

## REVIEW ARTICLE

# Contemporary threats of bacterial infections in freshwater fish

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#### **Abstract**

Changes occurring in freshwater ecosystems seem to be fundamental in the development of all microorganisms, including those pathogenic to fish. This has been especially evident in recent years during which dynamic variations in bacterial fish pathology have been observed. Gram-negative bacteria commonly known to be pathogenic to fish, like *Aeromonas* spp., *Flavobacterium* spp., *Pseudomonas* spp., and *Shewanella putrefaciens* are replaced by other species, which until now have not been known to be virulent or even conditionally pathogenic to fish. Nowadays, among these other species *Acinetobacter* spp., *Plesiomonas shigelloides*, *Sphingomonas paucimobilis*, and *Stenotrophomonas maltophilia* are the most frequently isolated from fish exhibiting clinical signs of disease. Two Gram-positive bacteria have become pathogens of particular importance in fish pathology in Poland: *Lactococcus garviae* and *Streptococcus iniae*. In addition, infections caused by the Gram-positive bacterium *Kocuria rhizophila* have appeared in recent years. This bacterium has not been known until now to be pathogenic to fish. Therefore, this infection could be called an emergent disease.

Keywords: freshwater fish, bacterial fish diseases, Poland.

#### Introduction

Water is an environment in which other organisms live besides fish, including many species of saprophyte bacteria inhabiting sediments and plants, as well as phyto- and zooplankton. Some of them colonise the skin, gills, and digestive tract of fish, living there as commensals, supporting digestion and having a beneficial effect on the immune system of these animals. These microorganisms could also threaten fish health and therefore are referred to as conditionally pathogenic. Interactions between fish, bacteria, and diseases are the subject of many studies conducted around the world. The great interest aroused by these topics indicates their important role in fish pathology. However, the development of disease is a complicated process, dependent not only on bacteria being capable of causing health disorders, but also on the immune status of fish, environmental conditions, and virulence of the disease agent. Therefore, changes occurring in freshwater ecosystems seem to be fundamental in the

development of any disease, including emerging ones (18).

The development of a particular fish disease depends largely on the climate conditions, prevailing in a given zone, region, or country. This means that dissimilar health problems occur in fish cultured in the Mediterranean Sea, in continental Europe and in Northern European countries. For example, in Scandinavian countries, one of the biggest health problems is posed by invasion of copepods and amoebic gill disease (AGD). Meanwhile, in Central Europe, infections caused by Aeromonas spp. are the most common among bacterial fish diseases, causing septicaemia (MAS), motile aeromonas (MAI), and furunculosis. aeromonas infection Infections caused by *Flavobacterium* spp. are also very often observed (30). In Poland, the epizootic situation regarding bacterial diseases in fish does not significantly differ from those presented by other continental European countries. Nevertheless, during recent years dynamic changes in the pathology of bacterial freshwater fish diseases have been observed. Gram-negative bacteria established in fact as pathogenic to fish, like *Aeromonas* spp., are displaced by infections caused by other species, hitherto not known to be pathogenic or even conditionally pathogenic to ichthyic species. Fish exhibiting clinical signs of the diseases often harbour the following microorganisms: *Acinetobacter* spp., *Kocuria* spp., *Plesiomonas shigelloides*, *Sphingomonas paucimobilis*, and *Stenotrophomonas maltophilia*.

The aim of this article is to present new potential bacteriological agents isolated from fish, which can cause diseases in freshwater fish cultures.

# Commonly known bacterial infections occurring in freshwater fish

Aeromonas infections. Almost every year health disorders in freshwater fish are recorded on many farms in Poland. The causes of the diseases may be various factors; however, the most important among bacterial infections are those caused by motile Aeromonas: A. hydrophila, A. sobria, and A. caviae. Clinical symptoms observed during health disorders vary depending on the type of disease. In cases of MAI, skin ulceration and gill and fin lesions are most frequently noticed. The disease can also become a systemic infection, and then it is called MAS. The other psychrophilic Aeromonas, Aeromonas salmonicida subsp. salmonicida, causes furunculosis in salmonids, which is manifested in ulcers on the skin. During infection, high mortalities reaching about 80% of stock are also observed (3).

Aeromonas spp. are commonly found in various environments including water, and therefore fish are constantly exposed to bacteria. Interaction with bacteria is especially dangerous under conditions of stress, which include unfavourable environmental conditions as well as human intervention in catching, sorting, and transporting of the fish. Aeromonas species are also part of the physiological microflora of the fish intestine (3).

According to the data collected during the last five years in Poland, health disorders caused by *Aeromonas* species were mostly observed in carp (*Cyprinus carpio* L.) and were usually manifested by skin lesions (MAI) in the form of ulceration (Fig. 1). Fish mortalities were also observed (26, data not published). Coinfections with other microorganisms like *Pseudomonas* spp., *Shewanella putrefaciens*, *Acinetobacter* spp. or *Stenotrophomonas maltophilia* were also found.

**Pseudomonas** infections. Pseudomonas spp. are widespread in the environment, forming a very large group of microorganisms. These psychrophilic bacteria develop well at low temperatures and are the dominant microflora. At higher temperatures (above 10°C), they are quickly replaced by competing, mesophilic microorganisms, including bacteria of the genus Aeromonas.



Fig. 1. Skin infection with *Aeromonas sobria* in carp (*Cyprinus carpio* L.) (photo: A. Pękala-Safińska)

P. fluorescens is the most important species in fish pathology and is very often associated with skin (Fig. 2) and fin disease. In some trout farms, throughout the year regardless of the water temperature, infections with P. fluorescens can cause sudden mortality, reaching even 100% of the rainbow trout population (data not published). Such a rapid disease course may be the effect of mutations, resulting in the appearance of a new property allowing the bacteria to adapt to new environmental conditions.



**Fig. 2.** Skin infection with *Pseudomonas fluorescens* in carp (*Cyprinus carpio* L.) (photo: A. Kozińska)

Other *Pseudomonas* (*P. putida* or *P. luteola*) are often isolated from internal organs of fish; however, these species are mostly an accompanying microflora (23). *Pseudomonas* spp. can cause strawberry disease in rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) (Fig. 3) and tench (*Tinca tinca*). Similar systemic infections with typical symptoms of septicaemia were also observed in crucian carp (*Carassius carassius*) and silver carp (*Carassius gibelio*) (1, 9, 23).



**Fig. 3.** Strawberry disease/infection with *Pseudomonas* spp. in rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) (photo: A. Kozińska, A. Pękala-Safińska)

Flavobacterium infections. Flavobacterium spp. naturally occur in the aquatic environment. These bacteria are also a part of the physiological gill microflora of healthy fish. However, the reservoirs of these bacteria as a source of potential disorders for fish have not been yet established (3). Flavobacteriosis is caused by three species of the genus Flavobacterium: F. columnare, which is the aetiological agent of columnaris disease, F. branchiophilum causing bacterial gill disease (BGD), and F. psychrophilum, which is associated with cold water disease (CWD) or rainbow trout fry syndrome (RTFS). Health disorders in cyprinids are caused by the first two species, while F. psychrophilum is mainly isolated from salmonids, although this species may also cause dangerous infections in cyprinids (28). Each of these diseases can be severe, and mortality can be 50% or even occasionally 80% of the fish population.

Relatively often during the last ten years, *Flavobacterium* spp. have been isolated from salmonids and cyprinids on Polish farms where clinical symptoms of the disease were observed in the fish (25) (Figs 4 and 5). The first documented outbreak of CWD in Poland was found in rainbow trout (4).



Fig. 4. Flavobacterium infection in rainbow trout (Oncorhynchus mykiss, Walbaum, 1792) (photo: A. Kozińska, A. Pękala-Safińska)

Acinetobacter infections. Bacterial fish diseases are generally caused by conditionally pathogenic microorganisms. One of them is Acinetobacter spp., which is widely dispersed in nature, including the aquatic environment. In recent years in Poland, these microorganisms have been isolated from trout and carp

relatively often (Fig. 6). Detection was in fish in which disease symptoms were observed in different seasons, most frequently in May and September (27). Clinically, depigmentation of the skin, loss of scales, exophthalmia with congestion of the eye, and gill petechiae were observed in infected trout. Haemorrhages in the skin and gill congestion were noted in infected carp, and post-mortem examination showed intestinal inflammation in both fish species. Disease symptoms were accompanied by mortalities, ranging from 5% to 20% (27).



Fig. 5. Rainbow trout fry infected with *Flavobacterium psychrophilum* (photo: A. Pekala-Safińska)

Infection with *Acinetobacter* spp. in most cases is mixed with other bacterial infection, mainly from the genus *Aeromonas* or *Chryseobacterium* spp.; however, the dominant flora in bacteriological examinations belonged to the genus *Acinetobacter*. It is worth noting that *Acinetobacter* spp. are generally regarded as carriers of antimicrobial resistance genes. Therefore, they can be very important in the spread of drug resistance in the environment (29).



**Fig. 6.** Infection with *Acinetobacter* spp. in carp (*Cyprinus carpio* L.) (photo: A. Pękala-Safińska)

**Shewanella putrefaciens infections.** For over 10 years, shewanelloses have been serious diseases of freshwater cultured fish. The aetiological agent,

Shewanella putrefaciens, is a halophilic bacterium and very well known as an important microorganism of the food spoilage process. It mainly putrefies fish stored at low temperatures, but also poultry meat and beef products (6, 15). This bacterium has also been isolated from marine and brackish waters, as well as from marine fish (2). The first bacterium isolations from diseased freshwater fish were described by Kozińska and Pekala (24). Infection rapidly spread and health disorders were noticed among different fish species. both cultured and ornamental: common carp (Cyprinus carpio L.), rainbow trout (Oncorhynchus mykiss, Walbaum, 1792), European eel (Anguilla anguilla), brown trout (Salmo trutta m. trutta), silver carp (Hypophthalmichthys molitrix), European whitefish (Coregonus lavaretus), sander (Sander lucioperca), ide (Leuciscus idus), common roach (Rutilus rutilus), zebrafish (Brachydanio rerio), slender (Pelvicachromis taeniatus), least killifish (Heterandria formosa), and koi carp (Cyprinus carpio L.) (36, 37).

Health disorders in cultured fish were noted mainly in spring, when water temperature rose to 7°C–10°C (34). Clinical signs, prevalently lethargy, darkening of the skin, skin lesions, and ulceration, have been observed in infected fish (Fig. 7). In post-mortem examination haemorrhage in the kidneys and spleen was noticed. The range of mortality varied from 40% to 85% in different fish species (34, 36).



Fig. 7. Shewanella putrefaciens infection in carp (Cyprinus carpio L.) (photo: A. Kozińska, A. Pękala-Safińska)

Fish infection with Gram-positive bacteria. In recent years, the number of infections caused by Gram-positive bacteria, especially in rainbow trout, has increased significantly in Poland (26). Among many species of Gram-positive microorganisms, two of them are of particular importance in the pathology of bacterial fish diseases: *Lactococcus garviae* and *Streptococcus iniae*. Both bacterial species cause serious health disorders in different species of freshwater and marine fish: rainbow trout and other

salmonids from the *Oncorhynchus* family, eels, and fish belonging to the *Ictaluridae* (catfish) and *Cichlidae* (tilapia) families (3).

The source of bacterial infection for fish can be both water and sediments (32). Although bacteria might be present in the environment and also in fish throughout the year, their related disorders occur during summer when the water temperature increases and reaches optimal values in the range of 18°C–25°C. Therefore, it is assumed that the temperature as well as the sanitary state of the aquatic environment are the most important factors conducive to the occurrence of disease symptoms (3).

The clinical symptoms of *L. garviae* and *Str. iniae* infections are usually similar and vary only slightly, depending on the fish species. In both infections, a typical clinical sign is exophthalmia. Furthermore, darkening of the skin, anal oedema, petechiae in the eyes, on the gill covers, and at the base of the fins are observed. Anatomopathological examination showed haemorrhages in the swim bladder, liver, spleen, and kidney, as well as stomach inflammation. During the process of disease development, the initial phase of infection is very characteristic. Then, fish exhibit nervous whirling rapid movements from the bottom to the surface or in the opposite direction, resulting from meningitis or encephalitis (12, 13).

In Poland, streptococcosis was not diagnosed until 2010, when cases of salmonids infections were described for the first time by Grawiński (16). Since then, a few outbreaks of streptococcosis have been observed in salmonid farms each year, always during summer (26, data not published).

### Emerging potential pathogens of freshwater fish

Serious new bacterial infections of freshwater fish have been observed for the last few years in Poland. These disorders can be described as emerging diseases. In order to define a disease as emerging, it must be possible to show that the disease or infection has appeared in a population for the first time, or may have existed previously, but is rapidly increasing in incidence or geographic range, or manifests itself in a new way (31). An example of such an emerging disease could be shewanellosis, a disease which appeared for the first time in 2004, causing serious health disorders in carp and rainbow trout (24). Then it spread rapidly to different fish species in different countries (34, 36, 37).

Considering these criteria, a few new infections or pathogens can be distinguished which potentially threaten fish health. Among Gram-negative bacteria isolated from both healthy and diseased fish, *Plesiomonas shigelloides* and *Stenotrophomonas maltophilia* have been diagnosed very often recently in our laboratory.

**Plesiomonas shigelloides infections.** Plesiomonas shigelloides, a microorganism belonging to the

Enterobacteriaceae family, has been considered a potential pathogenic agent for humans and animals. So far, only a few cases of health disorders observed in fish have been described in connection with isolation of *P. shigelloides*. Some of them inflicted high mortalities in salmonids, reaching 40% of the stock (8, 41). Among the clinical symptoms, cachexia of fish and redness of the anus were noted. Post-mortem examinations showed the presence of punctate petechiae on the peritoneum and the presence of exudative fluid in the body cavity. Bacterial examination of samples collected from diseased fish showed growth of *P. shigelloides* in monoculture or coinfections with *Flavobacterium* sp. and *Aeromonas hydrophila* (8, 41).

P. shigelloides was also isolated from African catfish (Heterobranchus bidorsalis), eels (Anguilla anguilla), and sturgeon (Acipenser sturio) (22). Symptoms of infection were similar to those described in rainbow trout and included reddening of the anus, petechial haemorrhages in the peritoneum, and accumulation of ascetic fluid in the peritoneal cavity. However, it is worth noting that P. shigelloides is considered a part of the physiological microflora of fish intestines (40). These data have been confirmed by the authors of this article during their experimental studies on sturgeons (data not published). In addition, P. shigelloides is often isolated from fish showing nonspecific clinical symptoms related to fish mortality. Bacteriological examination showed diverse microflora isolated from collected samples.

Stenotrophomonas maltophilia infections. Stenotrophomonas maltophilia is another species of bacteria currently often isolated from freshwater fish. This microorganism was considered a ubiquitous environmental bacterium isolated from bottom sediments as well as freshwater and salt water (11, 19). In a terrestrial environment, this bacterium was isolated from industrial and agricultural soils (38) and plant tissues (39). S. maltophilia is also involved in the degradation of various xenobiotic compounds and therefore plays an important role in biological purification processes (10). Due to its ability to produce phytohormones, this bacterium is used as a plant growth promoter and a biological agent for combating plant pathogens (33). S. maltophilia is currently classified as a multidrug-resistant microorganism which has internal resistance to a wide range of antibacterial agents. It is mainly associated with respiratory diseases of humans (7).

Available literature indicates only a few publications regarding infections of fish caused by *S. maltophilia*. Among them, the most important are those describing outbreaks of the disease observed in African catfish (*Heterobranchus bidorsalis*) and channel catfish (*Ictalurus punctatus*) (14, 17). During clinical examination of the fish, in both cases lethargy, depigmentation of the skin, focal haemorrhages and petechiae, as well as oedema in the body cavity were

observed. Post-mortem studies showed congestion of internal organs, petechiae on their surface, and intestines filled with gases. The mortality rate was 20% of the stock. These syndromes and health disorders were defined as infectious intussusception syndrome (IIS) (14).

Presently in Poland, *S. maltophilia* is very often isolated from the internal organs and skin of fish exhibiting non-specific symptoms of the disease. The health disorders described above are sometimes accompanied by fish mortality (data not published). *S. maltophilia* is often accompanied by numerous and diverse microflora when isolated. Therefore, the interpretation of the results of bacteriological examinations, and consequently, the determination of the impact of *S. maltophilia* on fish health could be difficult, and further studies are needed.

**Kocuria rhizophila infections.** An excellent example of an emerging pathogen for fish is *Kocuria rhizophila*. This is a completely new bacterium in the context of the group with proven pathogenic potential to salmonids (35).

Kocuria spp. are acknowledged skin commensals in mammals, also isolated from various environments like marine sediment, chicken meat, fresh water, or food (5, 21). These bacteria were also isolated from the trout gut where they composed the physiological microflora (20). The presence of Kocuria spp. in the internal organs of clinically healthy Baltic cod (Ghadus morhua) was confirmed by the author's own research (data not published).

During infections with *Kocuria rhizophila*, abnormal mortality (approximately 50% of the stock) was accompanied by pathological changes in external tissues and internal organs. Clinically, exophthalmia, swollen abdomen, increased skin melanisation as well as skin petechiae and focal lesions were often seen in moribund fish (Fig. 8). Post-mortem examinations showed inflammation of the intestine, liver congestion, and haemorrhages in the tail muscles, mainly in the caudal part (35). On account of the presented data, as well as the lack of information in available literature regarding the health impact of *Kocuria* spp. on fish, it should be assumed that this microorganism is conditionally pathogenic to fish.



**Fig. 8.** Exophthalmia observed in rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) during infection with *Kocuria rhizophila* (photo: A. Pękala-Safińska)

The appearance of a new species of microorganism or differentiation of extant bacteria is a natural consequence of the variability in a given ecosystem, affected by many factors. However, the appearance of new microorganisms in the environment is a natural consequence of its variability. The question remains open how these microorganisms will affect animals' and people's health in the future and in which direction their further development will go.

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