Fish: a potential source of bacterial pathogens for human beings

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ABSTRACT: Human infections caused by pathogens transmitted from fish or the aquatic environment are quite common and depend on the season, patients' contact with fish and related environment, dietary habits and the immune system status of the exposed individual. They are often bacterial species facultatively pathogenic for both fish and human beings and may be isolated from fish without apparent symptoms of the disease. The infection source may be fish kept for both for food and as a hobby. Human infections and intoxications with the following bacteria have been recorded: *Mycobacterium* spp., *Streptococcus iniae, Photobacterium damselae, Vibrio alginolyticus, V. vulnificus, V. parahaemolyticus, V. cholerae, Erysipelothrix rhusiopathiae, Escherichia coli, Aeromonas* spp., *Salmonella* spp., *Staphylococcus aureus, Listeria monocytogenes, Clostridium botulinum, C. perfringens, Campylobacter jejuni, Delftia acidovorans, Edwardsiella tarda, Legionella pneumophila*, and *Plesiomonas shigelloides*. Fish tissue histamine intoxications of people have frequently been described. The purpose of the present paper was to elaborate an overview of significant bacterial causative agents of human diseases transmitted from fish used as food or by handling them.

Keywords: risk assessment; food safety; the Czech Republic; mycobacteriosis; *Mycobacterium avium* complex

List of abbreviations used:

A. = *Aeromonas*, ***C***. = *Clostridium*, ***D***. = *Delftia*, ***E***. = *Escherichia*, ***Edw***. = *Edwardsiella*, ***Erys***. = *Erysipelothrix*, ***L***. = *Listeria*, ***M***. = *Mycobacterium*, ***Ph***. = *Photobacterium*, ***Pl***. = *Plesiomonas*, ***Salm***. = *Salmonella*, ***Staph***. = *Staphylococcus*, ***Strep***. = *Streptococcus*, ***V***. = *Vibrio*

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1. Introduction

Human infections caused by pathogens transmitted from fish or the aquatic environment are quite common depending on the season, patients' contact with fish and related environment, dietary habits and the immune system status of the exposed individual. They are often bacterial species facultatively pathogenic for both fish and man and may be isolated from fish without apparent symptoms of disease. The infection source may be fish kept either for food or as a hobby (Acha and Szyfres, 2003). Thorough anamnesis and microbiological examination are the prerequisites for correct diagnosis. However, quantification of the occurrence of these diseases is difficult because many cases, typically gastrointestinal illness, go unreported; the symptoms usually do not last long and are self-limiting in healthy people.

It can be extremely difficult to detect certain *in vitro* slow growing causative agents of diseases such as those of mycobacterial infections or infections caused by anaerobic pathogens. Mycobacterial infections are quite often misdiagnosed with subsequent inappropriate therapy (Kern et al., 1989; Harth et al., 1994; Ryan and Bryant, 1997). Consequently, the disease can last for years (Ang et al., 2000).

The purpose of the present paper was to elaborate an overview of significant bacterial causative agents of human diseases transmitted from fish used as food or by handling them.

2. Aetiology of human bacterial infections

Pathogenic and potentially pathogenic bacteria associated with fish and shellfish include mycobacteria, *Streptococcus iniae*, *Vibrio vulnificus*, *Vibrio* spp., aeromonads, *Salmonella* spp. and others (Lipp and Rose, 1997; Zlotkin et al., 1998; Chattopadhyay, 2000). People most often get infected as follows (Acha and Szyfres, 2003):

(i) through contact with infected fish while handling them, water or other constituents of fish life environment; the following cases of transmissions have been recorded so far: after injury by cleaning aquarium with bare hands (Alinovi et al., 1993), after exposure to fish tank water (Kern et al., 1989), by handling tropical fish ponds (Guarda et al., 1992), by contact with rare tropical fish (Bhatty et al., 2000), after injuries from fish, e.g. by thorns (Said et al., 1998), after fish bite (Seiberras et al., 2000), through contact with fish living in the wild (Darie et al., 1993), by contact with a fresh- or salt-water environment (Hayman, 1991; Jernigan and Farr, 2000), infection of young children who are in contact with a fish tank (Bleiker et al., 1996; Speight and Williams, 1997), through processing fish in the food industry and preparation of dishes (Notermans and Hoornstra, 2000) or

(ii) orally by consumption infected fish or related products or food contaminated with water or other constituents of water environment.

Apart from factors relating to the living environment (exposure), the development of an infectious disease is markedly affected by internal factors such as the physiological status of consumer, particularly by immunosuppression and stress as in the case of *HIV/AIDS* (Von Reyn et al., 1996).

2.1. Bacterial pathogens transmissible to human beings through contact with fish living in the wild and fish in aquacultures

2.1.1. *Mycobacterium* spp.

Mycobacteriosis is particularly significant among infections transmissible from fish to human beings. Mycobacteriosis of fish is a chronic progressive disease spread all over the world. The disease affects sea, brackish and fresh water fish kept as in aquacultures, as in aquariums and free nature (Wolf and Smith, 1999). Many mycobacterial species, both rapid and slow growing, have been isolated from affected fish tissues (Wayne and Kubica, 1986).

However, the most frequently detected mycobacterial species were the following: *Mycobacterium marinum*, *M. fortuitum* and *M. chelonae*. *M. avium*, *M. gordonae*, *M. abscesus*, *M. aurum*, *M. parafortuitum*, *M. poriferae*, and *M. triplex* have also been isolated from fish (Arakawa and Fryer, 1984; Arakawa et al., 1986; Bragg et al., 1990; Dalsgaard et al., 1992; Tortoli et al., 1996; Teska et al., 1997; Bruno et al., 1998; Talaat et al., 1999; Diamant et al., 2000; Lehane and Rawlin, 2000; Herbst et al., 2001; Perez et al., 2001; Lescenko et al., 2003).

Mycobacteriosis of aquarium fish in the Czech Republic is one of the most commonly diagnosed bacterial diseases (Navratil et al., 2000; Novotny et al., 2004). Gross changes may include emaciation, ascites, exophthalmos, and keratitis, changes in pigmentation and skin ulcerations (Wolf and Smith, 1999).

The significance of fish mycobacteriosis as zoonosis is evident from case reports published in scientific papers. Fish have been convincing sources of many cases of mycobacteriosis diagnosed abroad. Ninety-nine publications dealing with the infection of 652 cases of human beings with *M. marinum* appeared between 1966 and 1996. Of 193 infections with known exposures, 49% were associated with aquarium environment, 27% with injury by aquarium fish and 9% with injury during bathing in sea or brackish water (Jernigan and Farr, 2000). *M. marinum* often infects home aquarium hobbyists. With the expansion of aquaculture and popularity of recreational fishing in Australia, medical practitioners can expect to see more infections of this nature. Diagnosis and treatment may be difficult, especially in view of emerging antibiotic resistance in fish pathogens (Lehane and Rawlin, 2000).

Biopsy and histopathological examination should always be performed in injured aquarists; the presence of infectious granulomas can thus be revealed. The staining of histological specimens according to Ziehl-Neelsen (ZN) to detect mycobacteria is often negative as is the culture which should be performed under conditions allowing growth of atypical mycobacteria which usually require a lower *in vitro* culture temperature (about 25 to 30°C), through they can also grow when the temperatures reach about 37°C (Wayne and Kubica, 1986; Ang et al., 2000; Bhatty et al., 2000; Matlova et al., 2003). It is recommended to use biochemical examination or PCR detection for identification of the causative agent (Wayne and Kubica, 1986; Dvorska et al., 2001; Collina et al., 2002).

Combined therapy with tuberculostatic agents and antibiotics is necessary. A combination of claritromycin, ethambutol, and rifampin administered for 3 to 4 months is suitable for treating skin granulomas in the case of torpid lesions, these may be excised (Aubry et al., 2002; Lewis et al., 2003). Other medications used for the therapy of „fish tank granuloma“are cotrimoxazol-trimethoprim and minocycline which must be administered for 4 to 38 weeks (Ang et al., 2000).

2.1.2. *Streptococcus iniae*

Strep. iniae causes meningoencephalitis and death in cultured fish species (Bercovier et al., 1997) but may also be an emerging human pathogen associated with injury while preparing fresh aquacultured fish. Between 1995 and 1996 four cases of bacteraemia infections were identified among patients at a hospital in Ontario (Anonymous, 1996). Three were accompanied by cellulitis and the fourth with infective endocarditis, meningitis, and probable septic arthritis. *Strep. iniae* was isolated from all four patients and the cases were associated with previous injury during preparation of fresh, whole fish. Cases of cellulitis and lymphangitis of the hand and endocarditis in patients after skin injuries during the handling of fresh fish have also been described by Weinstein et al. (1996, 1997). The authors identified by pulsed – field gel electrophoresis (PFGE) analysis a clone of *Strep. iniae* that causes invasive disease in both humans and fish (Weinstein et al., 1997).

2.1.3. *Photobacterium damselae*

Ph. damselae (syn. *Vibrio damsela*) belongs among the important vibrios of medical importance. *Ph. damselae* sepsis was described by Perez-Tirse et al. (1993) in a 70-year old man after suffering a knife cut while filleting bluefish (*Pomatomus saltatrix*).

2.1.4. *Vibrio alginolyticus*

V. alginolyticus was described as the cause of *otitis media* in a child handling pressure-equalizing tubes. Infection with *V. alginolyticus* can occur even after mild, brief exposure to seawater. The interval between exposure to seawater and the onset of

clinical infection can be prolonged (Feingold and Kumar, 2004).

2.1.5. *Vibrio vulnificus*

V. vulnificus is a gram-negative bacterium which comprises two biotypes (Tison et al., 1982). Biotype 1 is an opportunistic human pathogen. Biotype 2 was considered an obligate eel pathogen but as reported by Amaro et al. (1995) it is also an opportunistic pathogen for humans and can be transmitted by water (Amaro and Biosca, 1996). The risk factors for severe *V. vulnificus* infections are chronic hepatic disease or immunodeficiency in patients (Ito et al., 1999; Osawa et al., 2002).

Primary septicaemia or the gastrointestinal illness in human beings may follow after ingesting raw seafood, particularly of ocean filter feeders (e.g. oysters and clams) and fish infected by *V. vulnificus.* For susceptible persons septicaemia is associated with a mortality rate greater than 50% and may be characterized by fevers, chills, and skin lesions like blisters, swelling, and purpuras (Kumamoto and Vukich, 1998; Osawa et al., 2002). Wound infections are seen after injury to the skin in a marine environment or from the exposure of pre-existing wounds to seawater (Kumamoto and Vukich, 1998; Borenstein and Kerdel, 2003; Calif et al., 2003). Dieng et al. (2001) described two cases of necrotising dermo-hypodermatitis due to *V. vulnificus* in fishermen after a penetrating scratch from a fish bone.

In the summer and autumn of 1996 and 1997, an outbreak of invasive *V. vulnificus* infection occurred in Israel in people who had recently handled fresh, whole fish purchased from artificial fish-ponds (Bisharat et al., 1999). Necrotising fasciitis of the knee at the site of amputation of a lower extremity caused by *V. vulnificus* after exposure to water from a tank containing fish was described by Miron et al. (2003). *V. vulnificus* can penetrate through to the organism after injury with quills and hard fish fin rays. Septicaemia can develop from the infection (Calif et al., 2003).

2.1.6. *Erysipelothrix rhusiopathiae*

This organism is a soil saprophyte which grows well in fish slime. *Erys. rhusiopathiae* has never been found in newly caught fish, but it regularly occurs in market fish, in fish boxes and on market floors in the summer months. *Erys. rhusiopathiae* was isolated from 60% of the retail cod samples and from 30% of the samples from herring (*Clupea harengus*) as described by Stenstrom et al. (1992). The bacterium is the causal agent of erysipeloid in fish handlers during the warm periods of the year (Chattopadhyay, 2000). In the study by Hjetland et al. (1995), fatal endocarditis after gutting an eel caused by *Erys. rhusiopathiae* infection was described. *Erys. rhusiopathiae* endocarditis was also found in a patient in Latin America after injuring his hand with a fish bone during fishing and cooking. The disease began in an erysipeloid form and developed into septicaemia and endocarditis (Rocha et al., 1989).

2.2. Foodborne pathogens associated with fish and fish products

From the standpoint of microbiology, fish and related products are a risk foodstuff group. Particularly *Clostridium botulinum* type E and *Vibrio parahaemolyticus* rank among pathogenic bacteria associated with fish. Other potentially pathogenic bacteria associated with fish and shellfish include *C. perfringens*, *Staph.* spp., *Salm.* spp., *Shigella* spp., *V. cholerae* and other vibrios. Outbreaks usually occur due to the ingestion of insufficiently heat-treated fish or products contaminated after/during their processing. Freezing fish and related products in the seawater, intensive handling, long-time transport or cooking in fishing containers straight on the deck contributes to their contamination with microorganisms.

Temperature and pH are limiting factors for the survival of bacteria in fish products; these facts are used during the processes of pasteurization and heat treatment, particularly of offal (Whipple and Rohovec, 1994). In the technology of marine animal processing by cooking, the following critical aspects of marine animals are significant: the duration of cooking, temperature of steam, water and other media used for the cooking, thickness of the cut cooked, accuracy of thermometer and other monitoring and timing devices.

Microbiological criteria for fish, fishery products, sea shellfish and molluscs have been elaborated both on international (Codex Committee on Food Hygiene) and European levels (competent European institution). The mandatory requirements on hygiene of foodstuffs are given in Regulation (EC) No. 852/2004 of the European Parliament and of

the Council of 29 April, 2004. Since this Regulation replaces Directive 93/43/EEC, the later should be repealed.

Microbiological criteria, including samples plans and methods of analysis, are laid down when there is a need to protect public health. Microbiological criteria for fish and fishery products include quantification of the counts of *Escherichia coli*, thermotolerant coliform, mesophilic aerobic bacteria and pathogenic *V. parahaemolyticus* is performed during the production. At the finished product stage, the measure monitored is the quantification of the count of *Staph. aureus* and detection of bacteria of *Salmonella* genus as their presence indicates recontamination of a finished product (Council Directive 91/493/EEC).

2.2.1. *Vibrio parahaemolyticus* and other vibrios

V. parahaemolyticus has been isolated from sea and estuary waters on all continents with elevated sea water temperatures. The agent's distribution shows marked seasonal variations in natural reservoirs. During cold months, it is found in marine sediment; during warm months, it is found in coastal waters, fish, and shellfish (Benenson, 1990). There have been a few reports of the isolation of *V. parahaemolyticus* from continental waters and fish in rivers or lakes. *V. parahaemolyticus* is frequently isolated from fish, molluscs, and crustaceans throughout the year in tropical climates and during the summer months in cold or temperate climates.

V. parahaemolyticus generally causes acute gastroenteritis that is self-limiting; however, several cases require hospitalization and, on rare occasions, septicaemia may occur. Fish food associated with illnesses due to consumption of *V. parahaemolyticus* includes fish-balls, fried mackerel (*Scomber scombrus*), tuna (*Thunnus thynnus*), and sardines (*Sardina pilchardus*). These products include both raw or undercooked fish products and cooked products that have been substantially recontaminated (Baffone et al., 2000).

V. parahaemolyticus was first identified as a foodborne pathogen in Japan in the 1950s (Fujino et al., 1953). The most affected by the disease are Japan, Taiwan, and other Asian coastal regions, though cases of disease have been described in many countries and on many continents. Generally no treatment other than re-hydration is required for food poisoning caused by *V. parahaemolyticus*. The use of antibiotics should be reserved for prolonged or severe cases.

Outbreaks of diarrhoea caused by *V. parahaemolyticus* have been demonstrated in Japan and Taiwan after the ingestion of undercooked fish and raw products Sashimi and Sushi (Anonymous, 1999; Vuddhakul et al., 2000). In the 1970s, *V. parahaemolyticus* was the cause of 14 outbreaks of gastroenteritis in USA (Barker et al., 1974). Between 1988 and 1997, 345 sporadic *V. parahaemolyticus* infections were reported in USA: 59% were gastroenteritis, 34% were wound infections, 5% were septicaemia, and 2% were from other exposures. Most of these outbreaks occurred during the warmer months and were attributed to seafood, particularly shellfish (Daniels et al., 2000). Cases of diseases caused by *V. parahaemolyticus* are occasional in Europe. During 20 years, only two cases of gastroenteritis were recorded in Denmark (Anonymous, 2001).

The interest in this organism has been widened by the finding that similar organisms, *V. alginolyticus* and group of F vibrios also cause serious disease in humans (Joseph et al., 1982). *V. parahaemolyticus* and *V. alginolyticus* strains were isolated from patients during an outbreak of an acute enteric disease in Vladivostok (Russia) in 1997 (Smolikova et al., 2001). Acute enteric infections caused by non-cholera vibrios were described in Russia also in 2000 (Boiko, 2000). Their etiological factors were *V. fluvialis* (30.3%), *V. parahaemolyticus* (27.3%), *V. costicola* (21.2%) and *Ph. damselae* (21.2%). As revealed in this study, the causative vibrios, with the exception of *V. costicola*, were more closely linked with the contamination of water than that of fish.

2.2.2. *Vibrio cholerae*

Cholera is a highly contagious disease, caused by infection of the small intestine with *V. cholerae* O1 and O139 and is characterized by massive acute diarrhoea, vomiting, and dehydration. Death occurs in severe and untreated cases. *V. cholerae* is often transmitted by water but fish or fish products that have been in contact with contaminated water or faeces from infected persons also frequently serve as a source of infection (Kam et al., 1995; Colwell, 1996; Rabbani and Greenough, 1999). The organism would be killed by cooking and recent cases of cholera in South America have been associated with the uncooked fish marinade seviche (*Cilus gilberti*) as

described by Kam et al. (1995). The infectious dose is relatively high and 10^6 to 10^8 organisms need to be ingested.

2.2.3. *Escherichia coli*

E. coli is a classic example of enteric bacteria causing gastroenteritis. *E. coli* including other coliforms and bacteria as *Staphylococcus* spp. and sometimes enterococci are commonly used as indices of hazardous conditions during processing of fish. Such organisms should not be present on fresh-caught fish (Chattopadhyay, 2000). The contamination of food of fish origin with pathogenic *E. coli* probably occurs during handling of fish and during the production process (Ayulo et al., 1994; Asai et al., 1999).

An outbreak of diarrhoeal illness caused by ingestion of food contaminated with enterotoxigenic *E. coli* was described in Japan (Mitsuda et al., 1998). The illness was strongly associated with eating tuna paste. Brazilian authors (Vieira et al., 2001) isolated 18 enterotoxigenic strains of *E. coli* (ETEC) from 3 of 24 samples of fresh fish originating from Brazilian markets; 13 of them produced a thermolabile enterotoxin.

The authors explained the presence of toxic strains of *E. coli* in samples collected from fish (not from water) from one fish market by a longer survival of bacteria on an adequate substrate, i.e. inside the living organism. The isolation of 317 *E. coli* isolates tested for thermostabile (ST) and thermolabile (LT) toxins has been described in another Brazilian study (Ayulo et al., 1994); only one produced ST and none produced LT toxin. Infection with verocytotoxin-producing strains of *E. coli* (VTEC) after ingestion of fish was recorded in Belgium (Pierard et al., 1999). An outbreak caused by salted salmon roe contaminated, probably during the production process, with enterohaemorrhagic *E. coli* (EHEC) O157 occurred in Japan in 1998 (Asai et al., 1999). The roe was stored frozen for 9 months but it appears that O157 could survive freezing and a high concentration of NaCl and retained its pathogenicity for humans (Semanchek and Golden, 1998).

2.2.4. *Aeromonas* spp.

Aeromonas spp. has been recognized as potential foodborne pathogens for more than 20 years. Aeromonads are ubiquitous in fresh water, fish and shellfish, and also in meats and fresh vegetables (Isonhood and Drake, 2002). The epidemiological results so far are, however, very questionable. The organism is very frequently present in many food products, including raw vegetables, and very rarely has a case been reported. A sepsis caused by *Aeromonas* is indeed dangerous (Lehane and Rawlin, 2000). The same *Aeromonas* species (primarily *A. hydrophila* HG1, *A. veronii* biovar sobria HG8/10, and *A. caviae* HG4) can cause self-limiting diarrhoea, particularly in children (Kirov et al., 2000). Up to 8.1% of cases of acute enteric diseases in 458 patients in Russia were caused by *Aeromonas* spp. (Pogorelova et al., 1995). In this study, *Aeromonas* spp. isolates with the same pathogenicity factors were isolated from river water in the Volga Delta, from fish, raw meat, and from patients with diarrhoea. Most *Aeromonas* spp. isolates are psychrotrophic and can grow at refrigerator temperatures (Fernandes et al., 1998). This could increase the hazard of food contamination, particularly where there is a possibility of cross-contamination with ready-to-eat food products.

Aeromonads (*A. hydrophila, A. sobria,* and *A. salmonicida*) can be causative agents not only of human enteritis (Sukroongreung et al., 1983), but also of a fatal septicaemia as recorded in a 15-year old healthy girl; the causative agent was *A. sobria* (Shiina et al., 2004). Aeromonads septicaemia most often caused by *A. hydrophila*, was described as a complication in 50 patients with liver cirrhosis (Qu et al., 2003) and eye infection in immuno-compromised patients (Tamura and Hida, 2003; Chou et al., 2004). Fatal bacterial pneumonia in a 5-year old child was also caused by *A. hydrophila* (Kao et al., 2003). *A. salmonicida* is the causative agent of the fish disease called furunculosis but human disease has not been described (Isonhood and Drake, 2002). Ouderkirk et al. (2004) described a case of meningitis caused by *A. veronii* biovar sobria associated with the use of leech therapy to salvage a skin flap after central nervous system surgery.

2.2.5. Salmonellosis

Fish and shellfish appear to be passive carriers of salmonella, demonstrate no clinical disease and can excrete *Salmonella* spp. without apparent trouble. The contamination of this organism derives from terrestrial sources and fish may serve as a vec-

tor for *Salmonella* spp. (Metz, 1980; Minette, 1986; Chattopadhyay, 2000). An outbreak of *Salm. blockley* infections following smoked eel consumption was described in Germany. The consumed eel came from four different local smokehouses, but could be traced back to fish farms in Italy (Fell et al., 2000). This outbreak indicates that eel may be a vector of *Salmonella* spp. infection and that the smoking process may not eliminate bacterial contamination from raw fish. *Salm. enterica* serotype Paratyphi B var. Java phage type Dundee was isolated from the stool of a 14-month old boy who suffered from diarrhoea, vomiting, and fever for two days. The same isolate was identified from the water of home fish tank (Senanayake et al., 2004). Fish was the vector of *Salmonella* spp. in this case.

Unusual *Salmonella* spp. serotypes were found in eight of 100 tropical aquariums sampled in Wales (Sanyal et al., 1987). In a Canadian outbreak of *Salm. enterica* serotype Paratyphi B linked to aquariums, five of seven cases were in children aged less than 10 years (Gaulin et al., 2002). Another outbreak caused by multidrug-resistant *Salm. enterica* subsp. *enterica* serotype Typhimurium DT104L was described in Singapore (Ling et al., 2002). Consumption of imported dried anchovy was found to be the cause of infection.

2.2.6. *Staphylococcus aureus*

Enterotoxins produced by *Staph. aureus* are another serious cause of gastroenteritis after consumption of fish and related products. In 3 of 10 samples of fresh fish, higher counts of *Staph. aureus* were detected than permitted by Brazilian legislation (Vieira et al., 2001). In the southern area of Brazil, *Staph. aureus* was isolated from 20% of 175 examined samples of fresh fish and fish fillets (*Cynoscion leiarchus*). However, only nine of 109 *Staph. aureus* isolates produced enterotoxins including enterotoxin A ($n = 4$), D ($n = 1$), and AB ($n = 4$) (Ayulo et al., 1994).

Staph. aureus has also been detected during the process of drying and subsequent smoking of eels in Alaska in 1993 (Eklund et al., 2004). During the process, *Staph. aureus* populations increased to more than 10^5 CFU/g of the analysed sample, after 2 to 3 days of processing. Subsequent laboratory studies showed that a pellicle (a dried skin-like surface) formed rapidly on the strips when there was rapid air circulation in the smokehouse and that bacteria embedded in/under the pellicle were able to grow even when heavy smoke deposition occurred. Elimination of pre-process drying and reduction in air flow during smoking resulted in smoke deposition before pellicle formation and enabled the product to reach levels of water-phase salt and water activity that inhibit the growth of *Staph. aureus*. In 1994, these modifications were then applied during processing at an Alaskan facility, and *Staph. aureus* could not be detected in the finished product (Eklund et al., 2004).

2.2.7. *Listeria monocytogenes*

L. monocytogenes is widely distributed in the general environment including fresh water, coastal water and live fish from these areas. Contamination or recontamination of seafood may also take place during processing (Huss et al., 2000). Moreover, *L. monocytogenes* is a psychrotrophic pathogen with the ability to grow at refrigerator temperatures (Bayles et al., 1996).

The outbreak of listeriosis related to vacuum-packed gravad and cold-smoked fish was described in at least eight human cases for 11 months in Sweden (Tham et al., 2000). Cold-smoked and gravad rainbow trout (*Oncorhynchus mykiss*) and salmon (*Salmo salar*) have been focused on during recent years as potential sources of infection with *L. monocytogenes*. Investigations have shown that up to 10% of retail vacuum-packaged products contain *L. monocytogenes* (Ericsson et al., 1997). However, there is evidence that there are differences in the human-pathogenic potential subtypes of *L. monocytogenes*. For example isolates representing 3 of 18 ribotypes found among isolates from the smoked fish industry had phenotypes indicative of attenuated virulence (Norton et al., 2001).

2.2.8. *Clostridium botulinum*

The main habitat of clostridia is the soil but they are also found in sewage, rivers, lakes, sea water, fresh meat, and fish (Haagsma, 1991). In the Czech Republic in 1990, two cases of death after consumption of bought herrings without previous heat treatment were recorded. *C. botulinum* type F was isolated from the leftovers of herrings (Sramova and Benes, 1998). Botulism caused by *C. botulinum* type B after eating fish salad was described by Weber et al. (1993). *C. botulinum* type E is found in marine and

lake sediments and in fish intestine; it does not grow or produce toxin in living fish but is carried passively. Commercial hot-smoking processes employed in five fish-smoking companies provided reduction in the numbers of spores of nonproteolytic *C. botulinum* of less than 10^3 (Lindstrom et al., 2003). Most critical are the hygienic conditions for handling the product after smoking. There is a risk of botulism due to the growth of *C. botulinum* type E in smoked fish. The use of preservatives for extending the shelf life of smoked fish is being investigated

The bacterium becomes a hazard when processing practices are insufficient to eliminate botulinal spores from raw fish, particularly improper thermal processing (Chattopadhyay, 2000). The growth of *C. botulinum* and toxin production then depends on appropriate conditions in food before eating: the temperature, oxygen level, water activity, pH, the presence of preservatives, and competing micro flora (Johnson, 2000). A problem with *C. botulinum* has been encountered with some traditional fermented fish products. These rely on a combination of salt and reduced pH for their safety. If the product has insufficient salt, or fails to achieve a rapid pH drop to below 4.5, *C. botulinum* can grow. Eight cases of type E botulism were described (Telzak et al., 1990). All eight patients had eaten uneviscerated, salted, air-dried whitefish known as kapchunka. There was no evidence that the fish had been mishandled, but a low salt environment in the viscera allowed the bacterium to multiply and to produce toxin.

2.2.9. *Clostridium perfringens*

C. perfringens, an important cause of both food poisoning and non-food-borne diarrhoeas in humans, was found in a number of fish owing to contamination with sewage, which is the main source of this organism (Chattopadhyay, 2000). *C. perfringens* was isolated from 37 faecal samples (38.9%) of cod. All isolates were *C. perfringens* toxin type A; the gene encoding for beta2 toxin was found in isolates from two cods (2.1%) (Aschfalk and Muller, 2002). *C. perfringens* was determined to be the causative organism in outbreaks of food poisoning after the consumption of a tuna salad (Khatib et al., 1994) and boiled salmon (*Salmo salar*) (Hewitt et al., 1986). The risk of such outbreaks may be reduced by rapid and proper cooling after heat treatment and before consumption of fish products.

2.2.10. *Campylobacter jejuni*

Campylobacteriosis due to consumption of fish and related products is very rare. Also in case-control studies, eating of fish has never been found a risk factor for campylobacteriosis. LoewenherzLuning et al. (1996) detected a prevalence of *C. jejuni* in food samples collected from retail business. *C. jejuni* was detected in 10.9% of the food samples (particularly in poultry, milk and dairy products). Comparatively low incidence of *Campylobacter* spp. was found in fish and meat products (2.3% and 0.8%, respectively). No *C. jejuni*/*C. coli* were found in any of the samples of vacuum-packaged, cold-smoked, or hot-smoked fish products in Finland (Lyhs et al., 1998). However, *C. jejuni* (O : 33) was described as the cause of enteric infection after the consumption of tuna salad (Roels et al., 1998). Contamination most probably occurred through cross-contamination with another food product, the hands of a food handler, or the work surface.

2.3. Other important bacterial species

2.3.1. *Delftia acidovorans*

A case of endocarditis caused by *D. acidovorans* (syn. *Comamonas acidovorans*, *Pseudomonas acidovorans*) in a 42-year old intravenous-drug abuser was described. This article appears to be the first detailed report of the isolation of this organism from a systemic clinical infection and its identification as a pathogen (Horowitz et al., 1990). Bacteraemia due to *Pseudomonas* spp. possibly associated with exposure to tropical fish was described by Smith and Gradon (2003).

2.3.2. *Edwardsiella tarda*

Human infections caused by *Edw. tarda* are considered rare and have only occasionally been described outside tropical or subtropical areas. Risk factors that are associated with *Edw. tarda* infections include in addition to contact with exotic animals (reptiles or amphibia) or pre-existing liver disease exposure to aquatic environments and dietary habits (raw fish ingestion). Infections associated with this species include bacterial gastroenteritis, wound infections (cellulitis or gas gangrene), and systemic diseases such as septicaemia, meningitis,

cholecystitis, and osteomyelitis (Janda and Abbott, 1993).

Septic arthritis of the knee caused by *Edw. tarda* after a catfish puncture wound was described by Ashford et al. (1998). Protracted diarrhoea in a 2-month old Belgian infant was associated with *Edw. tarda* as the only potential pathogen. The same organism was isolated from a tropical aquarium fish in the home of the patient (Vandepitte et al., 1983).

2.3.3. *Legionella pneumophila*

Legionnaire's disease is a respiratory illness transmitted solely by water and aerosols. The bacterium *Legionella* grows in natural waters but also in distribution systems and water and air conditioning systems and is inhaled through contaminated aerosols (Rose et al., 2001). Suzuki et al. (2002) described eight cases of community-acquired legionellas' pneumonia and in one case the legionnaire disease was associated with the work of the patient at a fish market.

2.3.4. *Plesiomonas shigelloides*

Pl. shigelloides is a facultative anaerobic bacterium from the family Vibrionaceae which is known as a causative agent in waterborne diseases. *Plesiomonas* spp. was isolated from 1.5% of fresh water fishes investigated in Okayama prefecture from 1987 to 1990 (Nakajima et al., 1991). The symptoms of *Pl. shigelloides* infection are diarrhoea (watery or bloody), fever, vomiting, and abdominal pain (Wong et al., 2000).

2.3.5. *Shigella* spp.

The genus *Shigella* is specific host-adapted to humans and higher primates, and its presence in the environment is associated with faecal contamination. *Shigella* spp. isolates have been reported to survive for up to 6 months in water (Wachsmuth and Morris 1989). *Shigella* spp. is the cause of shigellosis (earlier name was bacillary dysentery), which is an infection of the gut. The great majority of cases of shigellosis are caused by direct person-to-person transmission of the bacteria via the oral-faecal route. Also waterborne transmission is important, especially where hygiene standards are low. However, food, including seafood (shrimp-cocktail, tuna salads) has also been the cause of a number of outbreaks of shigellosis. This has nearly always been as a result from contamination of raw or previously cooked foods during preparation by an infected, asymptomatic carrier with poor personal hygiene.

2.4. Isolation of bacteria from fish products in the Czech Republic between 1999 and 2003

Fish and related products as foodstuffs found in circulation come under inspection by advisory bodies of the Ministry of Agriculture of the Czech Republic, particularly the State Veterinary Administration and the Czech Agriculture and Food Inspection Authority (CAFIA).

Between 1999 and 2003, CAFIA examined 219 samples of heat treated fish products mostly intended for ready-to-eat foodstuffs. In the five-year period investigated, only three (1.4%) samples did not meet the microbiological requirements for the grade. This means that the permissible values for the bacteria *Bacillus cereus*, aerobic mesophillic bacteria and yeasts set by the Czech National Legislature (Decree of the Ministry of Health No. 294/1997 Coll., incorporated in later regulations, replaced by the Decree of the Ministry of Health No. 132/2004 Coll., this year) were exceeded in three samples (Scandinavian herring salad with yoghurt, peppered fish, and fried fish fillets), but the highest limit values were not exceeded.

Permissible values indicate the acceptable level of risk and are set for foodstuffs produced from raw materials harmless to health, while adhering to the approved technology and using a system of critical check-points (Decree of the Ministry of Health No. 132/2004 Coll.).

The highest limit values indicate an unacceptably high level of risk of a threat to people's health, harmfulness to health or the spoiling of foodstuffs and its unfitness for purposes of human alimentation. Exceeding these highest limit values indicates that the foodstuff is harmful to health (Decree of the Ministry of Health No. 132/2004 Coll.).

Foodstuffs in which the permissible values have been exceeded, but the highest limit values set by the Czech legislature have not been exceeded (Decree of the Ministry of Health No. 132/2004

Coll.) are assessed as foodstuffs which do not meet the microbiological reuiqrements of the grade i.e. they have a reduced use value, a restricted use-by date or are unfit for the original use of the foodstuff.

2.5. Histamine fish poisoning

Histamine (or scombroid) poisoning is one of the most significant cause of illness associated with seafood, although frequently misdiagnosed as "*Salmonella* spp. infection". Histamine is formed in spoiling fish by certain bacteria that are able to decarboxylate the amino acid, histidine. Although some are present in the normal microbial flora of live fish, most of them seem to be derived from post-catching contamination on fishing vessels, at the processing plant or in the distribution system (Lehane and Olley, 2000). The fish are non-toxic when caught, but increase in histamine content as bacterial numbers increase. However, foods containing unusually high levels of histamine may not appear to be outwardly spoiled and cooking does not destroy the histamine (Lehane, 2000).

Improper storage of the fishes, usually at temperatures above 20°C, appears to be the most important predisposing factor. The organisms most commonly involved are *Proteus* spp., *Clostridium* spp., *Escherichia* spp., *Salmonella* spp., *Shigella* spp., *Morganella morganii*, followed by *V. parahaemolyticus* and *V. alginolyticus* (Russell and Maretic, 1986; Kim et al., 2001). The optimum temperature for supporting growth of prolific histamine formers was found 25°C, but at 15°C a significant level of histamine was still produced in fish muscle (Kim et al., 2001).

Scombroid poisoning is geographically diverse and many species have been implicated (Taylor et al., 1989; Lipp and Rose, 1997; Kim et al., 2001), namely: tuna (*Thunnus thynnus*), mahi-mahi (*Coryphaena hippurus*), bluefish (*Pomatomus saltatrix*), sardines (*Sardina pilchardus*), mackerel (*Scomber scombrus*), amberjack (*Seriola zonata*), and abalone (*Haliotis* sp.). Foods with histamine concentrations exceeding 50 mg per 100 g of food are generally considered to be hazardous (Taylor et al., 1989). The onset of the symptoms (rash, vomiting, diarrhoea, dyspnoea, headache, pruritus, hypotension, metallic or peppery taste in mouth) usually occurs within a few minutes after ingestion of the implicated food, and the duration of symptoms ranges from a few hours to 24 hrs (Russell and Maretic, 1986; Taylor et al., 1989; Becker et al., 2001). Histamine formation in fish can be prevented by the rapid cooling of fish after catching and adequate refrigeration during handling and storage (Gingerich et al., 1999; Kim et al., 2001).

A total of 22 cases of histamine fish poisoning after the consumption of tuna burgers, tuna salad and filets were reported in North Carolina from 1998 to 1999 (Becker et al., 2001). Tuna samples had histamine levels between 213 and 3 245 ppm (mg/l). Other products also commonly implicated in scombroid poisoning are smoked fish. One hundred and seven samples of smoked fish from Auckland (New Zealand) retail markets were investigated (Fletcher et al., 1998). Eight samples had histamine levels which exceeded 50 mg/kg but a hazard level of 200 mg/kg (346.4 and 681.8 mg/kg) was exceeded only in two samples.

3. Conclusions

Most outbreaks of food poisoning associated with fish derive from the consumption of raw or insufficiently heat treated fish, which may be contaminated with bacteria from water environment (*Vibrio* spp., *C. botulinum*) or terrestrial sources (*C. perfringens*, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *V. cholerae*), or fish products recontaminated after heat processing. Wound infections, caused particularly by mycobacteria, *Strep. iniae*, *Erys. rhusiopathiae* or *Ph. damselae* and *V. alginolyticus* are seen after injury during handling fish or after exposure of open wounds to water environment.

In the case of poor hygiene, the contamination of fish and fish products may increase due to unsanitary procedures, the rotation of the assigned duties of workers, and airborne microorganisms during packing of the product.

Hot smoking in mild conditions at a temperature in the fish not exceeding 65°C and a low concentration of salt does not inactivate all pathogens or inhibit bacteria during storage. Thus the required safety can be obtained only by using very fresh fish handled in hygienic conditions, controlling the processing and the plant hygiene at critical control points, and chilling of the product to about 2°C.

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