Artykuł przeglądowy ***Review***

Fish as a source of foodborne bacteria

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Summary

A significant part of the global population depends on fish for both survival and health. However, as industry and agriculture expand rapidly, more and more natural and artificial aquatic ecosystems become contaminated. It may not only have an impact on the health of fish, but also raise safety concerns regarding the human consumption of fish. Although fish and fish products are well known to have a great nutritional value, it is crucial to be aware of the risk associated with their consumption, as they are among the main sources of foodborne bacteria.

Keywords: foodborne bacteria, food safety, foodborne illness, seafood, fish market, food contamination

During the past few decades, the importance of water resources and the consumption of aquatic organisms as food sources have been increasing day by day (35). Over the last 50 years, the annual global consumption of seafood products per capita has more than doubled, from almost 10 kg in 1960 to over 20 kg in 2014 (29). According to the annual report published by European Market Observatory for Fisheries and Aquaculture (EUMOFA) in 2019, the average European eats 24.3 kg of fish and seafood per year. Owing to their nutritional value, fish and fish products are among the most traded food items in the world. The consumption of fish varies greatly across the EU, from 4.8 kg per person in Hungary to 56.8 kg in Portugal and 15 kg in Poland (24).

Fish are known to be the main source of polyunsaturated fatty acids (PUFA), specifically omega-3 PUFAs (70). N-6 and n-3 fatty acids influence eicosanoid metabolism, gene expression, and intercellular communication. The PUFA composition of human cell membranes depends to a great extent on nutrition, including an adequate fat intake. Therefore, appropriate amounts of dietary n-6 and n-3 fatty acids need to be considered in making dietary restrictions (67). Cold-water oily fish, such as salmon, anchovies, herring, mackerel, tuna, and sardines, have the highest levels of n-3 PUFAs among all fish and seafood products (63). In contrast, shrimp, lobster, scallops, tilapia, and cod have much lower levels of n-3 fatty acids. Nevertheless, fatty acids are not the only important nutrients in fish and fishery products. Fish are also a good source of easily digestible protein, and their amino acid profile usually contains most of the essential amino acids, which are required for a balanced diet (43). Proteins must first be digested in order for the constituent amino acids to be released. In a study by Boye et al. (10), protein quality indices for various food groups and food blends were compared. Among products of animal origin, fish had the highest protein digestibility (avg. 96%), compared to beef (92%), chicken (95%), and eggs (90%).

Fish is a food with excellent nutritive value, as it also provides a large variety of vitamins and minerals, namely vitamins A and D, magnesium, and phosphorus, which play a vital role in human health improvement (7).

However, fish and fish products, especially raw or undercooked products, have been involved in outbreaks associated with bacterial pathogens, biotoxins, histamine, viruses, and/or parasites (30). The Centers for Disease Control and Prevention (CDC) has released a study outlining fish-associated foodborne disease outbreaks over the past nearly two decades. The study shows that fish continue to be an important source of foodborne outbreaks, and the species most often linked to the outbreaks are tuna (37%), mahi-mahi (10%), and grouper (9%). Each year in the United States, 260,000 people become ill from contaminated fish. Between 1998 and 2015, fish consumption resulted in 4,815 illnesses, 359 hospitalizations, and four deaths in the US alone (8). At present, the most important pathogens causing bacterial infections in fish are *Flavobacterium* spp., *Chryseobacterium* spp., *Burkholderia* spp., *Staphylococcus* spp., *Streptococcus spp., Bacillus* spp., *Shewanella putrefaciens*, *Pasteurella* spp., *Klebsiella* spp., *Rhodococcus* spp., *Microbacterium* spp., and Enterobacteriaceae (60).

Food safety policy is to ensure a high level of protection of human health. According to the Food and Agriculture Organization of the United Nations (FAO), foodborne diseases due to microbial pathogens, biotoxins, and chemical contaminants in food represent serious threats to the health of thousands of millions of people (27). To ensure a better understanding of the distribution of foodborne diseases, it is essential to examine the prevalence of pathogens in all food products, including fish.

Aeromonas spp.

Aeromonas spp. are Gram-negative, non-spore-forming, rod-shaped, facultatively anaerobic bacteria that occur ubiquitously and autochthonously in aquatic environments (25). They are best known as agents of fish diseases, but motile species are now emerging as important opportunistic human pathogens (9). These bacteria have been recognized as a cause of various infections in human beings, from relatively mild illnesses, such as acute gastroenteritis, to life-threatening conditions, including septicemia, necrotizing fasciitis, and myonecrosis (36). Moreover, *Aeromonas* has been found to cause skin and soft tissue infections as a result of injuries when handling fish or working in aquaculture (9). An infection may present as a rash with crusting and erosions, pustules and abscesses, or cellulitis (14). Between January 2006 and December 2012, features of an *Aeromonas* spp. infection were studied in a series of 204 adult patients at the Hospital del Mar in Barcelona. Gastroenteritis was diagnosed in 160 (78.4%) patients, peritonitis in 20 (9.8%) patients, and skin and soft tissue infection in 12 (5.9%) patients (including six cases with ulcers on the legs and six with surgical or traumatic wound infection), bacteremia in 6 (2.9%) patients, and pneumonia in 6 (2.9%) patients. All patients with pneumonia died during hospitalization despite having been treated with appropriate antibiotics, and 50% of patients were admitted to the Intensive Care Unit (51). These data are alarming, considering the increase in resistance to antibiotics among *Aeromonas* spp. (59).

Shewanella spp.

Shewanella spp. are Gram-negative, non-fermentative, and oxidative bacilli that produce hydrogen sulfide gas and are widespread in the environment (56). *Shewanella* spp., especially *Shewanella putrefaciens*, play a predominant role in food spoilage processes, mainly the spoilage of frozen fish and meat products. Bacterial colonies or communities directly attached to a specific surface initiate the formation of biofilm, which is a major issue in food processing industries. Cross-contamination is the main cause of biofilm formation, and it occurs when food moves through some unhygienic surfaces, leading to biofilm production and contamination (38).

It has been reported that these pathogens can cause not only food poisoning (73), but also soft tissue infections, mostly after exposure to a marine environment or contaminated water (31). In 2012 in New Zealand, a 59-year-old man was diagnosed with lower limb soft tissue infection after fishing in seawater, and *Shewanella algae* was isolated from his blood and skin (42).

Staphylococcus spp.

The genus *Staphylococcus* comprises several species and subspecies (1), among which the most widely spread is *Staphylococcus aureus*. This bacterium is one of the most frequent causes of food poisoning and is often introduced during food processing, preparation, wrapping, mincing, and storage (2). Staphylococcal food poisoning (SFP), a frequent cause of foodborne diseases worldwide, is caused by the ingestion of staphylococcal enterotoxins (SEs). The clinical symptoms of SFPs include vomiting, abdominal pain, and diarrhea within 2-6 hours (49). The enterotoxigenic *S. aureus* strains are detected primarily in meat and dairy products, but some researchers have also reported these bacteria as commonly prevalent in fish products (4, 53). Moreover, Solano et al. (69) reported an outbreak of acute gastroenteritis due to staphylococcal food poisoning, which occurred in 2011 at a summer school held by a sports club in Barcelona.

Unfortunately, the danger of infections with *Staphylococcus aureus* and their potential effect on human health is increasing. The widespread use of antibiotics, and particularly inappropriate use or overuse, has facilitated the emergence of pathogens resistant to antibiotics, such as methicillin-resistant *S. aureus* (MRSA), which makes treating bacterial infections extremely complicated. The consumption of foods of animal origin contaminated with MRSA or MRSA preformed enterotoxins is a serious threat to the well-being of humans because of its numerous clinical implications (48).

In a study conducted from 2012 to 2015, 868 samples of airport-confiscated food samples, including fish samples, were examined. Microbiological tests revealed *S. aureus* in 15.7% of confiscated food items and MRSA in 3.0% (64). MRSA is not a normal microbiota of fish or shellfish, and contamination originates from the environment. It might occur in the harvest area or be the result of improper handling by fish handlers, processors, or consumers prior to consumption (72). A study by Fisher et al. (26) emphasizes that MRSA strains, especially community-associated ones (CA-MRSA), carry a broad set of virulence factors that contribute to their fitness, colonization, and virulence, which can lead to human foodborne infections from seafood (68, 72).

Streptococcus spp.

Streptococcus is a genus of Gram-positive coccus or spherical bacteria that belongs to the family Streptococcaceae in the order Lactobacillales (65). Streptococcosis can be caused by various bacterial agents. *Streptococcus parauberis*, *Streptococcus iniae*, *Streptococcus agalactiae*, and *Streptococcus dysgalactiae* are the most prominent species regardless of the geographical region (46).

Predominant symptoms of *Streptococcus* spp. infection in humans include sore throat, vomiting, fever, nausea, and rhinorrhea (74). In 2021, the Food and Agriculture Organization (FAO) of the United Nations reported on invasive diseases and illnesses linked to the consumption of raw freshwater fish in Southeast Asia. Problems caused by eating raw contaminated freshwater fish were first discovered in this region in 2015, when at least 146 people fell ill in Singapore. Some of them suffered serious consequences, including the amputation of limbs caused by severe blood poisoning. The case was later linked to Group B *Streptococcus* (GBS), and the specific strain responsible for the outbreak was sequence type 283 (ST283) (28).

Listeria spp.

Listeria spp. are small Gram-positive, rod-shaped, non-spore-forming, facultatively anaerobic, catalase-positive and oxidase-negative organisms (37). Genomic and phenotypic data clearly define a distinct group of six species (*Listeria* sensu stricto) that share phenotypic characteristics (e.g., ability to grow at low temperatures and flagellar motility). This group includes the pathogen *Listeria monocytogenes* (55).

With a mortality rate of roughly 24 percent, listeriosis mostly affects pregnant women, their fetuses, and immunocompromised people. It causes meningitis, septicemia, abortions, and neonatal deaths, as well as other symptoms (20).

In a study performed in Germany, 22 listeriosis outbreaks were noted between 2010 and 2021. They were probably related to eating smoked and graved salmon products. The study included 166 *Listeria monocytogenes* isolates from fish and fish processing environments, sampled during official food controls, and 259 clinical isolates from patients. Out of all identified patients, 7% were confirmed to have died from listeriosis (41).

In a study performed in Iran, a total of 264 samples of fresh and frozen fish and shrimp were collected from supermarkets and retail outlets. Twenty of them (7.6%) were positive for *Listeria* spp., including five isolates identified as *L. monocytogenes* (61).

In addition, 115 samples of fresh seafood were sampled and tested in India in 2009. Tests revealed that 24% of the samples were positive for *Listeria* spp., and *L. monocytogenes* was detected in 0.9% of all samples (57).

In 2004, Portuguese researchers performed a study in which several types of food products, including fish, were examined for the presence of *Listeria monocytogenes*. Out of 1,035 samples, 72 (7.0%) were positive, the majority being samples of raw products (milk, meat, fish, flour), although some heat-processed or fermented (ready-to-eat) foods were also positive (45).

Salmonella spp.

Salmonella is a facultatively anaerobic, non-sporulating, Gram-negative bacterium. It is mesophilic, with the growth range of 5°C to 46°C, and an ideal growing temperature between 35°C and 37°C. It is destroyed by pasteurization and temperature and is vulnerable to low pH (3).

According to the U.S. Food and Drug Administration (FDA), *Salmonella* has been found in many fish and shellfish, including ready-to-eat (RTE) seafood items, seafood products needing minimal cooking, and shellfish consumed raw.

In 2021, the Center for Disease Control and Prevention (CDC) investigated a multistate outbreak of *Salmonella* Thompson infections due to the consumption of seafood. A total of 115 people were infected, and 20 were hospitalized. Furthermore, an outbreak of *Salmonella* infections linked to frozen raw tuna was reported in 2019. According to the CDC report, this outbreak led to 15 persons falling ill and two being hospitalized (12).

The presence of these bacteria in seafood may result from contamination occurring in the natural aquatic environment, in aquaculture, or during processing. Concerns are also raised by the identification of *Salmonella* serovars multiresistant to antibiotics (50).

In 2017, a non-typhoidal *Salmonella* serovar, *S.* Urbana present in food, caused a disease outbreak, and the isolates showed resistance to streptomycin and intermediate resistance to tetracycline (13). Another serovar, *S.* Poona, caused multistate disease outbreaks in 2015, and the isolates were resistant to either tetracycline or nalidixic acid. In addition, the nalidixic acid-resistant isolate showed a decreased susceptibility to ciprofloxacin, a clinically important drug used against *Salmonella* infection in children (11).

Campylobacter spp.

The genus *Campylobacter* consists of several different species of clinical and economic importance, including *Campylobacter jejuni*, *Campylobacter fetus*, and *Campylobacter coli*. However, over 90% of documented cases of human campylobacteriosis worldwide were attributed to *C. jejuni* and *C. coli* (40).

The following characteristics of these bacteria determine their capacity to sicken the host: attachment through external membrane proteins, flagellins, and capsules; invasion and toxigenicity through the production of toxins; and the use of flagella for movement, which is necessary for colonization of the small intestine and subsequent translocation to the colon of the host (17).

Over 90% of documented cases of human campylobacteriosis worldwide were attributed to *C. jejuni* and *C. coli*, two of the many species that are known to cause disease in humans. Abdominal pains and watery or bloody diarrhea were the most common signs of infection, and they were frequently accompanied by additional symptoms, such as fever, vomiting, and headaches (34).

Campylobacteriosis is the most frequently reported bacterial diarrheal illness in the European Union (EU), with over 190,000 cases in humans reported each year, which cost public health systems and productivity 2.4 billion euros per year (22). According to European Union One Health Zoonoses Report, campylobacteriosis was the most reported zoonosis in 2020, with 120,946 cases, compared to more than 220,000 in 2019 (71).

Campylobacter was found in four fish species: perch, bream, roach, and rudd in Poland (18), in marine fishes, crustaceans, and molluscs in India (19), and in freshwater and salted fishes in Egypt (47).

Vibrio spp.

These Gram-negative bacteria belonging to the Vibrionaceae family are frequently found in aquatic environments, such as marine, estuarine, and aquaculture settings. Thus they are a common component of marine life and are often present in seafood species (24). *Vibrio parahaemolyticus* and *Vibrio vulnificus* are the two species that pose the greatest threat to public health.

The most common pathogen found in seafood is *Vibrio parahaemolyticus*. It is a natural member of the marine and estuarine community from which seafood is harvested, so limiting infections by this organism is very challenging. Unfortunately, there is practically no method to prevent *V. parahaemolyticus* from entering the food supply chain. Prevention of infections caused by these bacteria must rely on controlling the organism's growth after harvest (54).

Vibrio vulnificus is a particularly dangerous species of *Vibrio* spp. (39). It is the world's most lethal foodborne pathogen, with case fatality rates of 50% or more reported in numerous countries (54). A recent increase and geographical spread in reported infections, in particular wound cases, underlines the growing international importance of *V. vulnificus*, particularly in the context of coastal warming (6).

Common symptoms of foodborne infection by *Vibrio* include watery diarrhea, stomach cramping, nausea, vomiting, fever, and chills (21). Because many patients believe their illness is due to a virus or flu, it is difficult to estimate the number of cases of infection by *Vibrio* species that occur each year. The CDC estimates that there are almost 35,000 cases of *Vibrio parahaemolyticus* (86% foodborne); 22.5% of cases result in hospitalization, with four fatalities. The more dangerous, but fortunately less common, *Vibrio vulnificus* causes an estimated 96 cases (47% foodborne) each year in the United States (44).

Vibrio is an autochthonous inhabitant of aquatic environments, which, upon infection, spreads in the environment, where it acquires and transmits new genes, including antibiotic resistance. Consumers should avoid eating raw or undercooked seafood, which is a frequent source of potentially pathogenic vibrios. This helps prevent and control the spread of food-borne vibriosis (21).

Clostridium botulinum

Clostridium botulinum is a Gram-positive, slightly curved, motile, anaerobic, rod-shaped bacterium that produces heat-resistant endospores and causes botulism. In nature, soil and water frequently contain *Clostridium botulinum* (5). Although the bacteria and spores do not cause disease by themselves, they become pathogenic when they produce botulinum toxin. There are seven different types of *C. botulinum* (A, B, C, D, E, F, and G), and each differs from the others in terms of toxins it produces. A, B, E, and very infrequently F, can cause human botulism (66).

The common symptoms of foodborne botulism include a descending, flaccid paralysis of the muscles, including those of the respiratory system, double and blurred vision, vomiting, and nausea (62).

Fish, such as white fish, flounder, cod, rockfish, smoked fish, and others, were frequently discovered to contain spores and toxins of *Clostridium botulinum* type E (58).

The presence of these bacteria in fish may be due to direct contact with aquatic environments, as well as the ingestion of *C. botulinum* or spores present in contaminated sediments or feed (16). Studies of marine sediment samples from the Baltic Sea revealed a very high prevalence of *C. botulinum* type E, up to 100% (32, 33).

In November 2016, doctors in two countries detected five cases of botulism caused by *Clostridium botulinum* neurotoxin type E (BoNT E): three cases in men in Germany and two cases in a couple (a man and a woman) in Spain. All five persons had eaten salted and dried roaches (23).

Conclusion

Fish, like many other animal species, play a very important role as a source of bacteria causing foodborne diseases in humans. With the alarming increase in antibiotic resistance among pathogenic bacteria, an even greater emphasis should be placed on the prevention of infectious diseases in humans and animals. Since fish and fish products are potential sources of human pathogenic bacteria, the contamination of the natural habitat of fish may not only have a negative impact on the health of fish stocks, but also create public health problems. The presence of bacteria in fish and their habitat may be influenced by a number of variables, including human activities, contaminated water sources, sloppy handling, and shipping practices (52).

Sourcing fish from reliable suppliers, avoiding cross-contamination between raw and ready-to-eat foods, and preparing and storing food correctly, can prevent many foodborne diseases. Frequent inspections and long-term monitoring and evaluation of local markets by authorities can help minimize the spread of bacterial pathogenicity in humans. It may lead to the development of management plans that help in preventing disease outbreaks and preserve consumer health (15).

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