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Review paper

Bacterial hazards in fish meat: The aetiologic agents of foodborne diseases

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A b s t r a c t: Nowadays, consumers, fish industry professionals and scientists are increasingly directing their attention towards safety requirements associated with the consumption of fish due to the presence of bacterial hazards. There is no doubt that good knowledge and management of bacterial hazards associated with the consumption of fish are of great economic and medical importance. This study describes the bacterial pathogens that most often cause fishborne outbreaks of disease (Vibrio, Escherichia coli, Staphylococcus aureus, Salmonella, Aeromonas, Clostridium, Campylobacter, Listeria), as well as some individual disease outbreaks. In addition, the measures that can be implemented to reduce the risk of bacterial hazards related to consumption of fish are reviewed. The most important risk factor regarding bacterial zoonoses where fish was a vector was consumption of raw or undercooked processed fish, including cold smoked fish, but recontamination is also an important risk factor.
***Key words:** fish, microbiological safety, monitoring, zoonotic diseases, heat treatment.*

Introduction

The fish trade is bringing increasing economic benefits at the global level, but given the facts that natural resources are limited and that further increases in the amount of wild fish harvested is unsustainable, aquaculture is becoming one of the fastest growing industries in the production of food of animal origin. The rapid growth of aquaculture production is undoubtedly the result of increased demand for fish. One of the reasons is the well-known fact that fish meat is, to a much lesser extent, the cause of zoonotic diseases compared to the meat of other farm animals (*Baltic et al.,* 2009). In addition, processed fish products contain much fewer additives than meat products from other species (*Okanovic et al.,* 2013ab). It is well known that fish meat is a valuable source of nutrients that are present in optimal amounts for human requirements (*Cirkovic et al.,* 2011; 2012; *Ljubojevic et al.,* 2013abc) and the fact that this is a very high-quality food is the main reason for increased consumption of fish meat worldwide. Despite the global increase in the consumption of fish meat, average annual fish consumption per capita in Serbia is significantly lower than the global and European average – only 7 kg (*Janjic et al.,* 2015). Increased fish consumption and changed consumer habits will likely result from continuously promoting this highly valuable food.

Scientific research related to aquaculture is of increasing importance and objectives of this research are improved production (*Spriric et al.,* 2009; *Trbovic et al.,* 2009; *Ljubojevic et al.,* 2013d), as well as the preservation of sensory properties of fish meat (*Babic et al.,* 2009, 2014, 2015). A large number of authors from different countries have studied the chemical composition of fish meat, as well as its nutritive value (*Vranic et al.,* 2010; 2011; *Zsuzsánna et al.,* 2011; *Trbovic et al.,* 2013; *Ljubojevic et al.,* 2014; 2015; *Pavlicevic et al.,* 2014). Studies have also included the safety of fish meat, such as the content of chemical pollutants (heavy metals, persistent organic pollutants) in the fish (*Djinovic et al.,* 2010; *Trbovic et al.,* 2011; *Jankovic et al.,* 2012; *Djinovic-Stojanovic et al.,* 2013; *Djordjevic et al.,* 2013), as well as the microbiological quality of the fish meat (*Davies et al.,* 2001; *Cabrera-García et al.,* 2004; *Milijasevic et al.,* 2012). Today, the attention of consumers as well as fish industry professionals and scientists is increasingly directed towards the safety requirements associated with the consumption of fish meat, due to the presence of bacterial hazards. Taking these factors into account, there is a need to emphasise the bacterial pathogens that most commonly cause illness after consumption of fish meat.

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In addition, the measures that can be implemented to minimise the risk to human health of bacteriological hazards in fish meat are described in the present study. The main objectives of this study were to describe the most important bacterial hazards in fish meat, to point out the most important measures to ensure the safety of fish meat and to raise public awareness when it comes to the safety of fish meat.

Fish as the cause of foodborne diseases

The main causes of foodborne illness according to the available epidemiological data and risk analyses are microorganisms and microbial toxins (*Buncic and Katic*, 2011). Diseases caused by fish contaminated with microorganisms usually occur after consumption of inadequately heat-treated fish or fish products, including cold-smoked fish, which can be contaminated both during and after production. Outbreaks of foodborne illness, including food poisoning, associated with the consumption of fish and fish products have been recorded in almost all European countries (*EFSA,* 2014), as well as countries around the world. This indicates that the microbiological safety of fish meat on the market is a very important parameter in terms of public health, and there is great public interest because consumption of these products can lead to serious health problems. *Huss et al.* (2000a) found that about 12% of disease outbreaks caused by fish in the United States (US) were of bacterial aetiology (*Clostridium botulinum*, *Escherichia coli*, *Salmonella*, *Staphylococcus*, *Vibrio*, *Bacillus cereus*). *Shigella*, *Vibrio*, *Aeromonas*, *E. coli* and *Campylobacter* are pathogenic bacteria that can be transmitted to humans (*Djordjevic et al.,* 2012; *Raissy et al.,* 2014). *Listeria*, *Salmonella*, *Staphylococcus aureus* and *C. botulinum* type E are potential hazards in cold smoked fish (*Gram,* 2001; *Gram and Dalgaard,* 2002; *Dondero et al.,* 2004). Pathogens that are naturally found in water (*C. botulinum* type E, *Vibrio*, *Aeromonas*) or generally in the environment (*C. botulinum* types A and B, *Listeria monocytogenes*) can normally be found in both live fish and fish immediately after slaughter (*Huss et al.,* 2000a). However, if they are in insufficient numbers to cause illness, they do not pose a risk to consumers. Thus, the International Commission on Microbiological Specifications for Foods (ICMSF) found that food containing less than 100 *L. monocytogenes* g^{-1} is not a risk to the health of people who are not particularly vulnerable (*Vaz-Velho et al.,* 2001). A serious health concern exists when a very low infectious dose is capable of inducing disease, as is the case for *Salmonella, Shigella, E. coli* O157:H7, or in cases where there is potential for growth and the production of toxins (such as toxin production by *C. botulinum* type E) in raw or processed fish (*Huss et al.,* 2000a). It is known that fish meat is very susceptible to microbial deterioration/spoilage, mostly due to *Shewanella putrefaciens*, *Pseudomonas*, *Vibrio* and *Aeromonas* (*Milijasevic et al.,* 2010).

The health-hygienic properties of farmed fish meat are influenced by the entire production chain, from the environmental conditions in the pond, the quality of food used to feed fish, handling during harvesting, slaughter, evisceration and further processing to storage, transport and sale (*Orban et al.,* 2008; *Milijasevic et al.,* 2012), which forces producers along the chain to fulfil requirements associated with quality, and above all, consumer safety. It should be noted that modern principles that apply to food safety, including Good Hygienic Practice (GHP), Good Manufacturing Practice (GMP) and Hazard Analysis and Critical Control Points (HACCP) are required to improve the safety of fish meat for consumers. The main objective of HACCP is the control of biological, chemical and physical harmful agents that could be hazardous to human health (*Joffraud et al.,* 2001, 2006; *Djordjevic et al.,* 2006).

It is very often difficult to determine the exact number of people who suffer from a bacterial disease occurring as a result of fish meat consumption, since in most cases, especially when it comes to diseases of the gastrointestinal tract, they are not reported because symptoms usually do not last long in healthy individuals. In addition to exposure to environmental factors, internal factors, such as the physiological state of an individual, and especially their immunosuppression and stress status, contribute significantly to the development of infectious diseases. Temperature, water activity and pH are among the most important factors affecting the survival and growth of bacteria in fish meat and fish meat products, and this has led to these factors being used in different processes and heat treatments in order to ensure longer shelf life of fish meat, as well as to prevent the occurrence of foodborne illness in humans. Compulsory hygiene requirements for staff handling food are found in EU legislation (*European Council*, 2004). Microbiological criteria, including sampling plans and methods to be applied in the analyses are provided when there is a need to protect public health. Microbiological criteria for fish and fish meat products require quantification of *E. coli* and pathogenic *V. parahaemolyticus* and are implemented during production. At the end of the production cycle, monitoring measures are quantification of *S. aureus* and detection of

Salmonella, and their presence indicates recontamination of the finished product (*European Council*, 1991).

The microbiological quality of fish on the market is of concern for public health due to the incidence of foodborne diseases associated with the consumption of fish and fish products in European countries (*EFSA*, 2014). The European Union (EU) has established monitoring systems which include notification and management for biological hazards in foods including fish meat. The systems are supported by EU legislation and coordinated by the European Commission (EC), the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) (*Ribó et al.*, 2009). Collection of relevant data on biological hazards is crucial and these data contribute to risk assessments conducted by EFSA. Taking into account the opinion of the Scientific Committee on Veterinary Measures relating to Public Health, the EU Commission adopted microbiological criteria for foods (*European Commission*, 2005). Harmonisation of legislation in the field of food safety in Serbia with the EU includes the harmonisation of legislation on microbiological criteria for foods. In 2010, the Ministry of Agriculture, Forestry and Water Management adopted a new food hygiene regulation (*Serbia*, 2010) which is compliant with EU regulation (*European Commission*, 2005) and in accordance with Serbian food safety law (*Serbia*, 2009). Undoubtedly, though, there is still a need to establish permanent monitoring, improve the management of microbiological hazards, and utilise all other elements in risk analysis to make the Serbian food safety system function according to the principles of risk analysis.

The most important bacterial hazards isolated from fish

Vibrio

Consumption of raw or inadequately cooked fish infected with *Vibrio* can cause gastroenteritis in humans (*Raissy et al.,* 2012). *Vibrio parahaemolyticus*, in most cases, produces acute gastroenteritis which usually passes unreported and has a very short duration, but in some cases hospitalisation is necessary, and very rarely, it leads to septicaemia. *V. parahaemolyticus* is frequently isolated from fish and other aquatic organisms throughout the year in tropical areas and during the summer in areas with moderate or cold climates (*Cabrera-García et al.,* 2004). Some traditional Asian dishes such as fish-balls, fried mackerel, tuna and sardines have been associated with infections caused by *V. parahaemolyticus*. These meals can include both raw and undercooked fish and fish products, or heat-treated products which are subject to recontamination (*Baffone et al.,* 2005). *V. parahaemolyticus* was first established as a foodborne pathogen in Japan in the 1950s (*Fujino et al.,* 1974). In the suburbs of Osaka in Japan in October 1950, 272 patients developed gastritis and 20 people died as a result of the consumption of semi-dry small sardines known as shirasu. The occurrence of diarrhoea caused by *V. parahaemolyticus* was recorded in Japan and Taiwan after consumption of inadequately cooked fish and raw local dishes such as sushi and sashimi (*Vuddhakul et al.,* 2000). Disease caused by *V. parahaemolyticus* occurs most often in Japan, Taiwan and other Asian coastal regions, although cases of outbreaks of disease have been observed in many countries around the world. However, cases of disease caused by *V. parahaemolyticus* in Europe occur sporadically. During twenty years, only two cases of *V. parahaemolyticus* gastroenteritis were recorded in Denmark (*Joseph et al.,* 1982). *Smolikova et al.* (2001) isolated *V. parahaemolyticus* and *Vibrio alginolyticus* from patients during an outbreak of acute enteric diseases in Russia in 1997 and the *V. parahaemolyticus* isolates from humans produced thermostable exotoxin haemolysin. They proved that the alimentary toxico-infection was caused by *V. parahaemolyticus* O3:K6. Acute enteric disease was described in Russia in 1999 (*Boĭko,* 1999), wherein the aetiological agents were *V. fluvialis* (30.3%), *V. parahaemolyticus* (27.3%), *V. costicola* (21.2%) and *Photobacterium damselae* (21.2%) and according to the results of that study, vibriosis inducers, with the exception of *V. costicola*, were more associated with contamination of water than with contamination of fish. In the US, the occurrence of gastroenteritis in 14 people was recorded as a result of *V. parahaemolyticus* during the 1970s, and sporadic cases were recorded during the 80s and 90s, whereby 59% of the cases manifested as gastroenteritis, 34% as wound infections, 5% as septicaemia, and 2% as other symptoms, with most of these disease incidences occurring during the hot summer months and being associated with marine fish, and especially with molluscs (*Daniels et al.,* 2000). Normally, rehydration is sufficient for recovery after food poisoning caused by *V. parahaemolyticus*, and the use of antibiotics should be reserved for serious cases of illness that last a long time. *V. alginolyticus* has been described in fish and shellfish in Europe (*Di Pinto et al.,* 2005).

Vibrio cholerae

Cholera is a highly contagious disease caused by infection of the small intestine with *Vibrio cholerae* O1 or O139 and is characterised by severe acute diarrhoea, vomiting and consequent dehydration, while death may occur due to severe and untreated infections (*Colwell,* 1996). *V. cholerae* is mainly transmitted through water, although fish and fish products that have been in contact with contaminated water or faeces of infected persons can also be a source of infection (*Rabbani and Greenough,* 1999). *Kam et al.* (1995) recorded 12 outbreaks of cholera caused by *V. cholerae* biotype El Tor inaba, which occurred in Hong Kong for a period of three weeks in June and July of 1994. Only adults of both sexes were affected. Epidemiological research connected all cases with the consumption of seafood, including mussels, crabs and shrimp, and microbiological test results showed that contaminated sea water in the holding tanks used to keep the animals was most likely the source of the infection. Extensive control measures were implemented that involved a ban on the use of contaminated water in the tanks, rigorous microbiological control of used sea water, control of storage and more. These measures were enhanced through an active campaign and health education on food safety and personal hygiene and abruptly ended the epidemic.

Escherichia coli

The presence of *Enterobacteriaceae* in food and water is a common cause of diarrhoea and dysentery, especially among children, and *E. coli* is a classic example of a bacterium that leads to the onset of the disease. *E. coli* is the most common enterobacteria that causes gastrointestinal illness, and it is known that these diseases are the most common causes of mortality and morbidity in developing countries. It is important to note that in addition to other coliform bacteria, the presence of *E. coli* indicates poor hygienic conditions during processing of fish, because these microorganisms are not found on freshly harvested fish (*Milijasevic et al.,* 2012). Contamination of fish or fish products with pathogenic strains of *E. coli* most likely occurs during handling or processing of fish. Fish and fish products are often the vector of infection, especially if they are sold at local fish markets that do not meet the elementary hygienic conditions (*Vieira et al.,* 2001). Verocytotoxic *E. coli* (VTEC) are a group of *E. coli* which are characterised by the ability to produce verocytotoxins (synonym Shiga-like toxin). Most reported cases of human infection with VTEC are sporadic cases. The symptoms associated with VTEC infection in humans range from mild to bloody diarrhoea, which is usually accompanied by stomach cramps, commonly without the appearance of fever. VTEC can lead to haemolytic uremic syndrome (HUS), characterised by acute renal failure, anaemia and decrease in the number of platelets. HUS occurs in approximately 10% of patients infected with VTEC O157:H7 and this syndrome is the leading cause of acute kidney failure in children. Fish and fish products were the vector in 9.2% of foodborne outbreaks of VTEC associated disease in the EU in 2012 (*EFSA,* 2014). In Vietnam, *E. coli* was isolated from raw fish (*Dao and Yen,* 2006). *Mitsuda et al.* (1998) described disease that was manifested by diarrhoea, and caused by ingestion of food contaminated with enterotoxigenic *E. coli* (ETEC) in Japan. The outbreak was recorded in four Japanese primary schools in 1996 and spread to more than 800 people, while the disease itself was associated with tuna pâté. Examination of faeces from symptomatic patients revealed the presence of ETEC O25:NM that produced a heat stable toxin. *Vieira et al.* (2001) isolated 18 strains of ETEC in 3 of 24 samples of raw fish taken from the market in Brazil, 13 of which produced heat-labile enterotoxin. *Ayula et al.* (1994) also isolated (from 37.7% of samples) 317 *E. coli* isolates from fish in Brazil. *Asai et al.* (1999) described the occurrence of diseases caused by salted salmon roe most likely contaminated during the manufacturing process with enterohaemorrhagic *E. coli* O157:H7 in Japan in 1998. *Pierard et al.* (1999) recorded infection with VTEC as a result of fish consumption in Belgium.

Staphylococcus aureus

S. aureus is a pathogen of public health concern in fish and fish products (*Vieira et al.,* 2001). *Ayula et al.* (1994) concluded that much more care should be taken during harvesting and post-harvest handling of fish and seafood in order to reduce contamination with *S. aureus*. In research carried out by *Ayula et al.* (1994) on fish, 20% of 175 samples examined carried *S. aureus*, including 60% of the samples of shellfish meat. However, only 9 of 109 strains of *S. aureus* produced enterotoxins, including enterotoxin A (4), D (1) and AB (4). During growth in foods, many strains of *S. aureus* produce enterotoxins, which can cause staphylococcal food poisoning. Seven different enterotoxins have been identified: A, B, C1, C2, C3, D and E. Enterotoxin A is the most common enterotoxin involved in cases of *S. aureus* food poisoning (*Bergdoll,* 1990). In cases of food poisoning with *S.* aureus, vomiting and diarrhoea typically occur 2 to 6 hours after intake of food containing one or more enterotoxins (*Ayula et* *al.,* 1994). *Vieira et al.* (2001) detected, in 3 of 10 samples of fresh fish, higher counts of *S. aureus* than allowed according to Brazilian legislation. *Eklund et al.* (2004) studied *S. aureus* during the process of drying and smoking salmon in Alaska. During the process, the *S. aureus* counts increased to more than 105 CFU g^{-1}, after 2 to 3 days. Subsequent laboratory tests determined that there was rapid wrinkling of the skin on the smoked fish when there was strong air circulation in the smoking room, resulting in the bacteria being tied to or pulled into the creases, and thus, they were able to grow in spite of smoke deposition. The elimination of drying as a preceding process and reduction of the air flow during smoking resulted in deposition of smoke before creases formed on the skin and allowed the product to more quickly reach the stage where the salt and water activity inhibited the growth of *S. aureus*. This modification was then applied during fish drying and *S. aureus* was not isolated from the final product.

Salmonella

Salmonellosis in humans is usually characterised by acute occurrence of high temperature, fever, abdominal pain, nausea and vomiting after the incubation period, which lasts 12-36 hours (*Round and Mazmanian*, 2009). Symptoms are usually mild and in such cases the disease lasts only for several days, but the illness can be more serious in some patients, particularly when dehydration occurs, and it can lead to a lethal outcome. However, the mortality rate is usually very low, below 1% in patients who are diagnosed with *Salmonella* (*Day et al.,* 2011). *Friesema et al.* (2012) recorded a large number of cases of infection with *S.* Thompson in 2012, where the vector was smoked salmon, and some cases developed systemic infections such as septicaemia. Salmonellosis is also associated with long-term and sometimes chronic consequences, such as reactive arthritis. In the view of *Das et al.* (2010), since no clinical symptoms caused by *Salmonella* appear in fish, they are probably just passive carriers of *Salmonella*. However, the importance of fish in human salmonellosis is that they excrete *Salmonella* into the environment. *Fenlon* (1983) observed that aquatic birds carry *Salmonella* strains which can be found in the environment and, consequently, *Salmonella* can be isolated from pond-farmed fish (*Lotfy et al.,* 2011). In the Czech Republic and Latvia, *Salmonella* was not isolated in fish from streams, or farmed fish (*Hudecova et al.,* 2010; *Terentjeva et al.,* 2015). In contrast, in Germany, salmonellosis caused by *S.* Blockley where the vector consumed was smoked eel carrying *Salmonella*, originating from fish farm ponds in Italy as reported by *Fell et al.* (2000) who further stated that the smoking process does not eliminate bacterial contamination of raw fish. *Ling et al.* (2002) described illness caused by multi-drug resistant *S.* Typhimurium DT104L, after consumption of dried anchovies in Singapore. In Kenya and Malaysia, the presence of *Salmonella* in fish meat was associated with poor hygiene and unsanitary handling of fish during harvesting, processing and sale (*David et al.,* 2009; *Budiati et al.,* 2013).

Aeromonas

Foodborne gastroenteritis caused by *Aeromonas* (*Feldhusen,* 2000) is described in people of all ages and is particularly prevalent among vulnerable groups such as very young children and older immunocompromised people. *Aeromonas hydrophila* has been isolated from freshwater fish, crustaceans and molluscs (*Tsai and Chen* 1996; *Karabasil et al.,* 1999*; Djordjevic et al.,* 2012). *Karabasil et al.* (2002) studied motile *Aeromonas* in fish and other seafood products obtained from Belgrade retail markets in Serbia. They isolated nine motile *Aeromonas* from 78 fish/seafood products, three *A. sobria* and six *A. hydrophila*. Eight isolates were from freshwater fish and one from marine fish. Enteropathogenic strains are generally *Aeromonas veronii* sobria and *A. hydrophila*. *Aeromonas* can produce different exotoxins (*Karabasil et al.,* 2002) some of which are enterotoxins.

Clostridium

Khatib et al. (1994) reported that *Clostridium perfringens* was the causative agent of food poisoning after consumption of tuna salad. *Weber et al.* (1993) described a case of botulism after consumption of fish salads, and the causative agent was *C. botulinum* type B. *Telzak et al.* (1990) reported eight cases of type E botulism where the epidemiological survey found that all eight patients consumed kapchunka, fish that are not eviscerated, but are salted and air dried whole. There was no record that the fish had been prepared incorrectly, but nonetheless, the small amount of salt in the abdominal cavity and internal organs allowed the multiplication of bacteria and toxin production.

Campylobacter

The average incubation time for *Campylobacter* in humans is from two to five days. Mild or serious symptoms can appear in patients, and common clinical symptoms include watery and sometimes bloody diarrhoea, abdominal pain, fever, headache and nausea, while infections are usually self-limiting and only last for few days. In rare cases, extra intestinal infection or post-infection complications can occur, such as reactive arthritis and neurological disorders. It is important to note that *Campylobacter jejuni* is the most common predecessor of Guillain-Barré syndrome, a form of paralysis that can lead to respiratory disorders and severe neurological disorders and even death. *Campylobacter* is commonly found in various types of food, including meat, raw milk and dairy products, fish and fish products, a variety of seafood and fresh vegetables (*Kumar et al.,* 2001). The presence of *Campylobacter* has been recorded in fish and fish products from around the world (*Feldhusen,* 2000; *Raissy et al.,* 2014).

Listeria

Listeriosis occurs rarely in humans, but it is a very serious and severe zoonotic disease with high morbidity, a large number of hospitalisations, and a very high mortality rate in susceptible groups. Foods which are suitable for the growth of *Listeria* include thermally unprocessed food, food that is stored for a long time, food that is produced in unsanitary production facilities, and ready-to-eat prepared meals. Listeriosis is characterised by mild but also more severe symptoms such as meningitis, encephalitis, septicaemia, which usually occur in susceptible groups. The occurrence of *L. monocytogenes* in smoked salmon is very common (*Vaz-Velho et al.,* 2001; *Rotariu et al.,* 2014), and therefore is an issue of public health concern since this fish product is usually consumed without further heat treatment. *Kuzmanovic et al.* (2011) examined fish, fish products and seafood from the Serbian market, including chilled fresh fish, frozen foods (fish and seafood – cuttlefish, squid, octopus, clams, crabs and shrimp), breaded products, smoked fish, salted fish, heat-treated fish and canned fish for *Listeria*. *Listeria* was detected in 58 samples (12.34%) of fish, fish products and seafood. Among the isolates, nine (15.52%) were *L. monocytogenes* (1.92% of the fish harboured this species). Other *Listeria* species found were: *L. innocua* (8.51%), *L. welshimeri* (1.28%), *L. welshimeri/innocua* (0.21%), *L. grayi* (0.21%) and *L. seeligeri* (0.21%). The presence of other species of *Listeria* in fish and fish products indicates failures of GHP during production (*Round and Mazmanian*, 2009). The prevalence of *L. monocytogenes* recorded in freshwater farmed and wild fish harvested in Denmark and Finland was 8.6% and 14.6%, respectively (*Vogel et al.,* 2001 ; *Miettinen and Wirtanen,* 2005). During 2012, 17 EU member states reported information on the presence of *L. monocytogenes* in fish prepared for consumption and fish products (*EFSA,* 2014). Smoked fish was the product that was most commonly monitored, and most of the tests were carried out within the production plant. During 2012, the presence of *L. monocytogenes* in fish prepared for consumption was found in 12 out of 16 qualitative studies. In total, *L. monocytogenes* was found in 12.0% of the total surveyed 10,831 units, although the lack of representativeness must be noted when interpreting the overall results, since most of the samples were from Poland. Additionally, *L. monocytogenes* was recorded in 9 of 16 quantitative studies of fish stored for consumption in 2012, wherein a total of 6,141 units were tested, and in six studies, counts >100 cfu g^{-1} were obtained. *L. monocytogenes* counts of over 100 cfu g^{-1} were recorded in 1.4% of the fish samples tested by the counting method in 2012 (compared to 0.5% in 2011). However, this increase can be attributed to the large number of samples from Poland included in the study. Listeriosis associated with vacuum-packed, raw, thinly-sliced cold-smoked fish was described in at least eight people during 11 months in Sweden (*Tham et al.,* 2000). Cold-smoked, raw and thinly-sliced trout (*Oncorhynchus mykiss*) and salmon (*Salmo salar*) have been the focus of attention in recent years as potential sources of infection with *L. monocytogenes*. Studies have shown that up to 10% of vacuum-packed products in retail contain *L. monocytogenes* (*Norton et al.,* 2001). In the Czech Republic, two deaths from *Listeria* infection resulted from consumption of herring without heat treatment.

The importance of heat treatment and the cold chain

Heat treatment during the cooking process eliminates the risk of pathogens such as *Salmonella, Shigella* and *E. coli* (plus enteric viruses) which contaminate fish prior to harvesting and can pose a risk, since in some cases, a very low infectious dose is sufficient to produce disease (*Huss et al.,* 2000a). Therefore, the risk of these pathogens causing disease in humans is primarily related to the consumption of raw fish. *Gould* (1999) found that the combination of heat treatment at 90°C for a period of 10 minutes led to a 6 log reduction in numbers of spores of psychrotrophic *C. botulinum*, so this heat treatment is undoubtedly advantageous. Heat treatment at 70°C for 2 min ensures destruction of *L. monocytogenes* in fish (*Huss et al.,* 2000b). Moderate heat treatment is acceptable for products with a short storage time, or where any potential growth of pathogens can be prevented.

Cold-stored fish products can allow the survival or growth the survival of psychrotrophic pathogens (*Huss et al.,* 2000a). Reducing bacterial proliferation is achieved by rapid cooling of fish immediately after harvest, thus somewhat reducing the risk to human health. A range of pathogenic bacteria can be isolated from fresh fish, especially *L. monocytogenes, C. botulinum* types A and B, *C. perfringens*, *Bacillus*, and also bacteria that are transmitted/carried by humans or animals (*Salmonella, Shigella, E. coli, S. aureus*) and rarely *C. botulinum* type E and non-proteolytic types B and F, pathogenic *Vibrio* species, *A. hydrophila,* and *Plesiomonas shigelloides*.

The impact of fish packaging on the growth of microorganisms

The biggest concerns when packing fish under anaerobic conditions are *C. botulinum* type E and non-proteolytic type B and *L. monocytogenes* (*Baltic et al.,* 2009). *Milijasevic et al.* (2010) examined chemical, physico-chemical and microbiological changes in carp steaks packaged in a modified atmosphere during fifteen days of storage. They found that the rate of the chemical and microbiological breakdown of fish meat can be affected by the way the product is packaged (*Milijasevic et al.*, 2010). Modified atmosphere packaging has a synergistic effect on bacterial growth compared with vacuum-packaging, as the 40 to 100% CO_2 in the headspace gas inhibits microbial growth, primarily *Pseudomonas, Vibrio* and *Aeromonas*. This gas diffuses into the fish tissue, dissolves in the aqueous phase and creates carbonic acid, which slows down the oxidation process. Nitrogen can be used to replace oxygen in the package, as it slows down the formation of rancidity and inhibits the growth of aerobic microorganisms. Oxygen is also used in the modified atmosphere packaging technology for fish, as it inhibits the growth of *C. botulinum* type E, which is often found in fish (Özogul and Özogul, 2006; *Radetic et al.,* 2007). Modified atmosphere packaging significantly reduces the total number of *Enterobacteriaceae* on fish (*Milijasevic et al.,* 2010). *Babic et al.* (2009) found that the shelf-life of chilled fresh fish can be extended in vacuum or modified atmosphere packaging.

Measures to reduce biological hazards in fish meat

The most important risk factor when it comes to bacterial zoonoses, as is the case for parasitic zoonoses (*Novakov et al.,* 2012; *Cirkovic et al.,* 2013), is the consumption of raw or inadequately heat processed fish meat. In addition, recontamination after heat processing is a significant risk factor. It is worth noting that the number of cases of disease caused by consumption of fish as a source and/or vector of pathogenic bacteria is very low when compared with the number of cases that are caused by the consumption of poultry meat, pork, beef and meat obtained from other farm animals (*Newell et al.,* 2010). Reduction of the initial microbial contamination is one of the main strategies in most countries aiming to reduce the risk associated with fish meat, but if conditions are suitable for pathogen growth, the initial level of contamination is of relatively little significance (*Beuchat*, 2006). With this in mind, more attention must be paid to the role of temperature, water activity, pH and other extrinsic factors which can significantly affect the microbiological safety of fish meat, as well as the interaction between these parameters to reduce the occurrence and/or numbers of pathogenic bacteria to a minimum. Preventing pre-harvest contamination with pathogens that are naturally found in the aquatic environment is either very difficult or in many cases, impossible, while hazards associated with contamination or recontamination during the processing of fish can be controlled by applying GMP, GHP and suitable HACCP programs (*Panisello and Quantick*, 2001). In addition, there are methods to prevent the growth of biological hazards during transportation and storage of fish meat and fish meat products (*Sivertsvik et al.,* 2002). Hazard analysis and qualitative assessment of the risk of microbiological contamination of fish meat are very important and a connection between these measures and the frequency of disease outbreaks caused by fish meat that is microbiologically contaminated has been observed (*Huss et al.,* 2000a). Continuous medical education is certainly a key factor in fighting zoonotic infections, and experience in many countries has shown that successful implementation of control measures requires broad cooperation between medical and veterinary bodies, including government departments at all levels, and in addition, the cooperation of and constant communication with the public is necessary. However, avoiding consumption of raw or inadequately cooked fish is still the best measure to prevent fishborne disease caused by bacterial pathogens.

Conclusions

The following conclusions and recommendations can be made:

- The microbiological quality and safety of fish available on the market is very important from the standpoint of public health.
- Good knowledge and management of microbiological hazards associated with the consumption of fish meat is of great economic and health importance.
- Fish can be contaminated with bacterial pathogens pre- or post-harvest, depending on the pathogen.
- The most important bacterial hazards isolated from fish which cause foodborne outbreaks include *Vibrio*, *E. coli*, *S. aureus*, *Salmonella*, *Aeromonas*, *Clostridium*, *Campylobacter*, *Listeria*.
- The most important risk factor for bacterial foodborne disease where fish is the vector is the consumption of raw or inadequately heat processed fish.
- Fish and fish products are at risk of recontamination during processing, and so this aspect of fish and fish product hygiene must be strictly controlled.
- Continuous monitoring, including appropriate microbiological testing of fish meat and fish meat products in order to determine the presence of zoonotic bacteria, is necessary.

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