REVIEW ARTICLE

Major foodborne pathogens in fish and fish products: a review

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Abstract Fish plays an important role in the human diet, and there is an observed increase in the consumption of fish per capita in Europe. However, intensive growth of industry and agriculture may cause contamination of natural and humanmade aquatic environments, and may affect not only the health of fish, but also raise safety concerns with regard to fish used for human consumption. It is well known that fish and fish products are often associated with human diseases. Thus, it is necessary to study the prevalence of pathogens in fish to ensure the safety of fish products and environments. Microbial assessment of fish also gives additional information about the hygienic status of environments, including lakes, rivers, ponds, and fish farms. Detection of pathogenic microorganisms or changes in natural microflora in the water environment could be an important indicator of possible contamination. The aim of this review was to describe and discuss the five most relevant bacterial genera and species linked to aquatic environments—Vibrio spp., Listeria monocytogenes, Yersinia spp., pathogenic Salmonella serovars, and Clostridium botulinum—causing human foodborne diseases.

Keywords Foodborne pathogens · Prevalence · Fish · Aquatic environment

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Introduction

Fish plays an important role in the human diet. According to EUMOFA (2014), the consumption of fish per capita in Europe reached up to 24.5 kg in 2011. The consumption of fish and fish products in general has increased in Europe during recent years, while the Central and Eastern European countries are below the EU average in fish consumption (EUMOFA 2014). Fish and fish products are often associated with human disease, especially when raw or undercooked fish and fish products are consumed. The presence of different bacteria species including human pathogenic bacteria in fish can be linked to direct contact with a contaminated water environment and ingestion of bacteria from sediments or contaminated feed. Thus, bacteria detected in fish reflect the condition and safety of aquatic environments.

Some of the bacteria species can cause serious diseases in fish. An important example is *Yersinia ruckeri*, the causative agent of enteric red mouth disease (ERM) in rainbow trout, resulting in heavy commercial loses (Furones et al. 1993). However, from the point of public health, the types of bacteria transmitted through fish that can cause human diseases are important. The presence of human pathogenic microorganisms in fish and fish products may be affected by various factors, including cultural practices, environmental conditions, processing, and distribution of products. The most important fish pathogens can be generally divided into two groups: those native to natural freshwater habitats and those associated with water pollution. The bacterial species described in this paper, Vibrio spp., Listeria monocytogenes, Salmonella serovars, Clostridium botulinum, and Yersinia spp., represent both groups of bacteria mentioned above – native freshwater habitats and contaminants arising from different sources, including sewage and direct contamination by wild animals, livestock, and feed (Bottone et al. 2005;



Adgamov et al. 2013). In particular, the human pathogenic *Listeria monocytogenes* and *Yersinia* spp. were also identified as natural microflora of aquatic systems due to the ability to survive outside host organisms for a long time (Adgamov et al. 2013).

It is necessary to study the prevalence of pathogens in fish to ensure a better understanding of ecology and distribution of pathogens in the food chain. Limited studies on pathogens in freshwater fish have been conducted, and those studies mostly covered one or two pathogens over limited geographical areas. However, collection and systematization of the previous reported studies can be useful to understand better the epidemiology of certain foodborne pathogens over a geographical region. The aim of this review was to describe and discuss the aspects of epidemiology of the five most relevant bacterial genera and species (*Vibrio* spp., *Listeria monocytogenes*, *Yersinia* spp., *Salmonella* serovars, and *Clostridium botulinum*) causing human diseases through the consumption of contaminated fish and fish products.

Microflora of fish and fish environments

Fish from natural environments are known to harbour various bacterial species (Pillay 1990). Bacterial colonization can be observed on fish skin and gills due to constant exposure to contaminated water, while the digestive tract may be affected through contaminated feed or water. Contamination of fish muscles is also possible when immunological resistance is compromised (Guzman et al. 2004). Generally, a small number of microorganisms can be found on fish skin. As an example, studies from the UK reported that total bacteria count (TBC) on the skin of salmon (Salmo salar) varied from 10² to 10³ CFU/cm² (Horsley 1973). Meanwhile, a similar study carried out in Turkey revealed a higher number of 10¹–10⁷ CFU/cm² on salmon skin (Diler et al. 2000), and aerobic microorganisms were detected more often than anaerobic (Nedoluha and Westhoff 1997). It is generally accepted that bacteria found on fish skin are the same as those found in the contaminated water, including such genera and species as Aeromonas spp. (Aeromonas hydrophila, A. bestiarum, A. caviae, A. jandaei, A. schubertii, A. veronii), Flexibacter spp., Proteus spp., Providencia spp., Psychrobacter spp., Moraxella spp., Pseudomona fluorescens, Acinetobacter johnsonii, Alcaligenes piechaudii, Enterobacter aerogenes, Escherichia coli, Micrococcus luteus, and Vibrio fluvialis (Christensen 1977; Allen et al. 1983; Youssef et al. 1992; Diler et al. 2000; Gonzalez et al. 2000, 2001; Zmyslowska et al. 2001).

Usually, the muscles and internal organs of healthy fish are sterile. However, some studies reported the presence of bacteria (*Pseudomonas* spp. and *Vibrio* spp., including *V. fischeri*, *V. harveyi*, *V. pelagius*, *V. splendidus*) in the liver and kidneys of turbot (*Scophthalmus maximus*) (Evelyn and McDermott 1961; Toranzo et al. 1993; Apun et al. 1999). The highest

bacterial loads were observed in the gills and digestive tract of fish and can reach 10⁶ CFU/g and 10⁸ CFU/g, respectively (Trust and Sparrow 1974; Trust 1975; Campbell and Buswell 1983; Kamei et al. 1985).

Various factors including the season, part of the digestive tract of fish, and feeding type can affect the number of microorganisms detected. The minimum and maximum findings for specific bacteria were related to the changes of water temperature and were observed during winter and summer seasons (Diler and Diler 1998). Differences in the numbers of bacteria depending on the part of the digestive tract in fish and the aerobic bacteria count ranged from 5.5×10^3 to 5.0×10^4 CFU/g, and from 1.0×10^4 to 10×10^6 CFU/g in the stomach and intestines, respectively (Diler and Diler 1998). It has been observed that the number of microorganisms in the digestive tract depends on the type of fish feed, and the higher bacterial population was in detritus eaters than those in filter-feeding water (Balasubramanian et al. 1992).

Along with human non-pathogenic bacteria species and natural microflora of aquatic environments, pathogenic bacteria are also widely found in fish. According to the European Food Safety Authority, pathogens such as Campylobacter, Salmonella, Yersinia, E. coli, and Listeria monocytogenes are responsible for major foodborne outbreaks worldwide (EFSA and ECDC 2015). However, not all pathogens are associated with foodborne outbreaks through the consumption of contaminated fish and fish products. Meanwhile, some bacteria species, including L. monocytogenes, Vibrio spp., Salmonella, Yersinia spp., and C. botulinum, are of special interest. The emergence of these pathogens is described with a wide distribution in aquatic environments and also with high mortality rates in humans through resulting diseases such as listeriosis, botulism, and infection caused by V. vulnificus (Lindström et al. 2006; Lianou and Sofos 2007; Callol et al. 2015). Thus, along with nutritional benefits from the consumption of fish, the potential risk to human health exists.

Vibrio spp. in water environments and fish

Vibrio spp. are widely distributed in fish and fish environments. Various Vibrio spp. may cause serious disease both in wild and cultured fish, and the fish pathogenic species include V. ordalii (septicaemia in salmonids), V. anguillarum ("red pest" in eels), V. salmonicida (cold water vibriosis in fishes), V. vulnificus (warm water vibriosis in the European eel), V. viscous and V. wodanis ("winter ulcer disease" in Atlantic salmon) (Gauthier 2015; Callol et al. 2015).

Among *Vibrio* spp., *V. cholerae*, *V. vulnificus*, and *V. parahemolyticus* have been implicated in human vibrioses associated with the consumption of fish and shellfish. *Vibrio cholerae*, the etiologic agent of cholera, is autochthonous to various aquatic environments, but despite intensive efforts, its ecology and transmission via contaminated fish remains



unclear (Senderovich et al. 2010). Limited studies exist about the role of fish in V. cholerae caused disease; however, contamination of fish as high as 50 % was reported (Senderovich et al. 2010). More often V. vulnificus and V. parahemolyticus are associated with human vibriosis, and disease usually occurs due to the ingestion of insufficiently heat-treated fish or fish products (Iwamoto et al. 2010; Gauthier 2015; Callol et al. 2015). These bacteria may cause gastroenteritis and septicemia (primary) in humans and are of particular concern because of a high probability of death in immunocompromised patients (Gauthier 2015; Callol et al. 2015). Three biotypes (biotypes 1, 2, and 3) of V. vulnificus have been described of which biotype 1 has been isolated more frequently from water and humans. Meanwhile, biotype 2 was more often isolated from fish and humans (Gauthier 2015). V. vulnificus biotype 3 has not been associated with the consumption of seafood despite the observed linkage of bacteria isolates from human and fish tested with variable tandem repeat (VNTR) and multilocus sequence typing (MLST) methods (Broza et al. 2009; Mahmud et al. 2010). V. parahaemolyticus can be classified according to serotype, however, classifications based on the presence of particular genes have been made: V. parahaemolyticus strains are considered "pathogenic" if the thermostable direct hemolysin (tdh) and/or TDH-related hemolysin (trh) genes are present (Drake et al. 2007).

Vibrios are abundant in aquatic environments, and these bacteria were also observed on the skin, gills, and the intestinal tracts of fish or shellfish. The higher number of V. vulnificus and V. parahaemolyticus was described in fish intestines in comparison to water and sediment samples (Givens et al. 2014). Other factors such as water salinity and temperature may affect the prevalence of Vibrio spp. in fish and aquatic environments. Bacteria were more frequently found in warm and water with a lower salinity (Huehn et al. 2014). The prevalence of V. vulnificus was 37 % after testing of 242 fish samples comprising 28 fish species in a study carried out in Mexico. Moreover, the increase in prevalence of *V. vulnificus* up to 69 % was observed during summer period (Tao et al. 2012). Authors also described that the genetic diversity of bacteria strains studied with the amplified fragment length polymorphism (AFLP) method showed high genetic similarity and a Simpson's index of diversity of 0.991 (Tao et al. 2012). V. parahaemolyticus is often associated with the molluscan shellfish; however, a high prevalence of these bacteria is also observed in fish, and the pathogen was detected in more than 50 % of tested fish samples in Vietnam, Malaysia, and Indonesia (Nakaguchi 2013). Vibrios most frequently are found in marine fish; however, these pathogens are also observed in freshwater fish. As an example, 24 % of catfish and 40 % of red tilapia samples were contaminated with V. parahaemolyticus in a study from Malaysia (Noorlis et al. 2011).

In summary, *Vibrio* spp. are widely distributed in aquatic environments and are found both in marine and freshwater fish. These bacteria can contaminate fish and fish products

during improper handling, long-time transport, evisceration, and also cross-contamination from raw materials. *Vibrio* spp. can proliferate in food, and the level of bacteria in the final product may increase to such an extent that may present a health risk to consumers.

Aspects of epidemiology of Listeria spp. in fish and water environments

Listeria monocytogenes is the causative agent of listeriosis—a foodborne infection in humans. Despite its low incidence, the mortality rate in those who are susceptible, including immunocompromised individuals, may reach 20-40 % (Lianou and Sofos 2007). L. monocytogenes is ubiquitous in the environment and has been isolated from soil, silage, animal faeces, from fresh and marine waters, as well as from sediments. Listeria species can be found in different types of water sources, and these microorganisms were often isolated from polluted waters and from waters with high amounts of organic material, such as rivers and coastal areas (Embarek 1994). The prevalence of L. monocytogenes (6.6 %) in freshwater fish faeces was explained by contamination of the lake environment by the city sewage system (Ertas and Seker 2005). It is important to emphasize that the prevalence of L. monocytogenes correlates with the degree of human activity. The pathogen was not observed in freshwater streams, but was present in seawater fish farms (2 %), freshwater fish farms (10 %), in fish slaughterhouses (16 %), and in fish smokehouses (68 %) (Hansen et al. 2006). Recent studies described new Listeria species, such as L. floridensis, L. aquatica, L. cornellensis, L. riparia, L. grandensis (Den Bakker et al. 2014), L. weihenstephanensis (Lang Halter et al. 2013), and L. marthii (Graves et al. 2009) in environmental samples. The prevalence and virulence potential of these bacterial species in fish remains unclear, but their presence in water environments might serve as an indicator of possible contamination with L. monocytogenes (Wagner and Mc Lauchlin 2008).

The persistence of L. monocytogenes and other Listeria spp. in the environment, including water environments, depends on various factors. One of these factors is the ability of Listeria spp. to survive and multiply at very low temperatures. L. monocytogenes had the longest survival time both in water and sewage at 4 °C, where the maximum survival time of those microorganisms was from 120 to 141 days (Budzińska et al. 2012). Another important factor related to the survival of *Listeria* spp. is the ability of these microorganisms to form biofilms. Biofilms can be broadly defined as extracellular polymeric matrix-enclosed bacterial populations, adherent to each other and/or to surfaces or interfaces. Subsequently, in biofilms, bacteria are believed to be protected from various environmental stresses and have been shown to be less sensitive to antibiotics and disinfectants than planktonic bacteria (Costeron et al. 1995). Listeria can also



attach and develop biofilms on the indigenous zooplankton in ground water, making the removal of bacteria from zooplankton almost impossible (Koonse 2005).

Listeria monocytogenes has been found both in aquatic environments and in fish, and consequently, in various processed and unprocessed fish products, including frozen seafood, cold- and hot-smoked salmon, marinated fish, fermented fish, and fish salads (González-Rodríguez et al. 2002; Papadopoulos et al. 2010; Tocmo et al. 2014). Dhanashree et al. (2003) found that among different types of food samples, including milk, meat, and vegetables, only seafood was contaminated with L. monocytogenes (2/210, 0.95 %), indicating that seafood may pose a health risk to consumers. We should mention that L. monocytogenes often has been isolated from salt water fish and seafood since 1987 (Embarek 1994). The incidence of this bacteria varied from 4 to 12 % in studies conducted in the temperate climatic zone, whereas studies in the tropical zone revealed a lower prevalence of *Listeria* genus (0-2 %) (Embarek 1994). L. monocytogenes was detected in both saltwater and freshwater fish samples, and the prevalence of pathogen is shown in Table 1. It is interesting that seafood farms were described with potentially greater risk of Listeria contamination than inland fish farms due to contaminated surface waters entering such farms after heavy rainfall (Miettinen and Wirtanen 2005). Additionally, environmental conditions such as rainfall affecting the levels of Listeria in water were described by Thomas et al. (2012).

A low number of listeriosis outbreaks have been linked to the consumption of fish and fish products in comparison to other foods (EFSA and ECDC 2015). However, phenotypic and genetic characterization through subtyping analysis indicates fish as an important source of infection (Jami et al. 2014). L. monocytogenes is divided into at least 13 serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7), of which certain serotypes (1/2a, 1/2b, and 4b) are involved in the majority of human listeriosis cases (Liu 2006; Jami et al. 2014). These serotypes are also frequently found in fish. Johansson et al. (1999) reported that 86 % of the L. monocytogenes strains isolated from smoked fish in Finland belonged to serotype 1/2a and 14 % of the isolates to serotype 4b. L. monocytogenes serotype 1/2a was predominant (>95 %) in bacteria detected in smoked salmon in Ireland (Corcoran et al. 2006). Momtaz and Yadollahi (2013) described that L. monocytogenes serotype 4b was most frequently (66.66 %) detected in fresh fish samples. Other L. monocytogenes serotypes 1/2a and 1/2b were detected in 5.55 and 27.77 % of bacterial isolates, respectively. Genetic diversity of L. monocytogenes was described in a number of studies (Jami et al. 2014). Genetic characterisation methods such as pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) revealed the distribution of identical L. monocytogenes genotypes and sequence types (ST) in various foods including fish and infected humans (Markkula et al. 2005; Wang et al. 2012).

Listeria monocytogenes could be introduced into fish products during processing of raw meat and meat products. Raw fish material could also be an important source of contamination at processing facilities, resulting in subsequent contamination of products (Miettinen and Wirtanen 2005). Evisceration and scalding of the fish before marketing can contribute to the introduction of L. monocytogenes into the surroundings, leading to crosscontamination of fish, utensils, personnel, and environment (Papadopoulos et al. 2010). Duffes (1999) proved that L. monocytogenes could be transferred from the flesh to cut surfaces, equipment, and tables during salmon filleting, resulting in a potential source of contamination. Also, the chilling of fresh catfish fillets may represent the primary contamination risk (Chen et al. 2010). Despite the high prevalence of L. monocytogenes (23/30, 76.7 %) in the final product—catfish fillet, catfish skin, and intestines outside the environment and catfish processing facilities—the water and surface of the water holding tank were L. monocytogenes negative, indicating that live catfish was not the true source of Listeria contamination (Chen et al. 2010). Miettinen and Wirtanen (2005) isolated L. monocytogenes with a prevalence of 14.6 % from pooled unprocessed fresh rainbow trout samples. Moreover, authors observed that the prevalence of L. monocytogenes in rainbow trout samples varied depending on the sampling site. L. monocytogenes mostly was isolated from gills (43/510, 8.4 %), and only occasionally this pathogen was isolated from skin (1/510, 0.1 %) or from viscera (1/510, 0.1 %). Gills are an excellent tissue for bacterial growth because gills filter large amounts of contaminated water throughout the lifetime of fish (Miettinen and Wirtanen 2005). Effective cleaning and sanitation programs were suggested as important measures to avoid the contamination with L. monocytogenes on the surfaces and equipment in processing facilities, despite the high prevalence of this pathogen in fish and water (Miettinen and Wirtanen 2005).

In short conclusion, the prevalence of *L. monocytogenes* in aquatic environments correlates with the degree of human activity. High prevalence of *L. monocytogenes* together with the ability of these bacteria to survive in the environment indicate that fish can be a source of human listeriosis and potentially pose a public health concern. Therefore, the contamination of raw products could be an important factor, which contributes to the risks of broader contamination with *L. monocytogenes*, especially if products are consumed without prior thermal treatment.

Aspects of epidemiology of Salmonella in fish, fish products, and water environments

Salmonella is the second most common cause of human gastroenteritis (EFSA and ECDC 2015). Salmonella is a



Table 1 Prevalence of Listeria monocytogenes in various fish species

Fish species	Country/year	Sampling place	No. of tested samples/ No. of positive samples (%)	Reference
Whiting fish (Merlangius merlangus) European plaice (Pleuronectes platessa)	France/n.s.* Great Britain/n.s.	Commercial outlets	26/0 (0) 5/0 (0)	Davies et al. 2001
Atlantic salmon (Salmo salar)	Great Britain/n.s. Denmark/1998–1999	Norway and the Faroe Islands	5/0 (0) 185/16 (8.6)	Fonnesbech Vogel et al. 2001
Salmonidae	USA/1998	Smoked fish processors	102/8 (7.8)	Norton et al. 2001
Rainbow trout (Oncorhynchus mykiss)	Great Britain/n.s.	Commercial outlets	20/2 (10)	Davies et al. 2001
` ' '	Greece/n.s.	Retail outlets	71/0 (0)	Papadopoulos et al. 2010
	Portugal/n.s.	Commercial outlets	10/0 (0)	Davies et al. 2001
	Finland/2000	Fish farms in lakes and sea areas	103/15 (14.6)	Miettinen and Wirtanen 2005
European pilchard (Sardina pilchardus)	Portugal/n.s.	Commercial outlets	10/0 (0)	Davies et al. 2001
Catfish (Siluriformes)	USA/n.s.	n.s.	30/0 (0)	Chen et al. 2010
Gibel carp (Carassius gibelio)	Greece/n.s.	Retail outlets	65/0 (0)	Papadopoulos et al. 2010
Silver Carp (Hypophthalmichthys molitrix)	Iran/n.s.	Warm-water fish ponds in Guilan province	42/2 (4.76)	Razavilar et al. 2012
		Fish farm, freshly caught	39/1 (2.6)	Basti et al. 2006
Sardine (Sardina pilchardus) Croakers (Sciaenidae)	India/1997–2001	Retail outlets	15/0 (0) 11/0 (0)	Dhanashree et al. 2003
Mackerel (Scombridae)			14/0 (0)	
Pomfret (Bramidae)			12/0 (0)	
Flat fish (Pleuronectiformes)			35/1 (2.9)	
Caspian anadromous shad (Alosa kessleri)	Iran/n.s.	Caspian sea near the coast, freshly caught	28/0 (0)	Basti et al. 2006
Mullet dore (Liza aurata)	Iran/n.s.	Retail outlets	40/0 (0)	
Butter catfish (Ompok bimaculatus) Tengan (Aristichithys nobilis)	India/2007–2008	Retail outlets	7/0 (0) 7/0 (0)	Kakatkar et al. 2010
Hilsa (Tenulosa ilisha)			7/0 (0)	
Mangur (Clarias batrachus)			7/0 (0)	
Catla (Catla catla)			7/0 (0)	
Rohu (Labeorohita)			7/0 (0)	

^{*-} n.s. none specified

mesophylic organism and not a natural inhabitant of the aquatic environment. The presence of these microorganisms in aquaculture environments and products can be explained mainly by hygiene failures during production (Li et al. 2009; Budiati et al. 2013).

Salmonella was isolated from a variety of seafood, including fish, shrimp, clams, mussels, oysters, crabs, lobsters, squid, cuttlefish, and octopus (Kumar et al. 2009). The prevalence of Salmonella depends on the type of seafood, with the highest prevalence reported in molluscs, shrimp, clams, and various fish species. The reason for high prevalence of Salmonella in filter-feeding organisms is filtration of a large amount of water during their life cycle with accumulation of the pathogen in tissues (Kumar et al. 2009). Although environmental factors and human activity may influence the

prevalence of *Salmonella* in seafood, contamination of seafood may often occur from contaminated coastal areas and from contaminated surroundings where seafood was handled (Martinez-Urtaza et al. 2004).

Salmonella can be divided into more than 2,500 serovars (Agbaje et al. 2011). However, only certain serovars are described as dominant in fish and water environments. Various *Salmonella enterica* serovars, including *S. enterica* serovar Bareilly, ser. Braenderup, ser. Derby, ser. Irumu, ser. Georgia, ser. Lindenburg, ser. Nchanga, ser. Newport, ser. Ohio, ser. Othmarschen, ser. Rissen, ser. Riggil, ser. Takoradi, ser. Typhi, ser. Typhimurium, ser. Washington, ser. Weltevreden, and ser. Worthington, were detected in fish, shrimp, clams, mussels, oysters, crabs, lobsters, squid, cuttlefish, and octopus in India (Shabarinath et al. 2007; Kumar



et al. 2009). S. enterica serovar Weltevreden, ser. Rissen, ser. Typhimurium, and ser. Derby were confirmed as dominant in seafood samples. The presence of ser. Weltevreden was confirmed in fish, shrimp, crabs, and mussels, but not in other seafood (Kumar et al. 2009). This serovar has been reported as a frequent and increasing cause of human infection and is the predominant serovar in Malaysia, Thailand, and Vietnam (Ponce et al. 2008). Additionally, ser. Weltevreden was the most frequent serovar in imported seafood samples in the USA analysed by the Food Drug Administration (FDA) (Heinitz et al. 2000). This serovar was the predominant among 208 isolates from 5000 imported foods entering the USA in 2001 (Ponce et al. 2008). Other Salmonella including S. enterica serovar Typhimurium, ser. Enteritidis, ser. Typhi, ser. Paratyphi B, and ser. Newport were also mentioned as common serotypes isolated from seafood. S. enterica serovar Typhimurium and ser. Enteritidis were the predominate serotypes in human cases, whereas ser. Paratyphi B and ser. Typhi were found as a result of contamination during manual handling or sampling (Rahimi et al. 2013).

In a study by Hatha and Lakshmanaperumalsamy (1997), 14 out of 18 analysed fish samples were Salmonella positive. The highest prevalence was observed in the samples of Mugilidae 21/ 86 (24.4 %), Scopelidae 7/25 (28 %), and Trachnidae 7/26 (26.9 %) genera. The authors of the study proposed that the high lipid content in these types of fish may favour the growth of Salmonella. Seasonal variation may also increase or decrease the prevalence of Salmonella in fish. The prevalence of Salmonella in fish was significantly higher in the monsoon season (26.1 %) than in pre-monsoon and post-monsoon seasons (6.4 and 7.1 %, respectively). The lower temperatures, as well as the increased sewage and drainage inflow during the monsoon month stimulated the prevalence of Salmonella (Hatha and Lakshmanaperumalsamy 1997). The localization of Salmonella in fish may differ, and pathogens most frequently were isolated from the alimentary tract (64/150, 41.3 %), compared to other parts of fish—skin (48/150, 32 %) and gills (20/150, 26.7 %) (Hatha and Lakshmanaperumalsamy 1997). Youssef et al. (1992) also reported extended survival of Salmonella in the alimentary tract of catfish.

According to FAO (2010), *S. enterica* serovar Albany, ser. Agona, ser. Corvallis, ser. Stanley, ser. Bovismorbificans, and ser. Typhimurium were present in fish, fishery products, and aquaculture environments. Budiati et al. (2013) described the presence of *S. enterica* serovar Albany, ser. Agona, and ser. Stanley in catfish fed offal and ser. Corvallis in tilapia fed spoiled eggs. The presence of *S. enterica* serovar Albany, ser. Agona, and ser. Stanley in poultry and eggs has been also reported by Otomo et al. (2007) and Modarressi and Thong (2010). The feeding practice of fish can contribute to the prevalence of *Salmonella* in fish, and fish fed chicken eggs

and chicken offal exhibited significantly higher prevalence than those fed commercial feed (Budiati et al. 2013). The presence of *Salmonella* was also recognized in fish feed in Norway, which consisted of two main ingredients—fish meal and fish oil (Lunestad et al. 2007).

Salmonella can be isolated not only from seafood, but also from freshwater and freshwater fish. This can be explained by contamination of a water source, and poor hygiene during the capture, handling, and transportation of fish. Freshwater sources can be affected by stream water and groundwater, which was found to be contaminated with Salmonella that could be transmitted to ponds (Li et al. 2009; Budiati et al. 2013). Salmonella also can contaminate water sources because of poor sanitation and incorrect disposal of human and animal waste (Amagliani et al. 2012). Moreover, Salmonella can survive for 10 to 15 days in septic tank systems (Parker and Mee 1982) and also shows high survival rates in aquatic environments: up to 54 days in water and up to 119 days in sediment samples (Chao et al. 1987; Moore et al. 2003). The ability of Salmonella to form biofilms may also increase its survival in the environment, including freshwater reservoirs (Lapidot et al. 2006). Some studies report that Salmonella in biofilms is less sensitive to antimicrobial agents and disinfectants than planktonic bacteria (Janssens et al. 2008; Møretrø et al. 2009). Salmonella can persist for several months in biofilm during nutrient depletion as well as prolonged desiccation (White et al. 2006; Vestby et al. 2009).

The prevalence of *Salmonella* has been mostly studied in countries with a tropical climate, and only few studies are available for temperate climates. Summary of the studies on the prevalence of *Salmonella* in fish and certain types of seafood is presented in Table 2 and Table 3, respectively.

Genetic diversity of *Salmonella* serovars was described in a number of studies, and molecular methods such as random amplified polymorphic DNA (RAPD), repetetive sequence-based PCR (REP-PCR), enterobacterial repetitive intergenic consensus sequence-based PCR (ERIC-PCR), and PFGE have been implied for characterization of the *Salmonella* strains (Shabarinath et al. 2007; Ponce et al. 2008; Albufera et al. 2009). Identical or closely related *Salmonella* types from different foods including meat, poultry, and fish were observed, and despite the fact that data focusing on *Salmonella* genetic diversity with linkage to human salmoneliosis is limited, fish and fish products remain as important sources of *Salmonella* infection.

In conclusion, presence of *Salmonella* in fish is affected by various factors and both live and caught fish may be affected. The contamination of water and feeding practices with inappropriate and contaminated feed may influence the prevalence in live fish, while poor hygiene during the capture, handling, and transportation of fish may result in the contamination and high prevalence of *Salmonella* in fish intended for human consumption.



 Table 2
 Prevalence of Salmonella enterica serovars in fish

Fish species	Country/year	Sampling place	No. of tested samples/ No. of positive samples (%)	Salmonella enterica serovars	Reference
Tilapia (<i>Tilapia mossambica</i>)	Malaysia/2008–2009	Freshly caught and	32/14 (43.8)	Agona, Bovismorbificans, Corvallis,	Budiati et al. 2013
Catfish (Clarias gariepinus)			32/9 (28.1)	Albany, Agona, Corvallis, Stanley, Typhimurium	
Whiting fish (Merlangius merlangus)	France/n.s.*	Commercial outlets	26/0 (0)	ı	Davies et al. 2001
European plaice (Fleuronecies plaiessa)	Great Britain/n.s.		5/0 (0)	I	
European pilchard (Sardina pilchardus)	Portugal/n.s.		10/0 (0)	I	
Atlantic salmon (Salmo salar)	Great Britain/n.s.		2/0 (0)	I	
Rainbow trout (Oncorhynchus mykiss)	Great Britain/n.s.		20/0 (0)	I	
	Portugal/n.s.		10/0 (0)	I	
	USA/n.s.	Freshly caught	40/0 (0)	I	Pullela et al. 1998
Nile tilapia (<i>Tilapia nilotica</i>)	Egypt/n.s.		101/4 (3.9)	Typhimurium, Wangata, Newport	Youssef et al. 1992
	Kenya/2007		120/20 (31.7)	Typhimurium, Typhi, Enteritidis	David et al. 2009
Common carp (Cyprinus carpio)	Czech Republic/n.s.	n.s.	30/0 (0)	I	Hudecová et al. 2010
Silver carp (Hypophthalmichthys molitrix)	Iran/n.s.	Warm-water fish ponds in Guilan province	42/0 (0)	1	Razavilar et al. 2012
Golden grey mullet (Liza aurata)		Freshly caught Retail outlets	39/1 (2.7) 40/0 (0)	Dublin -	Basti et al. 2006
Caspian anadromous shad (Alosa kossleri)		Caspian sea near the coast, freshly caught	28/0 (0)	I	
Fish	Iran/2009–2011	Freshly caught	110/17 (10.4)	Typhimurium, Enteritidis, Typhi, Paratyphi B, Newport	Rahimi et al. 2013
	India/n.s.	Market and fish landing centre	30/10 (33)	Weltevreden, Worthington, Newport	Shabarinath et al. 2007
Butter catfish (Ompok bimaculatus) Tengan (Aristichithys nobilis)	India/2007–2008	Retail outlets	7/1 (14.3) 7/0 (0)	Oslo, Weltevreden –	Kakatkar et al. 2010
Hilsa (Tenulosa ilisha)			7/1 (14.3)	Oslo	
Mangur (Clarias batrachus)			7/2 (28.6)	Oslo, Weltevreden	
Catla (Catla catla)			7/2 (28.6)	Derby, Typhimurium	
Rohu (<i>Labeo rohita</i>)			7/2 (28.6)	Oslo, Typhimurium	
Gilthead seabream (<i>Sparus aurata</i>), Sea bass, (<i>Dicentrarchus labrox</i>)	Greece/n.s.	Retail outlets	75/1 (1.3)	n.s.	Alexopoulos et al. 2011

*- n.s. cnone specified



Type of seafood Country/year	Country/year	Sampling place	No. of tested samples/No. of positive samples (%)	Salmonella enterica serovars	Reference
Shrimp	India/2003–2006	India/2003-2006 Landing centres, retail outlets	86/23 (26.7)	Bareilly, Braenderup, Brancaster, Derby, Kottbus, Lindenburg, Mbandaka, Oslo, Rissen, Takoradi,	Kumar et al. 2009
	India/1990-1992 Retail outlets	Retail outlets	237/36 (15)	Typhi, Typhimurium, Weltevreden Senftenberg, Typhimurium, Weltevreden, Paratyphi B,	Hatha and Lakshmanaperumalsamy 1997
	Iran/2009-2011	Freshly caught	110/2 (1.8)	1ypiii Enteritidis	Rahimi et al. 2013
	India/n.s.*	Landing centres, retail outlets	27/5 (19)	Weltevreden, Worthington, Newport	Shabarinath et al. 2007
	Egypt/2009	Retail outlets	50/7 (14.0)	Typhimurium, Derby, Typhi, Paratyphi A, Abortus equi	Bakr et al. 2011
Clam	India/2003–2006	India/2003-2006 Landing centres, retail outlets	35/12 (34.2)	Bareilly, Brancaster, Derby, Emek, Irumu, Typhimurium, Virrhow, Weltermeden	Kumar et al. 2009
	India/n.s.		6/2 (33)	Weltevreden, Worthington, Newport	Shabarinath et al. 2007
Crab	India/1990-1992	Retail outlets	39/18 (30.8)	Weltevreden, Typhi, Paratyphi B, Mgulani, Senftenberg	Hatha and Lakshmanaperumalsamy 1997
	India/2003-2006	Landing centres, retail outlets	31/3 (9.6)	Mbandaka, Newport, Othmarschen	Kumar et al. 2009
	Iran/2009-2011	Freshly caught	42/0 (0)	I	Rahimi et al. 2013
Mussel	India/2003–2006	Landing centres, retail outlets	29/9 (31.0)	Derby, Lindenburg, Nchanga, Rissen, Typhi, Typhimurium, Weltevreden	Kumar et al. 2009
	Egypt/2009	Retail outlets	50/4 (8.0)	Typhimurium	Bakr et al. 2011
Oyster	India/2003-2006	India/2003-2006 Landing centres, retail outlets	24/3 (12.5)	Braenderup, Derby, Irumu, Mbandaka, Riggil	Kumar et al. 2009
	India/n.s.	Natural oyster bed	30/2 (7)	Weltevreden, Worthington, Newport	Shabarinath et al. 2007
	Egypt/2009	Retail outlets	50/4 (8.0)	Typhimurium, Derby, Paratyphi B, Infantis	Bakr et al. 2011
Lobster	India/2003-2006	India/2003-2006 Landing centres, retail outlets	21/1 (4.7)	Rissen	Kumar et al. 2009
	Iran/2009-2011	Freshly caught	(0) 0/89	I	Rahimi et al. 2013
Cuttlefish Octopus	India/2003–2006	India/2003–2006 Landing centres, retail outlets	19/2 (10.5) 18/3 (16.6)	Emek, Rissen, Riggil, Atakpame, Braenderup, Irumu, Virchow	Kumar et al. 2009
Squid			23/4 (17.3)	Bareilly, Ohio, Oslo, Typhimurium, Virchow	

*- n.s. none specified



Yersinia spp. in fish and water environments

According to current classification, the genus *Yersinia* belongs to the family *Enterobacteriaceae* in the class *Gammaproteobacteria* of the phylum *Proteobacteria* (Bottone et al. 2005). It currently includes 16 species, of which only three *Yersinia* species are known to be human pathogens, which can also cause disease in animals—*Y. pestis*, *Y. enterocolitica*, and *Y. pseudotuberculosis* (Brubaker 1991; Murros-Kontiainen et al. 2011a, b). Based on biochemical properties, *Y. enterocolitica* is divided into six biotypes of which biotypes 1B, 2, 3, 4, and 5 are considered human pathogens, but biotype 1A is known to be non-pathogenic (Wauters et al. 1987). Meanwhile, *Y. pseudotuberculosis* can be divided into four biotypes (1–4), and all bacteria strains are considered potentially pathogenic to humans (Tsubokura and Aleksić 1995).

Among the genus *Yersinia* species, *Y. ruckeri* is known as a fish pathogen and causes enteric red mouth disease (Ewing et al. 1978; Bottone et al. 2005). Bacteria belonging to the *Yersinia* genus, other than *Y. enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis* are often collectively called *Y. enterocolitica*-like species (Sulakvelidze 2000).

Members of the genus *Yersinia* can grow under aerobic and anaerobic culture conditions with optimum growth at 29 °C, and the acceptable range from 4 to 42 °C (Bottone et al. 2005). The ability of *Y. enterocolitica* to survive and multiply at very low temperatures is, therefore, of special interest. The adaptation of this microorganism to cold results from a combination of various factors that help to maintain the essential functions of cells during and after the cold shock. The cold adaptation process includes upregulation of specialized cold shock proteins and their encoding genes, fatty acid composition and compatible solutes that act as osmotic balancers in the cells (Bresolin et al. 2006; Palonen et al. 2010). It has been described that *Y. enterocolitica* can survive in stream water of 4 °C for up to 64 weeks and for up to 5 years in sterile water (Karapinar and Gonul 1991; Liao and Shollenberger 2003).

The survival of *Y. enterocolitica* in the environment, including water environments, is also increased by the ability of *Y. enterocolitica* to form biofilms (Ioannidis et al. 2014). The ability to form biofilms may allow microorganisms to persist in the environment and to resist desiccation and treatment with antimicrobial agents and disinfectants. Interestingly, it has been observed that the resistance of bacterial cells to antimicrobials in biofilms is significantly increased compared to what is normally seen with the same bacterial cells when not in biofilms (Mah and O'Toole 2001; Gilbert et al. 2002).

Yersinia spp. is common in the environment, with pigs, deer, rodents, and also birds known to be carriers of these human pathogenic and non-pathogenic bacteria (Bottone

et al. 2005). Rivers, lakes, and wells are occasionally contaminated with faeces from domestic or wild animals caused by leakage from septic tanks or open latrines in the surrounding farms or slaughterhouses, and resulting in commination of the environment with Yersinia spp. (Bottone et al. 2005). Pathogenic Y. enterocolitica and Y. pseudotuberculosis strains have sporadically been isolated from slaughterhouse facilities, water, and soil (Falcão et al. 2004; Jalava et al. 2006). However, most isolates of Yersinia spp. recovered from water were characterised as belonging to non-pathogenic Y. enterocolitica biotype 1A, or other non-pathogenic Yersinia species. In total, 26 % of samples collected from rivers in southwestern Ontario, Canada were positive for Yersinia spp., including Y. enterocolitica biotype 1A, Y. aldovae, Y. bercovieri, Y. frederiksenii, Y. intermedia, Y. kristensenii, and Y. mollaretii species (Cheyne et al. 2009). Yersinia spp. was also isolated from various water sources in Brazil (Falcão et al. 2004). The predominant bacterial species were Y. enterocolitica and Y. intermedia and 57 % of Y. enterocolitica strains belonged to human pathogenic biotype 2/O:5,27 or 3/O:5,27. Other Y. enterocolitica isolates (43 %) were confirmed as human non-pathogenic biotype 1A (Falção et al. 2004). Y. enterocolitica and Y. intermedia were also the dominant bacteria species isolated from water samples in India, but no human pathogenic bacteria strains were detected (Sinha et al. 2000). Despite this, the presence of Yersinia spp. in water is a matter of concern. It is worth mentioning that no significant correlation was observed among Y. enterocolitica strains and both total and faecal coliforms detected in river water (Massa et al. 1988). However, Yersinia could be a better indicator of faecal pollution due to its ability to survive in the environment.

A limited number of reports are available on the prevalence of *Y. pseudotuberculosis* in water samples. One of the studies revealed that *Y. pseudotuberculosis* was present in 21 % of 500 freshwater samples from 40 rivers in Japan. It has been described that *Y. pseudotuberculosis* also survived for several months in surface waters (Fukushima 1992).

Because of the wide distribution of *Y. enterocolitica* in water environments, these bacteria were also found in fish (Table 4). Out of 30 finfish and shellfish samples examined, only one was positive for *Y. enterocolitica* (Kishore et al. 2012). The other detected *Yersinia* isolates were identified as *Y. intermedia* (54 %), *Y. aldovae* (19 %), *Y. rohdei* (10 %), *Y. bercovieri* (5 %), *Y. kristensenii* (2 %), *Y. pseudotuberculosis* (2 %), and *Y. frederiksenii* (2 %) (Kishore et al. 2012). Despite this, contamination of fish with *Y. enterocolitica* can reach up to 90 % (Shanmugapriya et al. 2014). During a study performed in India, 20 % of the tested marine fish samples were contaminated with *Y. enterocolitica* (Akhila et al. 2013). A low number of *Yersinia* spp. isolates (6 out of 563 samples) were obtained in a study from Mexico (Salgado-Miranda et al. 2010).



 Table 4
 Prevalence of Yersinia enterocoltica in fish

Fish species	Country/year	Sampling place	No. of tested samples/ positive samples (%)	Reference
Rainbow trout (Oncorhynchus mykiss)	Great Britain/n.s. Portugal/n.s.	Commercial outlets	20/3 (15) 10/0 (0)	Davies et al. 2001
Finfish	India/2013	Fish markets	56/11 (20)	Akhila et al. 2013
Fish (no data about species)	India/2010-2011		20/18 (90)	Shanmugapriya et al. 2014
Butter catfish (Ompok bimaculatus), Tengan (Aristichithys nobilis), Hilsa (Tenulosa ilisha), Mangur (Clarias batrachus), Catla (Catla catla), Rohu (Labeo rohita)	India/2007-2008		42/0 (0)	Kakatkar et al. 2010
Whiting fish (Merlangius merlangus) European plaice (Pleuronectes platessa)	France/n.s. Great Britain/n.s.	Commercial outlets	26/0 (0) 5/0 (0)	Davies et al. 2001
Atlantic salmon (Salmo salar)			5/4 (80)	
Sardine (Sardina pilchardus)	Portugal/n.s.		10/0 (0)	

^{*-} n.s. none specified

Molecular typing methods such as AFLP and PFGE have been successfully applied to describe the genetic diversity of *Y. enterocolitica* strains (Fredriksson-Ahomaa et al. 2006). However, these reports are mainly focused on the one of human yersiniosis sources—pork—and identical bacteria genotypes were detected both in pork and infected humans. Genetic diversity of *Y. enterocolitica* strains isolated from fish was also studied, and ERIC-PCR, REP-PCR, and RAPD methods were used (Akhila et al. 2013; Shanmugapriya et al. 2014). However, the linkage between the consumption of contaminated fish and consumers remains unclear.

In summary, *Yersinia* spp. are widely found in aquatic environments. Human non-pathogenic bacteria strains found in water environments are described as predominant. However, human pathogenic *Y. enterocolitica*, *Y. pseudotuberculosis* are also frequently found in water environments, indicating that the safety of fish and fish products potentially may be affected. Therefore, the presence of yersiniae in fish reflects not only the condition and safety of aquatic environments, it also raises public health concerns with regard to safety of fish used for human consumption.

Clostridium botulinum in fish and water environments

Clostridium botulinum belongs to the genus Clostridium and is commonly associated with foodborne botulism. C. botulinum is widespread in nature and occurs naturally in soil and aquatic environments. C. botulinum is responsible for botulism due to the production of botulinum neurotoxin. Eight types (A, B, C, D, E, F, G, and H) of botulinum neurotoxins are currently recognized (Smith and Sugiyama 1988; Barash and Arnon 2014). The types A, B, E, F, and H are responsible for human botulism, while types C and D are responsible for botulism in various animal species. Type G has not been associated with any botulism cases until now (Carter and Peck

2014). The spore-forming nature of C. botulinum promotes survival of this organism in the environment. The prevalence of C. botulinum in water sediments and in fish can be influenced by various factors, such as geographical location, feeding habits of the fish species, types of samples and the detection method used. C. botulinum was found in various water sediments and fish samples (Johannsen 1962; Bott et al. 1966; Huss and Pedersen 1979; Huss 1980; Hielm et al. 1998a, b; Hyytiä et al. 1998; Fach et al. 2002; Nol et al. 2004; Merivirta et al. 2006; Leclair et al. 2012). Results of those studies have shown that the prevalence of C. botulinum, including types A, B, E, and F, in water sediment and in fish varies considerably (Table 5). High prevalence of C. botulinum type E in freshwater and sea sediments was found in Scandinavian countries, Greenland, and the Canadian Arctic (Johannsen 1962; Huss 1980; Hielm et al. 1998b; Leclair et al. 2012). Studies of marine sediment samples showed a very high prevalence of C. botulinum type E up to 100 % in the Baltic Sea (Huss 1980; Hielm et al. 1998b). Thus, the sea bottom sediment can be considered a major reservoir for C. botulinum type E in the Baltic Sea area. Huss and Pedersen (1979) suggested that fish and water currents can contribute to the spread of C. botulinum.

Various fish species from the Baltic Sea area, mainly Baltic herring, are used for commercial fishing. Hyytiä et al. (1998) studied the occurrence of *C. botulinum* type E in several nonfarmed fish species of commercial interest caught from the Baltic Sea. Of the non-farmed marine fish samples investigated, 23 % contained *C. botulinum* type E, and among these, the highest prevalence of *C. botulinum* type E (40 %) was found in Baltic herring (*Clupea harengus membras*) samples. It has been suggested that the occurrence of *C. botulinum* type E is higher in bottom feeding fish compared to pelagic fish (Huss and Pedersen 1979). The results by Hyytiä et al. (1998) support this suggestion, as they found that the plankton feeding vendace (*Coregonus*



 Table 5
 Prevalence of Clostridium botulinum in fish and in water sediment

Source	Country/year	No. of tested samples/ positive samples (%)	Туре	Reference
Fish from trout farms Sediment	Finland and Sweden/ 1995–1996	165/25 (15) 125/85 (68)	E E	Hielm et al. (1998a)
Baltic herring (Clupea harengus membras) Vendace (Coregonus albula)	Finland/1994-1996	53/21 (40) 50/5 (10)	E E	Hyytiä et al. (1998)
Tilapia (Oreochromis mossambicus)	USA/1999-2001	884/57 (7)	C	Nol et al. (2004)
European river lamprey (Lampetra fluviatilis)	Finland/2003-2004	67/1 (1.5)	E	Merivirta et al. (2006)
Freshwater fish	USA/1964	3240/536 (16.5)	E	Bott et al. (1966)
Freshwater fish Seawater fish	Northern France/2002	4/1 (25) 175/29 (16.6)	B (70 %), A (22.5 %), E (9.6 %)*	Fach et al. (2002)
Sediment		25/1 (4)		
Sediment	Baltic proper/1998	22/22 (100)	E	Hielm et al. (1998b)
Sediment	Denmark/1980	212/194 (92)	E	Huss (1980)

^{*} Type A, B, and E prevalence in 31 C. botulinum-positive freshwater fish, seawater fish, and sediment samples

albula) were less contaminated with C. botulinum type E, while higher prevalence was found in Baltic herring, which feed on both plankton and crustaceans close to the sea bottom. The lower occurrence of C. botulinum was found in European river lamprey (Lampetra fluviatilis). Merivirta et al. (2006) reported results on the prevalence of C. botulinum in European river lamprey obtained from Finnish rivers. Lampreys (n=67) were collected from 12 rivers flowing into the Gulf of Bothnia. Lampreys are usually caught during migration from the sea upstream to spawn. C. botulinum type E was detected in 1.5 % (1/67) of the samples.

A study by Hielm et al. (1998a) was conducted to investigate the prevalence and type distribution of C. botulinum in Finnish trout farm sediments and in fish harvested from the farms. The fish samples selected for this study belonged to four fish species—rainbow trout (Onchorhynchus mykiss), lake trout (Salmo trutta lacustris), sea trout (Salmo trutta trutta), and whitefish (Coregonus lavaretus). Out of 125 sediment samples and 165 samples of fish intestines tested, 68 and 15 %, respectively, were positive for C. botulinum type E. None of the types A, B, or F were detected in sediment or fish samples from trout farms. The results of this study indicated that the design of fish farms can influence the occurrence of C. botulinum in fish farm sediment. Also, it was observed that the level of C. botulinum type E was significantly lower in fish intestine samples in self-cleaning freshwater ponds than in other traditional earth pond farms. The prevalence of C. botulinum in fresh fish and sediment samples from northern France has been reported as 25 % of the evaluated sediment samples, 16.6 % of seawater fish, and 4 % of freshwater fish (Fach et al. 2002). From C. botulinum positive samples, type B was confirmed as most prevalent (70 %). The prevalence of type A was 22.5 %. Contrary to the study by Hielm et al. (1998a), Fach et al. (2002) detected the lowest

prevalence of type E (9.6 %) among *C. botulinum* positive fish and sediment samples.

Bott et al. (1966) examined the intestinal content of freshwater fish of various species from the Great Lakes for the presence of *C. botulinum* type E. Overall, 536 of 3240 (16.5 %) fish samples contained *C. botulinum* type E. Most of the fish samples belonged to species of alewife (*Pomolubus pseudoharengus*), creek chub (*Semotilus atromaculatus atromaculatus*), Great Lakes bloaterchub (*Coregonus hoyi*), Great Lakes cisco (*Coregonus artedii artedii*), smelt (*Osmerus mordax*), sucker (*Catostomus commersonnii commersonnii* and *Moxostoma macrolepidotum macrolepidotum*), trout perch (*Percopsis omiscomaycus*), and yellowperch (*Perca flavescens*). This study showed no correlation between *C. botulinum* type E and certain fish species.

Disease outbreaks in fish-eating birds have also been linked to ingestion of fish contaminated with *C. botulinum*. The Salton Sea avian botulism outbreak in 1996 was linked to fish as the source of *C. botulinum* type C toxin (Rocke et al. 2004). Nol et al. (2004) published a 3-year survey (1999–2001), finding that the prevalence of *C. botulinum* type C in freshly collected tilapia (*Oreochromis mossambicus*) was 7 % (57/884), and the prevalence of infected fish varied from year to year. Authors revealed that tilapia in the Salton Sea harbors *C. botulinum* capable of producing neurotoxin within their gastrointestinal tract. Therefore, tilapia was associated with *C. botulinum* type C avian botulism at the Salton Sea.

The occurrence of *C. botulinum* type E in fish leads to a high risk of fish product contamination. Several human botulism outbreaks have been reported worldwide due to consumption of fish products. Ninety-one case of botulism in Cairo, Egypt were caused by consumption of un-eviscerated, salted mullet fish (Weber et al. 1993). This outbreak was



associated with *C. botulinum* type E toxin. Lindström et al. (2006) reported a type E botulism outbreak that involved two persons in Finland, and was linked to vacuum-packed smoked whitefish. The temperature during the hot-smoking process of fish usually is not sufficient to destroy *C. botulinum* spores, thus vacuum-packaging of smoked whitefish and storage above 3 °C may contribute to the growth and neurotoxin production of *C. botulinum* type E. Based on this investigation, it was hypothesized that a failure to maintain the proper storage temperature at retail outlets or at home led to the type E neurotoxin presence in smoked whitefish, and the ingestion of neurotoxin caused botulism.

In conclusion, the prevalence of *C. botulinum*, mostly type E, in aquatic environments presents a risk that fish may harbor several types of *C. botulinum* and can be a source of foodborne botulism. The presence of *C. botulinum* in fish can be linked to direct contact with contaminated water environments and ingestion of *C. botulinum* spores from sediments or contaminated feed. *C. botulinum* in fish can pose a threat to public health, particularly when improper handling in fish processing or insufficient heat treatment fails to destroy all *C. botulinum* spores in the final product.

Conclusions

Fish is often contaminated with foodborne pathogens such as Salmonella, Listeria monocytogenes, Vibrio spp., Clostridium botulinum, and Yersinia spp., reflecting the microflora of the surrounding water. Contamination of the natural habitat of fish may affect not only the health of fish stocks, but also raise public health concerns as fish and fish products can be a potential source of human pathogenic bacteria. Various factors such as human activity, contaminated water sources, and poor hygiene during capture, handling, and transportation of fish could affect the prevalence of bacteria in fish and surrounding water. The hazard of these microorganisms is increased with the specific abilities of these bacteria to survive in the environment. The emergence of bacteria discussed is also based on the fact that fish and fish products often miss the heat treatment procedure before consumption, which has dramatic effects on human health. Pathogens via contaminated fish and fish products may enter the food chain, and processing of fish may lead to cross-contamination of premises, equipment, and end-product, facilitating the distribution of pathogenic bacteria. However, Good Hygienic Practice is a measure to avoid contamination and to provide the safety of fish and fish products.

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