Korea (32%) and Japan (22%), where seafood was responsible for 43% and 62%, respectively, of outbreak-related fatalities [10][11].

Hence, human and marine microbes have always been impacting each other through the course of time. This impact and relationship between the residents of coastal regions and marine microbes is even more important now, as the number of diseases and outbreaks caused by the marine microorganisms keep escalating with time. Hence, it is very. For that, it is essential to know about their source, route of transmission, possibility of outbreak, pathogenicity, and the biotic or abiotic factors affecting them. These are also required to ensure proper and safe marine environment, water quality etc. Nowadays, many countries have their own parameter microorganism for their oceanic zone to determine the seawater quality. Sea water quality determines and controls the overall biodiversity in the marine environment. So, this has a huge impact on the type of pathogens available in that environment.

Marine bacterial pathogens affect humans in a number of ways, including getting incorporated in the food sources or entering the body through fecal-oral route etc. Human bacterial infection from marine sources is getting more and more of an issue, as people is getting more involved in recreational activities related to oceans and seas, depending on foods from marine sources, using marine environment for commercial purposes etc. So, it has become important to know more about the origin, sources, transmission route etc of these bacterial pathogens. In this paper we have discussed the different bacterial pathogens for humans available in the marine environment, their source, occurrences, Biotic and abiotic factors impacting their growth and the threat emerging marine pathogens might have on human health.

Chapter 2: Emerging pathogenic bacteria in marine environment

Marine environment contains billions of microorganisms, including virus, bacteria, algae, fungi etc. We can also categorize them as non-pathogenic and pathogenic. In this paper, we are solely focusing on pathogenic marine bacteria. The source of these various species of pathogens ranges from indigenous sources contamination from different sources, such as, sewage, fecal, fishes and animals etc. In case of contamination, Foodborne illness is caused by fecal contamination of food, which is produced either directly by diseased or asymptomatic food handlers or indirectly by infected domestic animals reared for food. The commonly attributed to human activities at the coast line increase the level of pathogenic microbes. These pathogens can transmit to humans through direct contact, inhalation and consumption. In case of consumption of food, insanitary procedures during product handling are the most common source of enteric pathogen contamination in seafood. Another way is through fishes that are eaten raw, when they are harvested from polluted sea water. The pathogens found in the marine environment are responsible for a broad spectrum of acute and chronic human diseases like gastroenteritis, ocular and respiratory infections, hepatitis, myocarditis, meningitis, and neural paralysis. The origin of most marine pathogens is feces with only a very few “autochthonous” bacterial pathogens which are able to grow in the marine coastal environment. The route of transmission of these marine bacterial pathogens can also vary from fecal oral route to vehicle borne.

Table 1: Pathogenic Bacteria in Marine Environment

Group of Organism	Organism	Source	Disease	Transmission route	Occurrence	Reference
Rod(-ve)	*Aeromonas hydrophila*	Sewage contamination	Gastroenteritis, Dermohypodermitis	Fecal-oral, vehicle borne, zoonotic	South India, Southern Italy, South Coast Iran	[12][13][14][15][16][17][18][19][20][21]
	Vibrio cholerae	Inhabitant	Gastroenteritis, Dermohypodermitis	Fecal-oral, vehicle borne	France, Italy, Spain, USA, Philippines, Malaysia, Pakistan, Bangladesh	[12][13][22][23][24][18][19][21]
	Vibrio vulnificus	Inhabitant	Gastroenteritis, Wound infections, Dermohypodermitis	Fecal-oral, vehicle borne	Denmark, France, Italy, Spain, China, Taiwan	[12][13][24][25][26][27][28][29][30][31][32][21][33]
	Vibrio parahaemolyticus	Inhabitant	Gastroenteritis, Wound infections, Dermohypodermitis	Fecal-oral, vehicle borne	France, Italy, USA, China	[12][13][24][34][35][36][21][37][38]
	Vibrio alginolyticus	Inhabitant	Gastroenteritis, Wound infections	Fecal-oral, vehicle borne	Italy, Germany, Bangladesh	[12][13][24][34][39][21][40]
	Salmonella spp.	Fecal contamination	Gastroenteritis	Fecal-oral	EU, Portugal, Spain	[12][13][41][42][43][18][19]
	Shigella spp.	Fecal contamination	Gastroenteritis	Fecal-oral		[12][44]
	Escherichia coli	Fecal contamination	Gastroenteritis	Fecal-oral		[12][45][18][46][19][47]
	Plesiomonas shigelloides	Fecal contamination	Gastroenteritis	Fecal-oral		[13]
	Pseudomonas aeruginosa	Commonly found	Skin Disease, Ophthalmic Infection, Ear	vehicle borne	Japan, USA	[12][48][49][50][18][19][21]

			infection			
	Shewanella algae	Inhabitant	Spondylodiscitis, Acute exudative tonsillitis, Arthritis, Osteomyelitis, Ear infection, Bacteremia, Skin disease, Dermohypodermitis	Direct, vehicle borne	Italy, France, Denmark	[12][51][52][53][54][55][56][57][58][59][54]
	Shewanella putrefaciens	Inhabitant	Skin disease, Dermohypodermitis, Cerebral abscess, Bacteremia	Direct, vehicle borne	Denmark	[12][51][54][60][61][62][63][64]
Spiral rod(-ve)	*Campylobacter spp*	Commonly found	Gastroenteritis, Skin infection	vehicle borne	Finland, Spain, Italy, Iran, Netherlands	[13][65][66][67][68]
	Helicobacter pylori	Fecal contamination	Gastroenteritis	Fecal-oral	Italy, USA	[12][69][70][71][72]
Curved rod(-ve)	*Arcobacter butzleri*	Contamination animal	Gastroenteritis	Fecal-oral	Iran, Italy, Baltic Sea	[13][73][74]
	Arcobacter skirowii	Contamination animal	Gastroenteritis	Fecal-oral		
	Arcobacter cryaerophilus	Contamination animal	Gastroenteritis	Fecal-oral		
Rod(+ve)	Listeria monocytogenes	Sewage contamination	Gastroenteritis	Fecal-oral, vehicle borne	Egypt, New Zealand, Turkey	[75][76][77][78][79]
Cocci(+ve)	*Staphylococcus aureus*	Contamination	Gastroenteritis, Skin Disease, Ophthalmic Infection, Ear infection	Direct, vehicle borne	USA(Seattle, Florida, California)	[12][80][50][81][18][46][19][21]
Acid fast bacilli	*Mycobacterium marinum*	Commonly found	Skin Disease	Direct, vehicle borne	Australia, USA	[12][82][61][83][84][85]

Chapter 3: Factors affecting the survival of bacteria in seawater:

Because of the unique composition of seawater there are some microorganisms which can survive in marine environments. The microbes which can survive in the ocean must deal with different biotic and abiotic challenges such as grazing, solar radiation and temperature, organic matter availability, and particle load etc. There is increasing evidence that many pathogens found as pollutants in marine environments can survive harsh environmental conditions for prolonged periods of time in a spore-like, "viable but nonculturable" (VBNC) state. [86]. Pathogens indigenous the sea has evolved to their surroundings and their spread can be triggered by a variety of causes. Warm water temperatures, for example, appear to have a favorable influence on the quantity of human invasive pathogens, which tend to thrive best in a mesophilic environment. Furthermore, increased light can promote the development of marine heterotrophic bacteria by boosting nutrient availability through photochemical degradation of complex polymers to liberate organic metabolites, which increases nutritional availability.

Chapter 3.1: Abiotic factors affecting survival

i. **Solar Radiation:** Pathogenic bacteria can be destroyed by solar radiation. As UV radiation decreases with water depth and turbidity, bacteria and viruses in deeper water layers and particle rich water are less stressed by UV radiation than in clear surface water. [13]Survival of enteric bacteria in the sea is greatly affected both by UV and visible light. In fact, light is considered to be the single most important contributor to bacterial die-off in the sea, although its effects are restricted to shallow depths. A depth-dependent effect was also implicated in the importance of the radiation wavelength. Moreover, Fecal microorganisms differed in their sensitivity to light in seawater, and greater sunlight exposure was required to inactivate enterococci compared to fecal coliforms.[87] Sunlight can stimulate growth of marine indigenous heterotrophic bacteria by increasing nutrient availability by photochemical breakdown of complex polymers to release organic metabolites.[86].

ii. **Temperature:** Water temperature has a different effect on bacteria and viruses. Most pathogenic bacteria are more abundant at higher water temperature, because survival or even growth increases at higher temperature this is especially valid for the authouchthonous bacteria like vibrios. With decreasing water temperature, an increased fraction turns into a viable but non-culturable (VBNC) state, the infectivity and the survival rate decreases. By contrast, some enterobacteria survive longer at colder temperatures.[13]. Warm water temperatures appear to have a positive effect on the abundance of human invasive pathogens, which tend to have mesophilic growth optima. In temperate environments, the distribution of such pathogens is typically seasonal with peaks in both environmental abundance and human infection occurring during the warmer months. This has been demonstrated for human pathogenic

Aeromonas spp. *Shewanella algae* and vibrios including *V. cholera V. parahaemolyticus* and *V. vulnificus.*[86].

iii. Salinity: Enteric bacteria are subjected to an initial osmotic upshock when discharged into the sea, and their capacity to resist this through a variety of osmoregulatory systems may have a significant impact on their ongoing survival in the marine environment.[87]

E. coli survival in seawater/distilled water combinations at 37°C in varying ratios (0, 25, 50, 75, and 100% seawater) after 48 hours, showed that at a concentration of 25% seawater, exhibited the best survival rate (74%**).** Troussellier et al. studied the interactions between salinity, light, and the presence of organic matter. Salinity was shown to increase E. coli sensitivity (CFU) solely in the presence of light, independent of the presence of organic matter. [87]

iv. pH: Temperature, pressure, and the photosynthetic and respiratory activities of microorganisms all influence the pH of seawater, which typically varies between 7.5 and 8.5. The most suitable pH for E. coli survival was discovered to be 5. Furthermore, the adverse effects on E. coli survival are increased by the pH of seawater, which is typically about 8.[87]

v. Nutrient: It's a little-known fact that E. coli can grow in seawater nearly as well as it does on rich laboratory media in the presence of appropriate nutrient levels. E.coli may grow in seawater due to the availability of nutrients, but their absence does not always mean that non-growing cells will die. Interestingly, cells that were forced to thrive in nutrient-rich seawater were more vulnerable to mutations.[87]

Chapter 3.2: Biotic factors affecting survival

i. **Grazing and competition:** All pathogenic microorganisms are submitted to grazing in the marine environment to a certain extent. Most relevant grazers are flagellates, ciliates, zooplankton (cladocerans) and filter feeding bivalves, ascidians, polychaetes and sponges. Grazing implies ingestion and digestion of the microorganisms. Bacteria are to some extent eliminated by grazing.[13] Various studies indicate that the main predators of bacteria in the marine environment are protozoa. Using filters with different pore sizes and antibiotics to suppress indigenous bacterial activity, it was shown that bacterial competition, antagonism, and even bacterial predation were relatively unimportant in coliform removal. It was also shown that the reduction in E. coli populations paralleled an increase in the number of protozoa.[87]

ii. **Bacteriophages**: Bacteriophages can infect bacteria and lead (later on - and this time period is highly variable) to lysis and - thus elimination of the bacterial cells. Bacteria in the marine environment can either be infected before they entered the marine environment (e.g. in the intestine or the sewage treatment facility) or be infected in the marine environment. Due to their specificity, the host spectrum of a specific phage is very restricted, usually to a specific bacterial species. Since the probability to be infected by a virus of the right specificity is dependent on the abundance/density of a bacterial host, bacteria with a high abundance in the coastal environment - such as vibrios or *Aeromonas* sp. - are much more likely to be infected and killed by viruses in the marine environment. For bacteria unable to grow in the marine environment, infection by viruses of marine origin is not likely; by contrast the presence of bacteriophages specific for enterobacteria can be used as a tracer for fecal contamination in shellfish or in coastal water.[13]. Many studies detected coliphages in marine waters subject to sewage contamination. It was also shown that phages detected in seawater were active against E. coli, Aerobacter aerogenes, and Serratia marinorubra. The presence of coliphages was investigated in relation to their usage as indicators for fecal contamination and in many cases a positive correlation between fecal phages, enteric viruses, and other pathogens has been recorded. Nevertheless, the presence of enteric bacterial infectious phages does not necessarily indicate their actual activity in removing coliforms from marine water.[87]

iii. **Toxins and antibiotics:** Many studies throughout the 40s to 60s, showed some negative impact of sea water on marine bacteria. It was later found to be due to some toxins or antibiotics produced by marine microorganisms. There have been several studies that attempted to find out any sort of effect of sea water on marine bacteria, all of which concluded that, such sea water toxins and antibiotics, are not usually formed under natural conditions or are able to eliminate their target pathogen.

Chapter 3.3: Molecular mechanisms involved in seawater survival

i. Effect of specific mutations: Some pathogenic bacteria have effect of specific mutations. Past attention to the molecular mechanisms determining survival of enteric bacteria such as E. coli in artificial seawater or sterile seawater in the dark revealed very few genes crucial to seawater survival. Only six mutations were shown to have a significant effect. The most dominant among them was in rpoS. The other genes in which mutations were reported to be significant were otsA, relA, spoT (but only on top of a relA deletion) and one or both membrane porins ompC and ompF.[87]

ii. **Genes induced upon seawater exposure:** Another possible strategy in the search for genes playing a role in seawater survival is to look for those activated in response to seawater exposure. Such a study was conducted by Rozen et al. who screened a 687-member E. coli promoter:: luxCDABE fusion library. Twenty-two promoters were found to be activated in seawater amended by sufficient nutrients to allow bioluminescent expression but not growth.[87]

Chapter 4: Case study and emerging threat of Marine Pathogens

All of the known marine pathogenic bacteria pose a significant public health threat. Some pathogens occur naturally in marine waters. Others are carried into waterways after defecation/urination/shedding from human or animal hosts (e.g. via sewage effluent, agriculture and storm water runoff, sewage from ships, recreational population using the water, industrial processes, wildlife, septic tanks near the shore and urban development). Rivers discharging into coastal areas may carry abundant micro-organisms from these diverse sources. Contamination from human sources (e.g. fecal pollution) presents a greater risk to humans than contamination from animal sources because many animal pathogens are not infectious to humans. Risks to humans from pathogenic organisms are higher in areas with large population densities or with a significant tourism. Recently, different marine pathogens outbreaks are recorded in different areas of world.

Aeromonas hydrophila is a heterotrophic, Gram-negative, rod-shaped bacterium mainly found in areas with a warm climate. *Aeromonas spp.* is commonly found in ground water; drinking water at treatment plants, distribution systems, and reservoirs; and in clean and polluted lakes and rivers. So, it is contaminants in ocean which causes gastroenteritis, dermohypodermitis. *Aeromonas hydrophila* in fish, shrimp, lobster and crab caught off the south coast of Iran. A total of 541 samples including, 133 freshly caught fish of 4 different types including *Otollithes ruber, Famous argenteus, Parastromateus niger* and *Psettodes erumet,* 240 shrimp of 4 different species including *Penaeus monodon, P. semisulcatus, P. indicus,* and *P. merguiensis,* 108 lobster *(Panutirus homarus)* and 60 crab *(Panulirus homarus*) were collected in 3 provinces along Persian Gulf in the south coast of Iran.[14] Moreover, *Aeromonas spp* is also found in freshly caught finfish and prawn in South India and aquaculture production in China. [15][88]

Campylobacter infection is likely low, contaminated surface water may cause infection through swimming *campylobacters* are commonly found in natural waters, such as rivers, streams, lakes and ocean. *Campylobacter jejuni* and *C. coli* are leading causes of human bacterial gastroenteritis in industrialized countries. In 1998, in Finland, the number of reported *Campylobacter* cases exceeded that of salmonella for the first time. [68]. In addition, *Campylobacteria*, mainly *Campylobacter jejuni*, were found in 12% of bathing waters in a study in Finland.[12]

Helicobacter pylori, a gastric pathogen, is believed to be transmitted via the fecal-oral route as well as the oral-oral route. Its presence and viability in environmental waters is not well characterized. Two coastal beach sites were repeatedly positive in 2007. During this two year study, 137 samples were collected from a total of 23 sites from the Atlantic coast (Rehoboth Beach, Fenwick Island Beach, North Inlet Beach), Delaware Bay, Inland Bays (Rehoboth, Indian River, and Little Assawoman Bays; TowerRoadandHolt's Landing) and Broadkill River. [70]

Certain atypical bacteria have been reported to cause infections after contact with seawater. One such example is *M. marinum. Mycobacterium marinum* is a nontuberculous mycobacterium that causes infections in humans ranging from simple cutaneous lesions to debilitating disseminated infections. *M. marinum* is an endemic fish pathogen found in a variety of aquatic settings, such as fish tanks, swimming pools, and natural bodies of water. Infection is most often reported among fish fanciers and participants of waterborne activities chlorination has reduced the number of swimming pool acquired cases. While cases are rare, with an estimated annual incidence of 0.27 cases per 100,000 persons in the United States, outbreaks have been recently reported among patrons of seafood markets in New York City and at a fish farm in China [82]. Again, two cases of occupationally acquired *Mycobacterium marinum* infection in Chinese restaurant workers whose dealings with fish in Melbourne, Australia.[84]

Salmonella is the type of bacteria that's the most frequently reported cause of food-related illness. It can cause an upset stomach, diarrhea, fever, and pain and cramping in your belly. Salmonella may be found in water sources contaminated with the feces of infected humans or animals. Waste can enter the ocean water through different ways, including sewage overflows, sewage systems that are not working properly, polluted storm water runoff, and agricultural runoff. In September 1998, an outbreak of gastroenteritis occurred in a coastal Aboriginal community in the Northern Territory over a seven day period. An investigation was conducted by the Center for Disease Control, Territory Health Services. Thirty-six cases were detected and 17% (n=6) were hospitalized. *Salmonella chester* was isolated from eight of nine stool specimens. Sixty-two percent of cases interviewed (n=28) reported consumption of a green turtle (Chelonia mydas) within a median of 24 hours prior to onset of illness. [89]

Another case report discusses a rare presentation of salmonella bacteremia after an oral exposure to a sand dollar in a pediatric patient. A 2-year-old Hispanic male presented to the emergency department with a chief complaint of diarrhea and fever for 8 days after a family trip to Destin beach, Florida, during the sea turtle nesting season. The symptoms began a day after the patient took a bite on a sand dollar found on the beach that caused a small wound inside his cheek. The laboratory testing done in the emergency department was remarkable for blood and stool culture testing positive for non-typhoid salmonella. [90]

Shewanella algae, mainly found in marine environments, are a rare pathogen in humans, especially in healthy children. *Shewanella algae* and *Shewanella putrefaciens* increase in summer because there is more algal decomposition at this time. Other articles reported osteo-articular infections, arthritis and osteomyelitis by *S. algae.* Two other patients with lower leg ulcers had bacteraemia. In Denmark, *S. putrefaciens* and *S. algae* can be isolated from seawater with a salinity of 15–20%.[12][54]

Incidences of *Staphylococcus aureus* and its methicillin- resistant strains (MRSA) have risen worldwide, and thus increased understanding of the routes of human exposure. One approach to examining this question is to analyse beach sand and seawater. Mohammed et al. studied the survival of non-enteric pathogens in sand, investigating the factors affecting the survival and distribution of *S. aureus* and *P. aeruginosa*. Their results show that there is greater *S. aureus* and *P. aeruginosa* survival and proliferation in sterile beach sand than in seawater. Sand particles between 850 μm and 2 mm constituted the major micro-niche. A study in California detected *S. aureus* in samples of seawater (59%) and sand (53%). The significant explanatory variables for *S. aureus* in seawater were water temperature, or presence of enterococci in the seawater, and the number of swimmers.[12][81]

There is a clear impact of global warming on the incidence of *vibrios*. Ocean temperature rise increases the prevalence of cholera cases. In Germany, thirteen cases were reported between

1993 and 2013 [29]. In Sweden and Finland, 89 cases were described during summer 2014.[30]. Non-cholera *vibrios* are also concerned by this phenomenon, including at high latitudes[28][31], including lethal infections involving V. vulnificus.[32] A recent study showed the persistence of E. coli, enterococci and Bacteroidales HF183 in fresh and salt water for 3 to 5 days.[91]

Finally, from these case studies of human pathogen from marine environment represent the emerging public health risk. It needs more concern of doctors and researcher while diagnose a patient. These marine pathogens impose health risk to people who lives near beach or have an occupational relation with sea.

Chapter 5: Detection of pathogenic bacteria from marine environment

Positive identification of marine microorganisms at or above defined threshold concentrations is required for detection. For clinical reasons, detection is frequently adequate, but for environmental sample analysis, quantification of toxic populations is preferred. The approaches also range considerably in terms of implementation time and cost, thus the most precise method may not necessarily be the best option when making quick decisions.

The parameters that influence human exposure to the pathogen (e.g., availability, virulence/infectious dosage, way of exposure) and the method's restrictions (e.g., susceptibility, selectivity, dynamic range, expense) should be considered when selecting methods for monitoring and detecting pathogen populations. To identify and detect marine infections, scientists employ a variety of methods. Culture, immunology, and nucleic acid-based methods are the three primary types of methods used to identify and quantify microbial populations.

Table 2: Detection Methods of Pathogenic Bacteria

Name	**Sample**	**Method**	**Reference**
Campylobacter spp	marine recreational water	Culture based	[92],[86]
	Poultry	Modified RT-PCR followed by hybridization	
	Mussels and oysters	PCR and line-blot hybridization	
	Water, food	Real-time PCR	

	Food outbreaks—clinical isolated strains	PFGE (PulseNet)	
	Water, and waste water	Modified PCR	
Salmonella spp	Lagoon in brazil,	Culture based	[92],[86]
	Food (outbreak)	Real-time PCR	
	Waste samples	PCR following enrichment or directly from the sample	
	Water and Shellfish	PCR	
	Sewage polluted sea water	PCR	
	Water, and waste water	PCR	
	Harp Seals	ELISA	
Staphylococcus spp	Sea water	Culture based	[92],[86]
	Water/food outbreaks—clinical isolates	Colony hybridization (US-FDA established protocols)	
	Household waste sample	PCR following culture enrichment or directly from the sampl	
E. coli	North carolina and southern california coastal water	combined culture and PCR methods	[92],[86]
	Food	PCR following enrichment Colony hybridization following enrichment	
	Artificially contaminated food	Real-Time PCR (BAX rkit, Dupont)	
	Water	Real-Time PCR following enrichment with magnetic beads	
	Waste water	Multiplex PCR	
	Food outbreaks—clinical isolated strains	PFGE (Pulse net)	
	Wastewater wetlands	Real Time PCR	
Shigella spp	Algal mat sample	PCR	[92],[86]
	Sewage polluted seawater	PCR	
	Water	PCR	
Listeria monocytogenes	Food outbreaks—clinical isolated strains	PFGE/ PCR/ dot blot Hybridization	[86]
	Food	PCR ollowing culture enrichment	
	Household Waste sample	PCR following culture enrichment or directly from the sample	

Yersinia spp	Water/food outbreaks—clinical isolates	Colony hybridization/ Dot-blot hybridization	[86]
	Raw meat	Real time PCR	
	Household waste water samples	PCR following enrichment or directly from the sample	
	Water and waste water	PCR following culture enrichment	
Vibrio cholerae	Raw oyster	Real time PCR	[86]
	Waste water estuarine water	Multiplex PCR	
	sewage polluted sea	PCR	
	Seawater	PCR/Colony hybridization following enrichment	
	Seawater where v. cholerae is endemic (Bangladesh)	Immunofluorescence microscopy	
	Water/food outbreaks—clinical isolates	ELISA	
	Estuarine water	Agglutination following culture enrichment	
V. parahaemolyticus	Raw oysters	Colony hybridization following enrichment/ real time PCR following culture enrichment	[86]
	Sewage polluted water	PCR	
	Seawater	Most-probable-number PCR	
	Seafood	PCR with enrichment	
V. vulnificus	Seawater, sediment, fish	Hybridization following culture enrichment	[86]
	Oysters	PFGE	
	Eels	ELISA	
	water	Colony hybridization.	
Vibrio coralliilyticus	Coral reef	PCR	[93]

Chapter 6: Conclusion

This brief overview demonstrates that the pathogenic marine bacteria which are causing different diseases in human. In recent years, different changes of environmental factors give rise to different emerging pathogens in marine which has altered disease distribution in the world. Virulence of these waterborne pathogens varies greatly depending on environmental conditions and the type of pathogen. Moreover, transmission of waterborne infections to humans via seawater is a complex process depending on the type of pathogen, infectious dose, immune status of the human and a multitude of other factors. Certain microorganisms are developing chemical resistant increasingly. This produce the risks for sea users, whether professional sailors, recreational boaters or bathers. Doctors treating these people need to take into account the changes in microbial ecology for diagnoses and therapy orientation. This article will give physician a new idea of emerging disease from marine pathogens, improving diagnosis, and prevent marine diseases. Lastly, this field of study is still need more researches to develop, validated and standardized of rapid detection methods for the most relevant pathogenic bacteria in seawater and seafood. Otherwise, if the present condition is continued for some more years, emerging disease through marine will be a dangerous threat for public health.

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