

# 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 human-made 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 losses (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  $10^2$  to  $10^3$  CFU/cm<sup>2</sup> (Horsley 1973). Meanwhile, a similar study carried out in Turkey revealed a higher number of  $10^1$ – $10^7$  CFU/cm<sup>2</sup> 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., *Pseudomonas 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  $10^6$  CFU/g and  $10^8$  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  $5.5 \times 10^3$  to  $5.0 \times 10^4$  CFU/g, and from  $1.0 \times 10^4$  to  $10 \times 10^6$  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. parahaemolyticus* 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. parahaemolyticus* 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 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 multi-locus 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 cross-contamination 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 ( <i>Merlangius merlangus</i> )	France/n.s.*	Commercial outlets	26/0 (0)	Davies et al. 2001
European plaice ( <i>Pleuronectes platessa</i> )	Great Britain/n.s.		5/0 (0)	
Atlantic salmon ( <i>Salmo salar</i> )	Great Britain/n.s.		5/0 (0)	
	Denmark/1998–1999	Norway and the Faroe Islands	185/16 (8.6)	Fonnesbech Vogel et al. 2001
<i>Salmonidae</i>	USA/1998	Smoked fish processors	102/8 (7.8)	Norton et al. 2001
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	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 ( <i>Sardina pilchardus</i> )	Portugal/n.s.	Commercial outlets	10/0 (0)	Davies et al. 2001
Catfish ( <i>Siluriformes</i> )	USA/n.s.	n.s.	30/0 (0)	Chen et al. 2010
Gibel carp ( <i>Carassius gibelio</i> )	Greece/n.s.	Retail outlets	65/0 (0)	Papadopoulos et al. 2010
Silver Carp ( <i>Hypophthalmichthys molitrix</i> )	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 ( <i>Sardina pilchardus</i> )	India/1997–2001	Retail outlets	15/0 (0)	Dhanashree et al. 2003
Croakers ( <i>Sciaenidae</i> )			11/0 (0)	
Mackerel ( <i>Scombridae</i> )			14/0 (0)	
Pomfret ( <i>Bramidae</i> )			12/0 (0)	
Flat fish ( <i>Pleuronectiformes</i> )			35/1 (2.9)	
Caspian anadromous shad ( <i>Alosa kessleri</i> )	Iran/n.s.	Caspian sea near the coast, freshly caught	28/0 (0)	Basti et al. 2006
Mullet dore ( <i>Liza aurata</i> )	Iran/n.s.	Retail outlets	40/0 (0)	
Butter catfish ( <i>Ompok bimaculatus</i> )	India/2007–2008	Retail outlets	7/0 (0)	Kakatkar et al. 2010
Tengan ( <i>Aristichthys nobilis</i> )			7/0 (0)	
Hilsa ( <i>Tenulosa ilisha</i> )			7/0 (0)	
Mangur ( <i>Clarias batrachus</i> )			7/0 (0)	
Catla ( <i>Catla catla</i> )			7/0 (0)	
Rohu ( <i>Labeorohita</i> )			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; Møretør 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), repetitive 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 salmonellosis 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 (%)	<i>Salmonella enterica</i> serovars	Reference
Tilapia ( <i>Tilapia mossambica</i> )	Malaysia/2008–2009	Freshly caught and retail outlets	32/14 (43.8)	Agona, Bovismorbificans, Corvallis, Typhimurium	Budiati et al. 2013
Catfish ( <i>Clarias gariepinus</i> )			32/9 (28.1)	Albany, Agona, Corvallis, Stanley, Typhimurium	
Whiting fish ( <i>Merlangius merlangus</i> )	France/n.s.*	Commercial outlets	26/0 (0)	–	Davies et al. 2001
European plaice ( <i>Pleuronectes platessa</i> )	Great Britain/n.s.		5/0 (0)	–	
European pilchard ( <i>Sardina pilchardus</i> )	Portugal/n.s.		10/0 (0)	–	
Atlantic salmon ( <i>Salmo salar</i> )	Great Britain/n.s.		5/0 (0)	–	
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	Great Britain/n.s.		20/0 (0)	–	
	Portugal/n.s.		10/0 (0)	–	
	USA/n.s.		40/0 (0)	–	
Nile tilapia ( <i>Tilapia nilotica</i> )	Egypt/n.s.	Freshly caught	101/4 (3.9)	Typhimurium, Wangata, Newport	Pullela et al. 1998
	Kenya/2007		120/20 (31.7)	Typhimurium, Typhi, Enteritidis	Youssef et al. 1992
Common carp ( <i>Cyprinus carpio</i> )	Czech Republic/n.s.	n.s.	30/0 (0)	–	David et al. 2009
Silver carp ( <i>Hypophthalmichthys molitrix</i> )	Iran/n.s.	Warm-water fish ponds in Guilan province	42/0 (0)	–	Hudecová et al. 2010
		Freshly caught	39/1 (2.7)	Dublin	Razavilar et al. 2012
		Retail outlets	40/0 (0)	–	Basti et al. 2006
Golden grey mullet ( <i>Liza aurata</i> )		Caspian sea near the coast, freshly caught	28/0 (0)	–	
Caspian anadromous shad ( <i>Alosa kessleri</i> )	Iran/2009–2011	Freshly caught	110/17 (10.4)	Typhimurium, Enteritidis, Typhi, Paratyphi B, Newport	Rahimi et al. 2013
Fish					
Butter catfish ( <i>Ompok bimaculatus</i> )	India/n.s.	Market and fish landing centre	30/10 (33)	Weltevreden, Worthington, Newport	Shabarimath et al. 2007
Tengan ( <i>Aristichthys nobilis</i> )	India/2007–2008	Retail outlets	7/1 (14.3)	Oslo, Weltevreden	Kakatkarkar et al. 2010
Hilsa ( <i>Tenulosa ilisha</i> )			7/0 (0)	–	
Mangur ( <i>Clarias batrachus</i> )			7/1 (14.3)	Oslo	
Catla ( <i>Catla catla</i> )			7/2 (28.6)	Oslo, Weltevreden	
Rohu ( <i>Labeo rohita</i> )			7/2 (28.6)	Derby, Typhimurium	
Gilthead seabream ( <i>Sparus aurata</i> ), Sea bass, ( <i>Dicentrarchus labrax</i> )	Greece/n.s.	Retail outlets	7/2 (28.6)	Oslo, Typhimurium	Alexopoulos et al. 2011
			75/1 (1.3)	n.s.	

\* - n.s. none specified

**Table 3** Contamination of various seafood with *Salmonella enterica* serovars

Type of seafood	Country/year	Sampling place	No. of tested samples/No. of positive samples (%)	<i>Salmonella enterica</i> serovars	Reference
Shrimp	India/2003–2006	Landing centres, retail outlets	86/23 (26.7)	Bareilly, Braenderup, Brancaster, Derby, Kotibus, Lindenburg, Mbandaka, Oslo, Rissen, Takoradi, Typhi, Typhimurium, Weltevreden	Kumar et al. 2009
	India/1990–1992	Retail outlets	237/36 (15)	Senftenberg, Typhimurium, Weltevreden, Paratyphi B, Typhi	Hatha and Lakshmanaperumalsamy 1997
Clam	Iran/2009–2011	Freshly caught	110/2 (1.8)	Enteritidis	Rahimi et al. 2013
	India/n.s.*	Landing centres, retail outlets	27/5 (19)	Weltevreden, Worthington, Newport	Shabarath et al. 2007
	Egypt/2009	Retail outlets	50/7 (14.0)	Typhimurium, Derby, Typhi, Paratyphi A, Abortus equi	Bakr et al. 2011
	India/2003–2006	Landing centres, retail outlets	35/12 (34.2)	Bareilly, Brancaster, Derby, Emek, Irumu, Typhimurium, Virchow, Weltevreden,	Kumar et al. 2009
	India/n.s.		6/2 (33)	Weltevreden, Worthington, Newport	Shabarath 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
Mussel	Iran/2009–2011	Freshly caught	42/0 (0)	–	Rahimi et al. 2013
	India/2003–2006	Landing centres, retail outlets	29/9 (31.0)	Derby, Lindenburg, Nchanga, Rissen, Typhi, Typhimurium, Weltevreden	Kumar et al. 2009
Oyster	Egypt/2009	Retail outlets	50/4 (8.0)	Typhimurium	Bakr et al. 2011
	India/2003–2006	Landing centres, retail outlets	24/3 (12.5)	Braenderup, Derby, Irumu, Mbandaka, Riggil	Kumar et al. 2009
Lobster	India/n.s.	Natural oyster bed	30/2 (7)	Weltevreden, Worthington, Newport	Shabarath et al. 2007
	Egypt/2009	Retail outlets	50/4 (8.0)	Typhimurium, Derby, Paratyphi B, Infantis	Bakr et al. 2011
Cuttlefish	India/2003–2006	Landing centres, retail outlets	21/1 (4.7)	Rissen	Kumar et al. 2009
	Iran/2009–2011	Freshly caught	68/0 (0)	–	Rahimi et al. 2013
Octopus	India/2003–2006	Landing centres, retail outlets	19/2 (10.5)	Emek, Rissen, Riggil,	Kumar et al. 2009
			18/3 (16.6)	Atakpame, Braenderup, Irumu, Virchow	
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-Konttiainen 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 (Falcão 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 enterocolitica* in fish

Fish species	Country/year	Sampling place	No. of tested samples/ positive samples (%)	Reference
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	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 ( <i>Ompok bimaculatus</i> ), Tengan ( <i>Aristichithys nobilis</i> ), Hilsa ( <i>Tenulosa ilisha</i> ), Mangur ( <i>Clarias batrachus</i> ), Catla ( <i>Catla catla</i> ), Rohu ( <i>Labeo rohita</i> )	India/2007–2008		42/0 (0)	Kakatkar et al. 2010
Whiting fish ( <i>Merlangius merlangus</i> )	France/n.s.	Commercial outlets	26/0 (0)	Davies et al. 2001
European plaice ( <i>Pleuronectes platessa</i> )	Great Britain/n.s.		5/0 (0)	
Atlantic salmon ( <i>Salmo salar</i> )			5/4 (80)	
Sardine ( <i>Sardina pilchardus</i> )	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 non-farmed 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 (%)	Type	Reference
Fish from trout farms	Finland and Sweden/ 1995–1996	165/25 (15)	E	Hielm et al. (1998a)
Sediment		125/85 (68)	E	
Baltic herring ( <i>Clupea harengus membras</i> )	Finland/1994–1996	53/21 (40)	E	Hyttiä et al. (1998)
Vendace ( <i>Coregonus albula</i> )		50/5 (10)	E	
Tilapia ( <i>Oreochromis mossambicus</i> )	USA/1999–2001	884/57 (7)	C	Nol et al. (2004)
European river lamprey ( <i>Lampetra fluviatilis</i> )	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	Northern France/2002	4/1 (25)	B (70 %), A (22.5 %), E (9.6 %)*	Fach et al. (2002)
Seawater fish		175/29 (16.6)		
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 (*Pomolobus 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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## References

- Adgamov RR, Timchenko NF, Zaitseva EA, Pushkareva VI, Kolbasov DV, Egorova IY, Pukhovskaya NM, Musatov YS, Ivanov LI, Ermolaeva SA (2013) Ecological and genetic mechanisms of development of epidemiologically significant strains of saporonosis causative agents. *Biol Bull Rev* 3:125–138
- Agbaje M, Begum RH, Oyekunle MA, Ojo OE, Adembi OT (2011) Evolution of *Salmonella* nomenclature: acritical note. *Folia Microbiol* 56:497–503
- Akhila S, Shanmuga Priya S, Senthil Murugan T, Tha T (2013) Molecular diversity analysis of *Yersinia enterocolitica* isolated from marine marketed fish. *Int J Curr Microbiol App Sci* 2:204–214
- Albufera U, Bhugaloo-Vial P, Issack MI, Jaufeerally-Fakim Y (2009) Molecular characterization of *Salmonella* isolated by REP-PCR and RAPD analysis. *Infect Genet Evol* 9:322–327
- Alexopoulos A, Plessas S, Voidarou C, Noussias H, Stavropoulou E, Mantzourani I, Tzora A, Skoufos I, Bezirtzoglou E (2011) Microbial ecology of fish species growing in greek sea farms and their watery environment. *Anaerobe* 17:264–266
- Allen DA, Austin B, Colwell RR (1983) Numerical taxonomy of bacterial isolates associated with a freshwater fishery. *J Gen Microbiol* 129:2043–2062
- Amagliani G, Brandi G, Schiavano GF (2012) Incidence and role of *Salmonella* in seafood safety. *Food Res Int* 45:780–788
- Apun K, Yusof AM, Jugang K (1999) Distribution of bacteria in tropical freshwater fish and ponds. *Int J Environ Health Res* 9:285–292
- Bakr WMK, Hazzah WA, Abaza AF (2011) Detection of *Salmonella* and *Vibrio* species in some seafood in Alexandria. *J Am Sci* 7:663–668
- Balasubramanian S, Rajan MR, Raj SP (1992) Microbiology of fish grown in a sewage-fed pond. *Bioresour Technol* 40:63–66
- Barash JR, Aron SS (2014) A novel strain of *Clostridium botulinum* that produces type B and type H botulinum toxins. *J Infect Dis* 209:183–191
- Basti AA, Misaghi A, Salehi TZ, Kamkar A (2006) Bacterial pathogens in fresh, smoked and salted Iranian fish. *Food Control* 17:183–188
- Bott TL, Deffner JS, McCoy E, Foster EM (1966) *Clostridium botulinum* in fish from the Great Lakes. *J Bacteriol* 91:919–924
- Bottone EJ, Bercovier H, Mollaret HH (2005) Genus XLI. *Yersinia*. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT (eds) *Bergey's manual of systematic bacteriology. The proteobacteria. Part B. The gammaproteobacteria*, 2nd edn. Springer Press, New York, pp 838–848
- Bresolin G, Neuhaus K, Scherer S, Fuchs TM (2006) Transcriptional analysis of long-term adaptation of *Yersinia enterocolitica* to low-temperature growth. *J Bacteriol* 188:2945–2958
- Broza YY, Danin-Poleg Y, Lerner L, Valinsky L, Broza M, Kashi Y (2009) Epidemiologic study of *Vibrio vulnificus* infections by using variable number tandem repeats. *Emerg Infect Dis* 15:1282–1285
- Brubaker RR (1991) Factors promoting acute and chronic diseases caused by yersiniae. *Clin Microbiol Rev* 4:309–324
- Budiati T, Rusul G, Wan-Abdullah WN, Arip YM, Ahmad R, Thong KL (2013) Prevalence, antibiotic resistance, and plasmid profiling of salmonella in catfish (*Clarias gariepinus*) and tilapia (*Tilapia mossambica*) obtained from wet markets and ponds in Malaysia. *Aquaculture* 372:127–132
- Budzińska K, Wroński G, Szejniuk B (2012) Survival time of bacteria *Listeria monocytogenes* in water environment and sewage. *Pol J Environ Stud* 1:31–37
- Callol A, Pajuelo D, Ebbesson L, Teles M, MacKenzie S, Amaro C (2015) Early steps in the European eel (*Anguilla anguilla*)-*Vibrio vulnificus* interaction in the gills: role of the RtxA1<sub>3</sub> toxin. *Fish Shellfish Immunol* 43:502–509
- Campbell AC, Buswell JA (1983) The intestinal microflora of farmed dover sole (*Solea solea*) at different stages of fish development. *J Appl Bacteriol* 55:215–223

- Carter AT, Peck MW (2014) Genomes, neurotoxins, and biology of *Clostridium botulinum* group I and group II. Res Microbiol. doi:10.1016/j.resmic.2014.10.010
- Chao W, Ding R, Chen R (1987) Survival of pathogenic bacteria in environmental microcosms. Chin J Microbiol Immunol 20:339–348
- Chen BY, Pyla R, Kim TJ, Silva JL, Jung YS (2010) Prevalence and contamination patterns of *Listeria monocytogenes* in catfish processing environment and fresh fillets. Food Microbiol 27:645–652
- Cheyne BM, Van Dyke MI, Anderson WB, Huck PM (2009) An evaluation of methods for the isolation of *Yersinia enterocolitica* from surface waters in the Grand River watershed. J Water Health 7:392–403
- Christensen PJ (1977) The history, biology, and taxonomy of the *cytophaga* group. Can J Microbiol 23:1599–1653
- Corcoran D, Clancy D, O'Mahony M, Grant K, Hyland E, Shanaghy N, Whyte P, McLauchlin J, Moloney A, Fanning S (2014) Comparison of *Listeria monocytogenes* strain types in Irish smoked salmon and other foods. Int J Hyg Environ Health 209:527–34
- Costeron JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995) Microbial biofilms. Annu Rev Microbiol 49:711–745
- David OM, Wandili S, Kakai R, Waindi EN (2009) Isolation of *Salmonella* and *Shigella* from fish harvested from the Winam Gulf of lake Victoria, Kenya. J Infect Dev Countries 3:99–104
- Davies AR, Capell C, Jehanno D, Nychas GJE, Kirby RM (2001) Incidence of foodborne pathogens on European fish. Food Control 12:67–71
- Den Bakker HC, Warchocki S, Wright EM, Allred AF, Ahlstrom C, Manuel CS, Stasiewicz MJ, Burrell A, Roof S, Strawn L, Fortes ED, Nightingale KK, Kephart D, Wiedmann M (2014) Five new species of *Listeria* (*L. Floridensis* sp. nov., *L. Aquatica* sp. nov., *L. Cornellensis* sp. nov., *L. Riparia* sp. nov., and *L. Grandensis* sp. nov.) from agricultural and natural environments in the united states. Int J Syst Evol Microbiol. doi:10.1099/ijs.0.052720-0
- Dhanashree B, Otta SK, Karunasagar I, Goebel W, Karunasagar I (2003) Incidence of *Listeria* spp. in clinical and food samples in Mangalore, India. Food Microbiol 20:447–453
- Diler O, Diler A (1998) Quantitative and qualitative changes of the gastrointestinal microflora of pike-perch (*Stizostedion lucioperca* L. 1758) in Egirdir Lake. Turk J Vet Anim Sci 22:325–328
- Diler O, Altun S, Calikusu F, Diler A (2000) A study on qualitative and quantitative bacterial flora of the rainbow trout (*Oncorhynchus mykiss*) living in different fish farms. Turk J Vet Anim Sci 24:251–259
- Drake SL, DePaola A, Jaykus LA (2007) An overview of *Vibrio vulnificus* and *Vibrio parahaemolyticus*. Compr Rev Food Sci F 6:120–144
- Duffes F (1999) Improving the control of *Listeria monocytogenes* in cold smoked salmon. Trends Food Sci Technol 10:211–216
- EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) (2015) The European union summary report on trends and sources of zoonoses, zoonotic agents, and food-borne outbreaks in 2013. EFSA J 13(1):1–312
- Embarek PKP (1994) Presence, detection and growth of *Listeria monocytogenes* in seafoods: a review. Int J Food Microbiol 23:17–34
- Ertas HB, Seker E (2005) Isolation of *Listeria monocytogenes* from fish intestines and RAPD analysis. Turk J Vet Anim Sci 29:1007–1011
- EUMOFA (2014) European market observatory for fisheries and aquaculture products. The EU fish market. 2014 edn, pp 1–61
- Evelyn TPT, McDermott LA (1961) Bacteriological studies of freshwater fish. Isolation of aerobic bacteria from several species of Ontario fish. Can J Microbiol 7:357–382
- Ewing WH, Ross AJ, Brenner DJ, Fanning GR (1978) *Yersinia ruckeri* sp. nov., the redmouth (RM) bacterium. Int J Syst Bacteriol 28:37–44
- Fach P, Perelle S, Dilasser F, Grout J, Dargaignaratz C, Botella L, Gourreau JM, Carlin F, Popoff MR, Broussolle V (2002) Detection by PCR–enzyme-linked immunosorbent assay of *Clostridium botulinum* in fish and environmental samples from a coastal area in Northern France. Appl Environ Microbiol 68:5870–5876
- Falcão JP, Brocchi M, Proenca-Modena JL, Acrani GO, Correa EF, Falcão DP (2004) Virulence characteristics and epidemiology of *Yersinia enterocolitica* and *Yersinia* other than *Y. pseudotuberculosis* and *Y. pestis* isolated from water and sewage. J Appl Microbiol 96:1230–1236
- FAO (2010) FAO expert workshop on the application of biosecurity measures to control *Salmonella* contamination in sustainable aquaculture. FAO Fish Aquac Rep 937:1–37
- Fonnesbech Vogel B, Huss HH, Ojeniyi B, Ahrens P, Gram L (2001) Elucidation of *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. Appl Environ Microbiol 76:2586–2595
- Fredriksson-Ahomaa M, Stolle A, Korkeala H (2006) Molecular epidemiology of *Yersinia enterocolitica* infections. FEMS Immunol Med Microbiol 47:315–329
- Fukushima H (1992) Direct isolation of *Yersinia pseudotuberculosis* from fresh water in Japan. Appl Environ Microbiol 58:2688–2690
- Furones MD, Rodgers CJ, Munn CB (1993) *Yersinia ruckeri*, the causal agent of enteric redmouth disease (ERM) in fish. Annu Rev Fish Dis 3:105–125
- Gauthier DT (2015) Bacterial zoonoses of fishes: a review and appraisal of evidence for linkages between fish and human infections. Vet J 203:27–35
- Gilbert P, Allison DG, McBain AJ (2002) Biofilms *in vitro* and *in vivo*: do singular mechanisms imply cross-resistance? J Appl Microbiol 92:98–110
- Givens CE, Bowers JC, DePaola A, Hollibaugh JT, Jones JL (2014) Occurrence and distribution of *Vibrio vulnificus* and *Vibrio parahaemolyticus* – potential role for fish, oyster, sediment and water. Lett Appl Microbiol 58:503–510
- Gonzalez CJ, Santos JA, Garcia-Lopez ML, Otero A (2000) Psychrobacters and related bacteria in freshwater fish. J Food Prot 63:315–321
- Gonzalez CJ, Santos JA, Garcia-Lopez ML, Gonzalez N, Otero A (2001) Mesophilic aeromonads in wild and aquacultured freshwater fish. J Food Prot 64:687–691
- González-Rodríguez MN, Sanz JJ, Santos JA, Otero A, García-López ML (2002) Foodborne pathogenic bacteria in prepackaged fresh retail portions of farmed rainbow trout and salmon stored at 3°C. Int J Food Microbiol 76:135–141
- Graves LM, Helsel LO, Steigerwalt AG, Morey RE, Daneshvar MI, Roof SE, Orsi RH, Fortes ED, Milillo SR, den Bakker HC, Wiedmann M, Swaminathan B, Sauters BD (2009) *Listeria marthii* sp. nov., isolated from the natural environment, Finger Lakes National Forest. Int J Syst Evol Microbiol 60:1280–1288
- Guzman MC, Bistoni MA, Tamagninii LM, Gonzales RD (2004) Recovery of *Escherichia coli* in fresh water fish, *jenynsia multidentata* and *Bryconamericus iheringi*. Water Res 38:2368–2374
- Hansen CH, Vogel BF, Gram L (2006) Prevalence and survival of *Listeria monocytogenes* in Danish aquatic and fish processing environments. J Food Prot 69:2113–2122
- Hatha AAM, Lakshmanaperumalsamy P (1997) Prevalence of *Salmonella* in fish and crustaceans from markets in Coimbatore, South India. Food Microbiol 14:111–116
- Heinitz ML, Ruble RD, Wagner DE, Tatini SR (2000) Incidence of *Salmonella* in fish and seafood. J Food Prot 63:579–592
- Hielm S, Björkroth J, Hyytiä E, Korkeala H (1998a) Prevalence of *Clostridium botulinum* in finnish trout farms: pulsed-field gel electrophoresis typing reveals extensive genetic diversity among type E isolates. Appl Environ Microbiol 64:4161–4167

- Hielm S, Hyytiä E, Andersin B, Korkeala H (1998b) A high prevalence of *Clostridium botulinum* type E in finnish freshwater and baltic Sea sediment samples. *J Appl Microbiol* 84:133–137
- Horsley RW (1973) The bacterial flora of the Atlantic salmon (*Salmo salar* L.) in relation to its environment. *J Appl Bacteriol* 36:377–386
- Hudecová K, Buchtová H, Steinhauserová I (2010) The effects of modified atmosphere packaging on the microbiological properties of fresh common carp (*Cyprinus carpio*). *Acta Vet Brno* 79:93–100
- Huehn S, Eichhorn C, Urmsbach S, Breidenbach J, Bechlers S, Bier N, Alter T, Bartelt E, Frank C, Oberheitmann B, Gunzer F, Brennholt N, Böer S, Appel B, Dieckmann R, Strauch E (2014) Pathogenic vibrios in environmental, seafood and clinical sources in Germany. *Int J Med Microbiol* 304:843–850
- Huss HH (1980) Distribution of *Clostridium botulinum*. *Appl Environ Microbiol* 39:764–769
- Huss HH, Pedersen A (1979) *Clostridium botulinum* in fish. *Nord Vet Med* 31:214–221
- Hyytiä E, Hielm S, Korkeala H (1998) Prevalence of *Clostridium botulinum* type E in finnish fish and fishery products. *Epidemiol Infect* 120:245–250
- Ioannidis A, Kyratsa A, Ioannidou V, Bersimis S, Chatzipanagiotou S (2014) Detection of biofilm production of *Yersinia enterocolitica* strains isolated from infected children and comparative antimicrobial susceptibility of biofilm versus planktonic forms. *Mol Diagn Ther* 18:309–314
- Iwamoto M, Ayers T, Mahon BE, Swerdlow DL (2010) Epidemiology of seafood-associated infections in the United States. *Clin Microbiol Rev* 23:399–411
- Jalava K, Hakkinen M, Valkonen M, Nakari UM, Palo T, Hallanvuo S, Ollgren J, Siitonen A, Nuorti JP (2006) An outbreak of gastrointestinal illness and erythema nodosum from grated carrots contaminated from birds in Norway. *J Wildl Dis* 18:247–248
- Jami M, Ghanbari M, Zunabovic M, Domig KJ, Kneifel W (2014) *Listeria monocytogenes* in aquatic food products—a review. *Compr Rev Food Sci F* 13:798–813
- Janssens JCA, Steenackers H, Robijns S, Gellens E, Levin J, Zhao H, Hermans K, Keersmaecker D (2008) Brominated furanones inhibit biofilm formation by *Salmonella enterica* serovar Typhimurium. *Appl Environ Microbiol* 74:6639–6648
- Johannsen A (1962) *Clostridium botulinum* in Sweden and the adjacent waters. *J Appl Bacteriol* 26:43–47
- Johansson T, Rantala L, Palmu L, Honkanen-Buzalski T (1999) Occurrence and typing of *Listeria monocytogenes* strains in retail vacuum-packed fish products and in a production plant. *Int J Food Microbiol* 47:111–9
- Kakatkar AS, Gautam RK, Nagar V, Karani M, Bandekar JR (2010) Incidence of foodborne pathogens in freshwater fish from domestic markets of Mumbai. *Fish Technol* 47:95–200
- Kamei Y, Sakata T, Kakimoto D (1985) Microflora in the alimentary tract of the tilapia: characteristics and distribution of anaerobic bacteria. *J Gen Appl Microbiol* 31:115–124
- Karapinar M, Gonul SA (1991) Survival of *Yersinia enterocolitica* and *Escherichia coli* in spring water. *Int J Food Microbiol* 13:315–319
- Kishore P, Lalitha KV, Joseph TC, Thampuran N (2012) Biotyping and antibiotic resistance profile of *Yersinia enterocolitica* associated with seafoods from south-west coast of India. *Fish Technol* 49:64–71
- Koonse B (2005) Good aquaculture practices for farmers – an update. *FAO Fish Proc* 1:76–77
- Kumar R, Surendran PK, Thampuran N (2009) Distribution and genotypic characterization of *Salmonella* serovars isolated from tropical seafood in cochin, India. *J Appl Microbiol* 106:515–524
- Lang Halter E, Neuhaus K, Scherer S (2013) *Listeria weihenstephanensis* sp. nov., isolated from the water plant lemna trisulca taken from a freshwater pond. *Int J Syst Evol Microbiol* 63:641–647
- Lapidot A, Romling U, Yaron S (2006) Biofilm formation and the survival of *Salmonella typhimurium* on parsley. *Int J Med Microbiol* 109:229–233
- Leclair D, Farber JM, Doidge B, Blanchfield B, Suppa S, Pagotto F, Austin JW (2012) Distribution of *Clostridium botulinum* type E strains in Nunavik, Northern Quebec, Canada. *Appl Environ Microbiol* 79:646–654
- Li TH, Chiu CH, Chen WC, Chen CM, Hsu YM, Chiou SS, Chiou CS, Chang CC (2009) Consumption of groundwater as an independent risk factor of *Salmonella* Choleraesuis infection: a case control study in Taiwan. *J Environ Health* 72:28–31
- Lianou A, Sofos JN (2007) A review of the incidence and transmission of *Listeria monocytogenes* in ready-to-eat products in retail and food service environments. *J Food Prot* 70:2172–98
- Liao CH, Shollenberger LM (2003) Survivability and long-term preservation of bacteria in water and in phosphate-buffered saline. *Lett Appl Microbiol* 37:45–50
- Lindström M, Vuorela M, Hinderink K, Korkeala H, Dahlsten E, Raahenmaa M, Kuusi M (2006) Botulism associated with vacuum-packed smoked whitefish in Finland, June–July 2006. *Euro Surveill* 11(29):3004
- Liu D (2006) Identification, subtyping, and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *J Med Microbiol* 55:645–659
- Lunestad BT, Nesse L, Lassen J, Svihus B, Nesbakken T, Fossum K, Rosnes JT, Kruse H, Yazdankhah S (2007) *Salmonella* in fish feed; occurrence and implications for fish and human health in Norway. *Aquaculture* 265:1–8
- Mah FC, O’Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol* 9:34–39
- Mahmud ZH, Wright AC, Mandal SC, Dai J, Jones MK, Hasan M, Rashid MH, Islam MS, Johnson JA, Gulig PA, Morris JG Jr, Ali A (2010) Genetic characterization of *Vibrio vulnificus* strains from tilapia aquaculture in Bangladesh. *Appl Environ Microbiol* 76:4890–4895
- Markkula A, Autio T, Lundén J, Korkeala H (2005) Raw and processed fish show identical *Listeria monocytogenes* genotypes with pulsed-field gel electrophoresis. *J Food Prot* 68:1228–1231
- Martinez-Urtaza J, Saco M, de Nova J, Perez-Pioneiro P, Peiteado J, Lozano-Leon A, Garcia-Martin O (2004) Influence of environmental factors and human activity on the presence of *Salmonella* serovars in a marine environment. *Appl Environ Microbiol* 70:2089–2097
- Massa S, Cesaroni D, Poda E, Tronchetti LD (1988) Isolation of *Yersinia enterocolitica* and related species from riverwater. *Zentralbl Mikrobiol* 14:3575–81
- Merivirta OL, Lindström M, Björkroth KJ, Korkeala H (2006) The prevalence of *Clostridium botulinum* in European river lamprey (*Lampetra fluviatilis*) in Finland. *Int J Food Microbiol* 109:234–237
- Miettinen H, Wirtanen G (2005) Prevalence and location of *Listeria monocytogenes* in farmed rainbow trout. *Int J Food Microbiol* 104:135–143
- Modarressi S, Thong KL (2010) Isolation and molecular subtyping of *Salmonella enterica* from chicken, beef, and street foods in Malaysia. *Sci Res Essays* 5:2713–2720
- Momtzag H, Yadollahi S (2013) Molecular characterization of *Listeria monocytogenes* isolated from fresh seafood samples in Iran. *Diagn Pathol* 8:149
- Moore BC, Martinez E, Gay JM, Rice DH (2003) Survival of *Salmonella enterica* in freshwater and sediments, and transmission by the aquatic midge *Chironomus tentans* (chironomidae: diptera). *Appl Environ Microbiol* 69:4556–4560
- Møretro T, Vestby LK, Nesse LL, Hannevik S, Kotlarz K, Langsrud S (2009) Evaluation of efficiency of disinfectants against *Salmonella* from the feed industry. *J Appl Microbiol* 106:1005–1012
- Murros-Konttinen A, Fredriksson-Ahoma M, Korkeala H, Johansson P, Rahkila R, Björkroth J (2011a) *Yersinia nurmii* sp. nov. *Int J Syst Evol Microbiol* 61:2368–2372

- Murros-Konttinen A, Johansson P, Niskanen T, Fredriksson-Ahomaa M, Korkeala H, Björkroth J (2011b) *Yersinia pekkanenii* sp. nov. Int J Syst Evol Microbiol 61:2363–2367
- Nakaguchi Y (2013) Contamination by *Vibrio parahaemolyticus* and its virulent strains in seafood marketed in Thailand, Vietnam, Malaysia, and Indonesia. Trop Med Health 41:95–102
- Nedoluha PC, Westhoff D (1997) Microbiological analysis of striped bass (*Morone saxatilis*) grown in a recirculating system. J Food Prot 60:948–953
- Nol P, Rocke TE, Gross K, Yuill TM (2004) Prevalence of neurotoxic *Clostridium botulinum* type C in the gastrointestinal tracts of tilapia (*Oreochromis mossambicus*) in the salton Sea. J Wildl Dis 40:414–419
- Noorlis A, Ghazali FM, Cheah YK, Tuan Zainazor TC, Ponniah J, Tunung R, Tang JYH, Nishibuchi M, Nakaguchi Y, Son R (2011) Prevalence and quantification of *Vibrio* species and *Vibrio parahaemolyticus* in freshwater fish at hypermarket level. Int Food Res J 18:689–695
- Norton DM, Mccamey MA, Gall K, Scarlet JM, Boor KJ, Wiedman M (2001) Molecular studies on the ecology of *Listeria monocytogenes* in the smoking fish processing industry. Appl Environ Microbiol 67:198–205
- Otomo Y, Abe K, Odagiri K, Shiroto A, Takatori K, Hara-Kudo Y (2007) Detection of *Salmonella* in spent hens and eggs associated with foodborne infections. Avian Dis 51:578–583
- Palonen E, Lindström M, Korkeala H (2010) Adaptation of enteropathogenic *Yersinia* to low growth temperature. Crit Rev Microbiol 36:54–67
- Papadopoulos T, Abraham A, Sergelidis D, Kirkoudis I, Bitchava K (2010) Prevalence of *Listeria* spp. in freshwater fish (*Oncorhynchus mykiss* and *Carassius gibelio*) and the environment of fish markets in Northern Greece. J Hell Vet Med Soc 61:15–22
- Parker WF, Mee BJ (1982) Survival of *Salmonella* Adelaide and fecal coliforms in coarse sands of the swan coastal plain, Western Australia. Appl Environ Microbiol 43:981–986
- Pillay TVR (1990) Fish and public health and disease. In: Pillay TVR (ed) Aquaculture, principles and practices. Fishing News Book, Farnham, UK, pp 174–215
- Ponce E, Khan AA, Cheng C, Summige-West C, Cerniglia CE (2008) Prevalence and characterization of *Salmonella enterica* serovar Weltevreden from imported seafood. Food Microbiol 25:29–35
- Pullela S, Fernandes CF, Flick GJ, Libey GS, Smith SA, Coale CW (1998) Indicative and pathogenic microbiological quality of aquacultured finfish grown in different production systems. J Food Prot 61:205–210
- Rahimi E, Shakerian A, Falavarjani AG (2013) Prevalence and antimicrobial resistance of *Salmonella* isolated from fish, shrimp, lobster, and crab in Iran. Comp Clin Path 22:59–62
- Razavilar V, Khani MR, Motalebi AA (2012) Bacteriological study of cultured silver carp (*Hypophthalmichthys molitrix*) in Gilan province, Iran. Iran J Fish Sci 12:689–701
- Rocke TE, Nol P, Pelizza C, Sturm K (2004) Type C botulism in pelicans and other fish-eating birds at the Salton Sea. Stud Avian Biol 27:137–140
- Salgado-Miranda C, Palomares E, Jurado M, Marin A, Vega F, Soriano-Vargas E (2010) Isolation and distribution of bacterial flora in farmed rainbowtrout from Mexico. J Aquat Anim Health 22:244–247
- Senderovich Y, Izhaki I, Halpern M (2010) Fish as reservoirs and vectors of *Vibrio cholerae*. PLoS One 5, e8607
- Shabarinnath S, Kumar HS, Khushiramani R, Karunasagar I, Karunasagar I (2007) Detection and characterization of *Salmonella* associated with tropical seafood. Int J Food Microbiol 114:227–233
- Shanmugapriya S, Senthilmurugan T, Thayumanavan T (2014) Genetic diversity among *Yersinia enterocolitica* isolated from chicken and fish in and around Coimbatore City, India. Iran J Publ Health 43:835–844
- Sinha I, Choudhary I, Viridi JS (2000) Isolation of *Yersinia enterocolitica* and *Yersinia intermedia* from wastewaters and their biochemical and serological characteristics. Curr Sci 79:510–513
- Smith LDS, Sugiyama H (1988) Botulism: the organism, its toxins, the disease, 2nd edn. Springfield, Illinois, p 139
- Sulakvelidze A (2000) *Yersinia* other than *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis*: the ignored species. Microbes Infect 2:497–513
- Tao Z, Larsen MA, Bullard SA, Wright AC, Arias CR (2012) Prevalence and population of *Vibrio vulnificus* on fishes from the northern Gulf of Mexico. Appl Environ Microbiol 78:7611–7618
- Thomas DJ, Strachan N, Goodburn K, Rotariu O, Hutchison ML (2012) A review of the published literature and current production and processing practices in smoked fish processing plants with emphasis on contamination by *Listeria monocytogenes*. Final FSA report [http://foodbase.org.uk/admintools/reportdocuments/775-1-1323\\_FS425012.pdf](http://foodbase.org.uk/admintools/reportdocuments/775-1-1323_FS425012.pdf). Accessed 25 Jan. 2015
- Tocmo R, Krizman K, Khoo WJ, Phua LK, Kim M, Yuk HG (2014) *Listeria monocytogenes* in vacuum-packed smoked fish products: occurrence, routes of contamination, and potential intervention measures. Compr Rev Food Sci F13:172–189
- Toranzo AE, Novoa B, Romalde JL, Nunez S, Devesa S, Marino E, Silva R, Martizen E, Figueras A, Barja JL (1993) Microflora associated with healthy and diseased turbot (*Scophthalmus maximus*) from 3 farms in Northwest Spain. Aquaculture 114:189–202
- Trust TJ (1975) Bacteria associated with the gills of salmonid fishes in freshwater. J Appl Bacteriol 38:225–233
- Trust TJ, Sparrow RAH (1974) The bacterial flora in the alimentary tract of freshwater salmonid fish. Can J Microbiol 20:1219–1228
- Tsubokura M, Aleksic SA (1995) Simplified antigenic scheme for serotyping of *Yersinia pseudotuberculosis*: phenotypic characterization of reference strains and preparation of O and H factor sera. Contrib Microbiol Immunol 13:99–105
- Vestby LK, Moretto T, Langsrud S, Heir E, Nesse LL (2009) Biofilm forming abilities of *Salmonella* are correlated with persistence in fish meal- and feed factories. BMC Vet Res 5:20
- Wagner M, McLaughlin J (2008) Biology. In: Liu D (ed) Handbook of *Listeria monocytogenes*. CRC Press, Washington, pp 3–27
- Wang Y, Zhao A, Zhu R, Lan R, Jin D, Cui Z, Wang Y, Li Z, Wang Y, Xu J, Ye C (2012) Genetic diversity and molecular typing of *Listeria monocytogenes* in China. BMC Microbiol 12:119
- Wauters G, Kandolo K, Janssens M (1987) Revised biogrouping scheme of *Yersinia enterocolitica*. Contrib Microbiol Immunol 9:14–21
- Weber JT, Hibbs RG Jr, Darwish A, Mishu B, Corwin AL, Rakha M, Hatheway CL, El-Sharkawy S, El-Rahim SA, Al-Hamd MF, Sarn EJ, Blake PA, Tauxe RV (1993) A massive outbreak of type E botulism associated with traditional salted fish in Cairo. J Infect Dis 167:451–454
- White AP, Gibson DL, Kim W, Kay WW, Surette MG (2006) Thin aggregative fimbriae and cellulose enhance long-term survival and persistence of *Salmonella*. J Bacteriol 188:3219–3227
- Youssef H, El-Timawy AK, Ahmed S (1992) Role of aerobic intestinal pathogens of fresh water fish in transmission of human diseases. Food Control 4:34–40
- Zmyslowska I, Lewandowska D, Nowakowski T, Kozłowski J (2001) Occurrence of bacteria in water and in vendace (*Coregonus albula*) during rearing in tanks. Pol J Environ Stud 10:51–56