The Emergence of Pathogenic Bacteria in Coldwater Ichthyofauna: A Review

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Abstract: Pathogenic bacteria are the most common causes of serious illness outbreaks in wild and farmed coldwater fish around the world, and they have socioeconomic consequences. Pathogenic bacteria can also cause zoonotic illnesses and infection in both humans and fish. Coldwater fisheries that are susceptible to bacterial infections include *Schizothorax*, Rainbow trout, and Salmonids. At temperatures ranging from 5 to 14°C, coldwater bacterial pathogens proliferate and cause epidemics. Furunculosis, motile aeromonas septicemia, coldwater illness, columnaris, bacterial gill disease, vibriosis, edwardsiellosis, and pseudomonasis are the most common gram-negative diseases in coldwater fisheries. A few gram-positive bacterial pathogens, *Streptococcus agalactiae*, produced streptococcosis in hill stream fisheries, while *Lactococcus garvieae* caused lactococcosis. The bacteria that cause disease in coldwater fisheries are mainly opportunistic pathogens while few are obligate pathogens. This review discusses the bacterial agents, and diseases caused by them with emphasis on the bacterium-host interactions by compiling some published literature scattered over the past decades.

Keywords: Coldwater ichthyofauna • Pathogenic bacteria • Gram-positive bacteria • Gram-negative bacteria • Bacterial diseases.

Introduction

The Earth is home to several coldwater streams, rivers, lakes, and reservoirs that hold a variety of fish fauna. Coldwater fishes include a wide variety of fishes which required temperature lower than 25°C to survive for an extended period (Singh and Sarma, 2020). In the coldwater environment, ichthyofauna interacts with a wide variety of microflora including bacteria, viruses, fungi, and parasites which intern diseases in fish. Pathogen and host interact in a trade-off that is influenced by environmental conditions like poor water quality, temperature variation, nutritional deficits, overcrowding, disturbance, parasitism, and infections that bring about natural stress to fish and stimulate diverse pathogenic diseases (Gnanagobal and Santander, 2022). Even so, pathogenic bacteria are the main causative agents of infection in both wild and cultured ichthyofauna worldwide due to their high stress tolerance potential. The development of specific bacterial pathogenic illnesses in wild fish is mostly dependent on changing climatic circumstances and human activities and it is nearly impossible to use vaccination against disease outbreaks in wild coldwater fish due to the large volume of water bodies. In this way, outbreaks may result in economic losses in a region. Cultured fish practice has a high risk of originating bacterial zoonotic diseases in part because of overcrowding, unhygienic feed, and poor maintenance.

Pathogens detection, isolation, and identification

Since the microbial population is so diverse thus, difficult to detect pathogens at the species level. Nowadays, the polyphasic approach overcomes the challenge of isolating, identifying, and classifying pathogenic bacteria.

Pathogenic detection tests are performed on gills, skin mucus, liver, kidney, and spleen samples (Xue et al., 2022). Specific and general agar media are used to isolate and replicate the bacterium respectively. The identification procedure begins with fundamental identification methods such as Gram staining and acid-fast staining, followed by biochemical tests (Pavlinec et al., 2022; Ganesan et al., 2023) for clarification of discovered samples. DNA extraction (Duchaud et al., 2018) followed by polymerase chain reaction (real-time, multiplex, capillary, BOX PCR) (Yu et al., 2004; Singh et al., 2010; Lievens et al., 2011; Law et al., 2014; Sheikhi et al., 2020) used to amplify the 16S rDNA (Chen et al., 2022) with the help of a universal primer. The amplified sample is analysed by gel electrophoresis (Rao et al., 2022) followed by gene sequencing (Duchaud et al., 2007; Stenholm et al., 2008; Wang et al., 2022) and finally sequence is compared with the NCBI database to classify the detected species. Gram-positive fish pathogens are also identified by MALDI-TOF mass spectroscopy (Assis et al., 2017; Piamsomboon et al., 2020; Ashfaq et al., 2022). Thus, the application of molecular techniques allows a better knowledge of morphology and identification of the pathogenic species for subsequently developing control measures against infections.

Pathogenic bacteria of coldwater fisheries

A dynamic change in pathology has led to the discovery of many new bacterial species that cause coldwater fish diseases in recent years. The genera of gram-negative bacteria such as *Pseudomonas, Edwardsiella, Aeromonas, Flavobacterium, Vibrio*, and gram-positive bacteria such as *Mycobacteria, Renibacteria* and *Streptococcus* include the major fish pathogens causing diseases in the ichthyofauna of aquaculture (Pereira et al., 2022). Alterations in the temperature (Guijarro et al., 2015), pH, oxygen and various pollutants may lead the bacteria to colonize, penetrate the host tissues, express their genes and cause infection (Hansen & Olafsen, 1999; Nematollahi et al., 2003). The epidermis, gills, and gastrointestinal (GI) tract are the three principal routes of pathogenic infection in fish (Ringø et al., 2010).

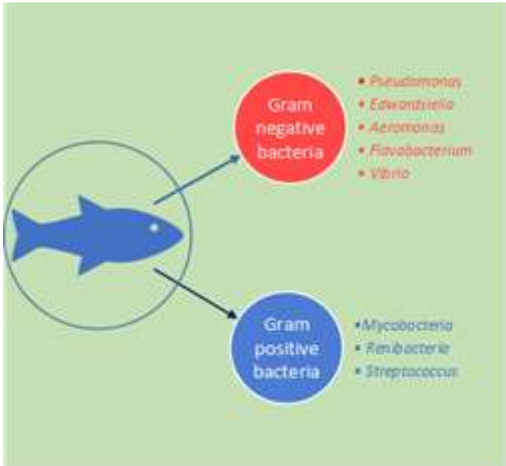

Fig. 1: Common bacterial pathogens of Coldwater ichthyofauna.

Gram-negative pathogenic bacteria

Aeromonas**:** The genus *Aeromonas* is gram-negative rods with positive oxidase activity and facultative anaerobic properties (Martínez-Murcia et al., 2016) having 36 species described so far, of which more than half are pathogenic (Fernández-Bravo & Figueras, 2020). *Aeromonas salmonicida* is only non-motile (Park et al., 2020) and psychrophilic primary fish pathogen that grows between the optimal temperature of 22 to 25°C causes furunculosis in a variety of wild and cultured fishes whereas a motile group of *Aeromonas* (*hydrophila, sobria, veronii*, and *caviae*) are mesophilic, required optimal temperature

35-37°C (Parker & Shaw, 2011) and responsible for motile aeromonas septicemia (MAS) disease (Sudheesh et al., 2012; Ozturk & Altinok, 2014). *Aeromonas veronii*, commonly reported in Chinese farms, was isolated from infected Crucian carp (*Carassius auratus gibelio*) (Chen et al., 2019). *Schizothorax* species have been reported to have furunculosis in Wular Lake, Kashmir with maximum infection during winter (Chalkoo et al., 2007). *Schizothorax prenanti*, an important coldwater fish in China, is also infected with *Aeromonas hydrophila* (Zheng et al., 2016; Ye et al., 2018). The aerolysin (toxin) gene from *Aeromanas hydriphila* was successfully cloned and expressed in *E.coli*, suggesting that aerolysin could be used as an immunoassay for *A. hydrophila* control and vaccination (Singh et al., 2010).

Flavobacterium**:** *Flavobacterium psychrophilum* is a psychrotrophic, pathogenic bacterium of Salmonids (Álvarez et al., 2008) and Rainbow trout (*Oncorhynchus mykiss*) (Starliper, 2011) having symptoms of open lesions on an external body surface developed cold water disease (CWD) (Macchia et al., 2022). CWD is most prevalent and serious when the water temperature is below or equivalent to 10°C (Ozturk & Altinok, 2014) and recognized in France, Germany, UK, Finland, Denmark, Norway, Switzerland, Japan and Korea (Nematollahi et al., 2003). The other species of the *Flavobacterium* like *columnare* and *branchiophilum* cause columnaris (Evenhuis et al., 2018) and (water temperature range of 12-14°C or above) (Ozturk & Altinok, 2014) and bacterial gill disease (BGD) in Salmonids respectively (Starliper, 2011). The outbreaks of columnaris disease in the infected gills of farmed *Catla catla* for the first time were identified in India (Verma & Rathore, 2013). Salmonid fish farms in Switzerland reported high fish mortality due to bacterial cold water disease (CWD) (Vallejo et al., 2021) and rainbow trout fry syndrome (RTFS) triggered by *Flavobacterium psychrophilum* (Strepparava et al., 2013). Over the past 20 years, Ayu (*Plecoglossus altivelis*), an amphidromous fish, have lost a large number to CWD when infected with *Flavobacterium psychrophilum* (Nakayama et al., 2016). *Flavobacterium bernardetii*, a new species from coldwater farms has recently been found in infected Rainbow trout (*Oncorhynchus mykiss)* in Turkey (Saticioglu et al., 2021).

Vibrio**:** Coldwater vibriosis (CWB) is caused by *Aliivibrio salmonicida*, which penetrates fish bloodstreams quickly and causes haemorrhagic septicaemia in Rainbow trout (*Oncorhynchus mykiss*) (Nørstebø et al., 2018). It has been reported that the obligate Eel pathogen *Vibrio vulnificus* biotype-2 remained in a non-culturable state at low temperatures but recovers its culture ability upon increasing the incubation temperature to 5°C from 25°C (Biosca et al., 1996).

Edwardsiella**:** *Edwardsiella tarda* is a gram-negative, motile and rod-shaped bacterium that caused edwardsiellosis (haemorrhagic septicaemia) mainly in freshwater and marine fish species worldwide (Mohanty & Sahoo, 2007) but rare supportive literature found in the case of a coldwater infection. There has been a recent study in Sichuan Province which reported acute septicaemia caused by *Edwardsiell tarda* in two coldwater culture farms of Ya-fish *(Schizothorax prenanti*) that is endemic to China (Zhou et al., 2016).

Pseudomonas: *Pseudomonas* genera cause pseudomoniasis disease in freshwater and saltwater fish (Ozturk & Altinok, 2014). *Pseudomonas koreensis* isolated from a sick eye of *Tor putitora* was found to be pathogenic to fish and capable of causing histopathological abnormalities in golden mahseer fingerlings (Shahi & Mallik, 2014). In general, *Pseudomonas* infection caused ragged fins and tail disease in both young and adult fishes reported in West Bengal, India (Sen et al., 2018). *Pseudomonas* genera are also found predominant in Tibetan *Schizothorax o'connori* but designated as probiotic micro-intestinal flora (Shang et al., 2020). Recent research found *Pseudomonas* phage AIIMS-Pa-A1 in the coldwater of the Ganga, which is beneficial against drug-resistant *Pseudomonas aeruginosa* (Rathor et al., 2022). Bacteriophages against *Pseudomonas plecoglossicida* have also been found in Ayu and *Plecoglossus altivelis* fishes in Japan (Park et al., 2000).

Acinetobacter lwoffii was discovered in diseased fish of the *Schizothorax* genus, which is a group of gram-negative, aerobic, nonmotile bacillus with seven named and nine unnamed genomospecies. (Cao et al., 2018). An experimental study in Turkey concluded that *Arcobacter cryaerophilus* (Gram-negative, spiral-shaped rods and motile bacterium) at low temperature cause various clinical abnormalities and affects the biochemical nature of blood, peroxide and lipids contents in Rainbow trout (Aydin et al., 2009).

Gram-positive pathogenic bacteria

***Streptococcus*:** *Streptococcus* is distinguished by its gram-positive status confirmed purple/blue colour when stained with Gram's stain and caused streptococcosis (Yanong & Francis-floyd, 2013). A serious infectious disease with unknown aetiology has been observed in Ya-fish (*Schizothorax prenanti*) farms in Sichuan Province caused by *Streptococcus agalactiae* (Group B streptococci) (Geng et al., 2012). *Streptococcus agalactiae* had recognised as a prominent pathogenic bacterium of farmed coldwater fishes in China including *Tilapia* (Razzak et al., 2017; Zhang et al., 2018), *Schizothorax prenanti*, and *Schizopygopsis pylzovi*, typically associated with septicaemia and meningoencephalitis diseases (Deng et al., 2019). In China, there had been several outbreaks of *Streptococcus agalactiae* infection reported in bighead Carp (*Aristichthys nobilis*) (Zhang et al., 2018). *Lactococcus garviae* and *Streptococcus iniae* are reported pathogenic in Poland (Pękala-Safińska,2018; Gatesoupe,2008). *Streptococcus pyogenes* species was found in Rainbow trout raised at various trout hatcheries of Azad Jammu and Kashmir, Pakistan (Kousar et al., 2020). *Streptococcus* species infections were also found in Nile tilapia raised in Lake Sentani, Papua, Indonesia (Anshary et al., 2014).

***Mycobacteria*:** Mycobacteria are pleomorphic, non-motile rods with acid-fast, aerobic in nature measured 0.2-0.6 µm in wide and 1-10 µm in long and have an apparent cell wall that contains long-chain fatty acids (Gauthier & Rhodes, 2009). *Mycobacterium* species that have been identified as a significant source of morbidity and mortality in the wild and farmed aquaculture industries include zoonotic pathogens, which are rarely reported in coldwater fisheries.

***Renibacteria*:** *Renibacterium sulmoninurum* is the most prevalent etiological agent caused bacterial kidney disease (BKD) seen in wild and cultured Salmonid fish around the world (Evenden et al., 1993). Recently, BKD report from a hatchery in Colorado, USA, demonstrated that *Renibacterium sulmoninurum* transmitted vertically in Cutthroat trout

(*Oncorhynchus clarkii)* (Riepe et al., 2023).

The other pathogenic bacteria like *Listeria monocytogenes*, in Finland (Autio et al., 1999), *Lactococcus garvieae in trout*, (Castro et al., 2017), *Clostridium perfringens* in *Schizothorax*, *Cyprinus carpio* (Wani et al., 2018) has also found a greater degree of contamination.

Conclusion

Despite probiotic bacterial microflora, fish diversity is threatened by pathogenic bacterial flora thus interactions between fish, bacteria, and the aquatic environment is a big concern around the world. Pathogenic outbreaks in fishes have been reported from different parts of the world of which *Schizothorax*, Rainbow trout and Salmonids are the most affected groups. The discovery of a *Pseudomonas* phage in coldwater revealed a rare infection of *Pseudomonas* pathogenic genera. Under the current climate-changing scenario and declining water quality by anthropogenic activities have led to an increase in the pathogenic bacterial diversity, thus creating a threat to the fish population throughout the coldwater ecosystems.

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