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The occurrence of enteric bacteria in marine environment and pollution.

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ABSTRACT

The microbiological quality of the marine environment affects the microbiological quality of all fishery products. This study is a review of the occurence of enteric bacteria in freshly harvested fishery products dependent on the quality of marine environment from which these products are harvested. The control of marine environment from pollution as measured by the index of fecal coliform bacteria is necessary. Otherwise, our fishery products can be contaminated from the polluted marine environment. Environmental factors are predominant for the quality of seafoods.These factors consist of human and animal sources of enteric bacteria in the marine environment. Enteric bacteria in polluted marine environment can contaminate migratory fish species, mobile fish species as well as sedentary shellfish. Molluscs such as oysters, mussels, cockles, and clams filter and concentrate environmental microorganisms in high numbers in their tissues. These microorganisms may be active when the molluscs are eaten raw. Some enteric pathogenic bacteria can be found in polluted marine environment are *Clostridium* spp. such as *C. perfringens*, *C. botulinum*, *Salmonella* spp. such as *Salmonella typhi*, *Shigella* spp., *Vibrio* spp. such as *V. parahaemolyticus*, *Vibrio chlorea*, *Escherichia* spp. such as *E. coli*. These pathogens can also be found in live fish and shellfish. Furthermore, the microorganisms found on seafood may cause various illnesses and death as well. The safety of various seafood products varies according to the origin of the fish, microbiological ecology of the product, contamination level, handling, processing practices and preparations before consumption. Marine environment must be protected from the pollution to limit disease outbreaks of enteric pathogenic bacteria.

Introduction

Microbial pollution is a serious ecological and public health concern in marine coastal zones used for recreation, especially those located near highly polluted areas. It is believed that human activities can accelerate the rate of nutrient input into water ecosystems. Once discharged into the sea, surface runoff and insufficiently treated urban, municipal, domestic, and industrial wastes may encourage intense growth of microplankton or become sources of infectious microorganisms (Fleisher et al. 1993). Pathogenic bacteria can be discharged into the sea through submarine sewage outfalls, being a possible health risk to consumers of fishery products (Skanavis and Yanko, 2001). Bivalves are filter feeders; they may accumulate pathogenic microorganisms from waters impacted by sewage pollution (Ward and Hackney, 1991). Fish microflora is strongly affected by natural occurring factors, such as environment, human made factors such as farming, industries and sewage treatment plant that are in contact with marine habitats. The presence of fecal indicator bacteria in fish suggests that the pathogenic bacteria such as *Salmonella, Shigella*, enteropathogenic *E. coli*, *Pseudomonas aeruginosa*,

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Streptocoocus feacalis and others may be present and therefore can infect aquatic microorganisms including fish (Al-Bahry et al. 2009). International laws provide for studies on fecal coliforms and fecal streptococci in seawater in order to assess their quality and suitability for recreational use. The enteric commensal saprophytes are indicators of fecal contamination and, therefore, their presence of pathogenic intestinal microbes (Gabutti et al. 2000). Although, concentrations of enteric microorganisms in seas are often reduced as a result of dilution, the environmental factors, salinity and sunlight, some pathogens survive long enough in sufficiently high concentrations to cause infection and disease in bathers and to contaminate shellfish (Skanavis and Yanko, 2001). The presence of pathogens and indicator bacteria in the water samples show us a seasonal effect as they tend to be more abundant during the months with higher temperature (Holvoet et al. 2014). This review covers of indicator microorganisms whose presence indicates the pathogenic microorganisms that are routinely monitored for environmental pollution of seawater and fishery products.

Indicator microorganisms

Many coastal communities and regions are currently attempting to adress water quality problems associated with primarily non-point sources of pollution (Lipp et al. 2001). Sea water which is heavily polluted with untreated sewage effluents, municipal sewage disposal, and recreational activities, are contaminated with coliforms, *Vibrio* and *Pseudomonas*. Isolated bacteria are mostly pathogenic microorganisms including *Vibrio, Pseudomonas*, coliforms, *Salmonella* and *Shigella* (Santhiya et al. 2011).The fecal coliform group of bacteria has been used as indicators of water quality with respect to the presence of human pathogens (Abreu-Acosta and Vera, 2011). While several bacteria are currently used as indicator organisms for fecal contamination, the ideal indicator bacterium should be: 1) present in intestinal tracts of warm-blooded animals; 2) present when pathogens are there, and absent in uncontaminated samples; 3) present in greater numbers than the pathogen; 4) able to survive similarly to pathogens in the environment; 5) be unable to multiply in the environment; 6) detected and quantified by easy, rapid and inexpensive methods; and 7) non pathogenic (Ishii and Sadowsky, 2008).

The presence of indicator-microorganism in water is evident that the water is polluted with fecal material from humans or other warm-blooded animals. This kind of pollution means that any normal flora and pathogenic microorganisms that occur in the intestinal tract of these animals may also be found (Al-Bahry et al. 2009). The high prevalence of enteroviruses in seawater also suggests a chronic pollution problem and potential risk to humans (Lipp et al. 2001). The indicator- microorganisms are coliform bacteria that are divided into fecal coliform and nonfecal coliform. The fecal type is *Escherichia coli* which is normal flora found in the human intestinal tract and other warm-blooded animals. The nonfecal type includes *Enterobacter aerogenes* which is commonly distributed in nature and occasionally found in the intestinal tract of warm blooded organisms (Al-Bahry et al. 2009). The enterococci have been considered as better indicators than coliform bacteria, because their survival rate is somewhat higher and to be similar to that of viruses. However, *Esherichia coli* is more stable than that of enterococci, and its survival as culturable cells was similar to that of the polioviruses. Therefore it is concluded that *E.coli* is a better indicator of fecal pollution (Dan et al. 1997). Fecal streptococci that are facultative anaerobes are associated with the gastrointestinal tracts of humans and animals. The term enterococci associated almost exclusively with animal gastrointestinal tracts. Alternative indicators are reported as the obligate anaerobes, *Bifidobacterium, Bacteriodes* and *C. perfringens* (Ward and Hackney, 1991). Several indicator bacteria in wastewater, estuarine receiving water and its oysters has been examined for their ability to predict the presence and levels of faecal contamination and enteric viruses in oysters. *Clostridium perfringens* is an indicator significantly associated with the presence of enteric viruses in oysters (Chung et al. 1998). Curiel-Ayala (2012) reported that three microorganisms (*Enterococcus, Clostridium perfringens, Staphylococcus aureus*) were as part of a set of indicators in the routine microbiological evaluation. Hughes and Thompson (2004) described the distribution of sewage pollution markers (fecal coliform, *Clostridium perfringens*, fecal sterols) in seawater and marine sediments. In sediment cores, both *C. perfringens* and fecal coliform concentrations decline with the distance from the outfall, through *C. perfringens* persists at greater depths in the sediment. Sewage contamination is limited to the immediate vicinity of the sewage outfall. Nevertheless, a sewage treatment plant can be installed to reduce this contamination further.

Bacterial pathogens in marine environment

Bacterial pathogens are a leading cause of gastroenteritis world wide. Some bacterial pathogens in raw sludge and marine environment have been presented as total coliforms, faecal coliforms, *Enterococci*, faecal streptococci, *Escherichia coli, Salmonella, Clostridium perfringens, Campylobacter jejuni, Listeria monocytogenes, Yersinia enterocolitica, Vibrio Aeromonas* etc. (Sidhu and Toze, 2009).

In recent years the incidence of vibriosis has greatly increased, raising the concern among consumers about the innocuity of certain food products (Garrido-Maestu et al. 2014). The genus *Vibrio* consists of more than 70 species that are mostly in aquatic environments and inhabit marine animals. Thirteen diffrent species have been associated with human pathogenesis, however, the majority of human *Vibrio* infections are associated with three species: *Vibrio chlorea, Vibrio parahaemolyticus* and *Vibrio vulnificus* (Neogi et al. 2010). The *Vibrio* group includes causative agent of *Vibrio chlorea* and other human pathogenic species like *Vibrio vulnificus* and *Vibrio parahaemolyticus* but also other non-harmful species. *Vibrio chlorea* can pose a public health risk when it is ingested via untreated water, contaminated seafood (raw or under cooked or exposure of skin wounds to seawater (Whittman and Flick, 1996; Chavez et al. 2005). *Vibrio alginolyticus* has been categorized as a human pathogen since 1979 and it has been shown to result in endophthalmitis, otitis media and food poisoning in infected patients. Therefore it is important to identify these 4 Vibrio species for risk assessment (Wei et al. 2014). The experiments illustrated how disinfection of seawater, most probably by generating more easyly degradable substrates and removing predators and competitors, can alter the growth potential with regard to rapid multiplying heterotrophic bacteria, including *Vibrio parahaemolyticus* and *Vibrio chlorea* (Wennberg et al. 2013). The role of zooplankton in the seasonal distrubition of *Vibrio parahaemolyticus* and the significance of bacterial indicators of pollution in the incidence of this halophile were examined. The highest counts of this bacterium occured in autumn. *Vibrio parahaemolyticus* was distributed homogeneously in the seawater and was found to be isolated with plankton samples (Venkateswaran et al. 1989). Several *Vibrio* species known to be pathogenic for humans have been isolated from bivalves. These bacteria are a part of the natural estuarine microflora and may be accumulated by shellfish during feeding. A number of deaths caused by *V. vulnificus* have been linked to the consumption of raw oysters (Ward and Hackney, 1991).

Clostridium perfringens is a microbiological marker for fecal contamination, as it is of intestinal origin (Curiel-Ayala, 2012). *C. perfringens* as an anaerobic bacteria never occured in the surface waters in vegetative or spore forms, even if the waters were extremely polluted by domestic or industrial activities. Vegetative forms occured only in the bottom samples but spore forms which are more resistant to various environmental effects occured in all depths except for the surface (Bezirtzoglou et al. 1997). Spores of *C. perfringens* survive longer than the coliforms. High numbers of *C. perfringens* were detected in marine sediments near the sewage outfall, areas with high *C. perfringens* levels were associated with known sewage sludge dumping. *C. perfringens* may be a suitable indicator for sewage solids in marine sediments (Skanavis and Yanko, 2001).

Staphylococcus aures has been regarded as an effective indicator of human pollution in order to define the quality of seawater. The presence of *S. aureus* in marine coastal environments has been related to the number of bathers and may be considered as a risk indicator for skin, eye and ear diseases (Charoenca and Fujioka, 1995; Gabutti et al. 2000).

Salmonella are considered typical intestinal organisms, being able to colonize the gastrointestinal tract of animals and humans. Even though *Salmonella* is not a spore-former heat treated products may be contaminated by this bacterium (Jones and Richardson, 2004). *Salmonella* is one of the most important food borne zoonotic pathogens. These bacteria are virtually absent in food producing animals and domestically produced food, including products from fish. Some *Salmonella* species can be found in animal feed and its ingredients, as well as in the feed production facilities. Thus fish feed are shown to occasionally harbour *Salmonella* originating from ingredients or the processing environment (Lunestad et al. 2007). *Salmonella enterica* is the leading cause of foodborne illness in the United States and raw shellfish consumption is a commonly implicated source of gastrointestinal pathogens (Morrison et al. 2011).

Escherichia coli has been widely used as an indicator of fecal contamination since it is a part of the common intestinal flora of endothermic animals and is easy to cultivate (Scott et al. 2002). *E. coli* species has very low tolerance to seawater, however their number is high in the tested samples because of constant discharge of seawage into the sea (Vieira et al. 1998). In order to use *E.coli* as an indicator bacterium in seawater knowledge of survival must be acquired as it is not a halophilic microorganisms. Loss rate of indicator microorganisms pose an interesting problem especially in coastal waters as many factors affect the survival times of *E. coli* such as temperature, light, salinity, predation,nutrients and pollutants (Sinton et al. 2002). Enterohaemorrhagic *E. coli* O157: H7 can colonize the gastrointestinal track of mammals. Prolonged survival for more than 6 months can be expected in winter (Avery et al. 2004).

Thermophilic *Campylobacter* are widespread in the environment and are commonly found in surface water and sewage sludge. *Listeria* is present in raw and treated sewage sludge. It is also found in water, soils, feces of healty indivudials, fish, processed seafoods (Kilinc, 2001; Sahlstrom et al. 2004).

Yersinia enterocolitica are being isolated from soil, water, animals and variety of foods. *Yersinia* spp. that cause human yersiniosis (Bercovier et al. 1980). This bacterium is associated with gastroenteritis in humans and known to be present in sewage sludge (Sahlstrom et al. 2004). This suggests that *Y. enterocolitica* can potentially survive well in fish at low temperatures. The association of human illness with consumption of *Y. enterocolitica*-contaminated food, animal wastes, and unchlorinated water is well documented (Aulisio et al. 1982).

Shigella is one of the major causes of reported gastric illnesses worlwide and is regulary found in sewage. Humans are the main sources of *S. sonnei* which is reported to be the predominant cause of shigellosis outbreaks (Sahlstrom et al. 2004).

Control of bacterial pathogens

Fecal wastes enter water bodies by direct discharge, through surface run-off and/or seepage. However, managing and reducing fecal pollution levels in water are challenging because there are many host sources that are difficult to identify (Fu et al. 2011). The ecological and survival characteristics of bacterial pathogens vary under different environmental conditions, indicating that probably no single indicator organisms can predict the presence of all enteric pathogens for all types of water and different host associated fecal pollution. Application of conventional and alternative fecal indicators such as fecal anaerobes (genera *Bacteriodes* and *Bifidobacterium*) has greatly enhanced our abilities to predict and reduce the health risks associated with waters (Savichtcheva and Okabe, 2006). If pathogens are discharged into bodies of water, mortality rates are affected by many physical and biological factors, including nutrients, sunlight, temperature, salinity, predators, toxicity, settling/scouring, and aftergrowth (Kim and Hur, 2010). Many of the directives that relate to the prevention of pollution or the improvement of fresh water also relate to lake waters since lake water ultimately inherit much of the pollution that enter into fresh water (Bezirtzoglou et al. 1997).

Bivalves can be cleansed of most microbiological contaminants by placing them in water that is free of the microorganisms to be removed and under conditions where the shellfish will actively feed. This process is known as relaying and requires 15 days for the bivalves to cleanse, provided the water temperature remains above 10˚C and salinity remains at an acceptable level for the species (Ward and Hackney, 1991).

For prevention of water- borne diseases, regular monitoring of coastal waters is strongly recomended, including testing for total and fecal coliforms and enterococci (Wheeler et al. 2002). It is recomended that quantitative sanitary profiling of shellfish waters and cost-benefit appraisal over long- term planning horizons are considered as part of sewage investment programmes under the Water Framework Directive. This would allow greater scope to secure protection and improvement of shellfish water quality (Campos et al. 2013).

Conclusions

The safety of various seafood products varies according to the origin of the fish, microbiological ecology of the product, contamination level, handling, processing practices and preparations before consumption. Marine environment must be protected from the pollution to limit the disease outbreaks of enteric pathogenic bacteria.

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