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A Review on Columnaris disease in freshwater farmed fish

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Abstract

Columnaris disease is one of the most common infectious bacterial diseases of freshwater fishes infecting both cultured and wild fish species. It has a large economic impact on fish production because of the extensive and rapid rate of mortality that it causes. Research on columnaris has revealed that *Flavobacterum columnare* has a broad host range and geographic distribution, and contains important virulence factors that enhance bacterial survival and pathogenesis in hosts. Transmission of columnaris disease is more efficient in higher temperatures, high density and physical skin damage. *F.columnare* causes acute to chronic infections and typically affects the gills, the skin and fins with corrosion of the dorsal and tail fins, and this progresses to external infection in which gray spots or yellowed areas of erosion appear, generally surrounded by a hyperemic reddened zone. Management of production variables such as feed consumption, pond depth, ammonia levels and stocking events which associate with columnaris disease outbreaks is mandatory. Fish can be protected from subsequent *F. columnare* infections by activating the adaptive immune system with bath immunization and oral immunization incorporated into fish feed protects against columnaris. External treatments are possible only in early stages of the disease with drugs such as Oxytetracycline, when the infection is still superficial. The present review aims to provide an overview of the identification, pathology, diagnosis and virulence factors of *F. columnare* in fish, and describe recent strategies for developing prevention against columnaris.

Keywords: Columnaris, Farmed fish, *Flavobacterium columnare*.

1. Introduction

The fisheries and aquaculture sectors have nowadays been producing more food for the world, with a rapid expansion taking place. Global aquaculture production hit a record high of more than 90 million tons in 2012. Global fisheries and aquaculture production has become a valuable source of cheap protein and income for many people. Aquaculture is probably the fastest growing food producing sector and accounts for almost 50% of the world's fish that is used for food. Africa's contribution to the world aquaculture in 2012 amounts to 1,485,367 tons. Ethiopia is endowed with huge water resources and the status of aquaculture is advancing with promising activities. The country has the potential of producing 40-50 thousand tons of fish annually (FAO, 2014).

However, the development and sustainability of the sector could be threatened by harmful practices, poor management and diseases. Outbreaks of classical or emerging diseases can have a severe economic impact on the fish industry. Disease problems in fish are complex and result from the interaction of the disease agent, the fish, and the aquatic environment as well. Fish diseases can be broadly classified as either noninfectious or infectious based upon etiology. Noninfectious causes of disease may include environmental stressors such as water temperature changes, chemical pollutants, and poor management practices. Examples of poor management practices include overcrowding, malnourishment, improper diet, transportation effects, and lack of attention to water

quality. Infectious causes of disease are diverse and include parasites, fungi, viruses, and bacteria. Bacteria that have been characterized as major piscine pathogens usually include *Flavobacterium*spp., *Edwardsiella*spp., and *Aeromonas*spp. (Tripathi, 2005).

Flavobacteriumcolumnare, the etiologic agent of columnaris disease, is one of the most common bacteria infecting different fish species. Columnaris is an infectious bacterial disease of freshwater fish leading annually to severe financial and material losses at fish farms worldwide. The disease is caused by *F. columnare*, a member of Bacteroidetes, which is a major bacterial pathogen of farmed freshwater fish around the world. It is known that *F. columnare* can survive outside the fish host for long periods and may respond to stressful conditions by entering into a viable but non-dividing state. It has a large economic impact on fish production because of the extensive and rapid rate of mortality that it causes *(*Arias *et al*., 2012).

The clinical signs of the disease begin with corrosion of the dorsal and tail fins, and this progresses to external infection in which gray spots or yellowed areas of erosion appear, generally surrounded by a hyperemic reddened zone, in the cranial region, body surface and gills. In these locations, there is progressive necrosis involving the epidermis, dermis and musculature. *F. columnare* infects several fish species ranging from catfish and tropical aquarium fish to saimonids in the warm water period. Columnaris disease is transmitted by contact or by propagules shed into the water. Diagnostic procedures were described to identify *F. columnare*that used biochemical and cultural characteristics that were unique to the bacterium. More specific and sensitive diagnostic techniques have been developed, but these techniques are primarily restricted to experimental use (Griffin, 1987; Welker *et al*., 2005).

2. Columnaris disease

2.1. Etiology and Pathogen Characteristics

The causative agent of columnaris disease is *F. columnare*. Davis described the disease and reported large numbers of slender, motile bacteria present in the lesions although unsuccessful in cultivating the etiological agent. Column-like structures formed by these bacteria were evident upon examining a wet mount preparation of these lesions (Davis, 1922).

The taxonomic status of the pathogen has changed several times since the pioneering work of Davis. The causative bacterium has been referred to by different names including *Bacillus columnaris, Flexibactercolumnaris, and Cytophagacolumnaris.* Finally, in 1996, the bacterium received its current name, *Flavobacteriumcolumnare*whichwas adopted following molecular characterization of archived strains.*Flavobacteriumcolumnare* belongs to the family Flavobacteriaceae (Triyanto and Wakabayashi, 1999).

*Flavobacteriumcolumnare*is a long, slender, non-flagellated Gram-negative rod, 0.3 to 0.7 µm wide x 3 to 10 µm long, which exhibits gliding motility on solid surfaces. Colonies on cytophaga agar are flat, yellow, rhizoid, strongly adherent, and spread across solid media surfaces forming irregular margins. The bacteria form columnar aggregates on infected tissue that are often referred to as “haystacks.” The temperature range for growth of *F. columnare*is reported to be between 4ºC and 37ºC with 25ºC being the optimum. Growth is strictly aerobic, and the bacterium is nonhalophilic. The physiological characteristics of *F. columnare*include: strict aerobic growth; no acid produced from carbohydrates; and catalase positive; reduces nitrate to nitrite; and hydrogen sulfide is produced. Chondrotin AC lyase, an enzyme produced by *F. columnare*degrades polysaccharides, particularly those found in cartilaginous connective tissue. The bacterium produces a capsule and the thickness of the capsule appears to be correlated with virulence. High virulence strains have a thick 120-130 nm capsule, while strains with low virulence have a thinner 80-90 nm capsule, as observed by transmission electron micrography (Decostere*et al*., 1999).

A method for the differentiation of *F. columnare*from other morphologically similar bacteria was advised by Griffin. The “Griffin screen” consists of five characteristics that separate *F. columnare*from other yellow pigmented Gram negative aquatic bacteria: the ability to grow in the presence of neomycin sulfate and polymyxin B; colonies on Cytophaga agar plates typically rhizoid and pigmented pale yellow; production of gelatin degrading enzymes; binding of Congo red dye to the colony; and production of chondroitin sulfate A degrading enzymes (Griffin, 1992).

2.2. Epidemiology

2.2.1. Host susceptibility

Columnaris disease has been documented from different fish species throughout the world, including the commercially important species: channel catfish (*Ictaluruspunctatus*), common carp (*Cyprinuscarpio*), goldfish (*Carassiusauratus*), tilapia (*Oreochromis*spp.), and brown trout (*Salmotrutta*). The disease also infects important recreational species such as the largemouth bass (*Micropterussalmoides*), bluegill *(Lepomismacrochirus*), and crappie (*Pomoxisnigromaculatus*, *P. annularis*) and numerous ornamental species that are popular in the aquarium trade. It has been produced experimentally in channel catfish, koi carp, rainbow trout, tilapia and zebra fish (Darwish*et al.*, 2011).

2.2.2. Distribution

F. columnare is distributed worldwide in fresh water sources and may infect many different wild and cultured freshwater fish species. This disease also attacks many tropical freshwater aquarium fish. In the channel catfish (*Ictaluruspunctatus*) industry in the United States, *F. columnare* is the second most prevalent bacterium, after *Edwardsiellaictaluri*, to cause disease and mortality, with yearly losses estimated at 30 million dollars. This organism can also be part of the bacterial microbiota of freshwater fish, eggs and the rearing waters the fish live in (Suomalainen*et al*., 2005; Kunttu*et al.*, 2009).

Flavobacterumcolumnare is capable of surviving in sterile river mud. Apparently, mud slurry often contains sufficient nutrients to maintain viability of *F. columnare* longer than sterile river water. In this case, however, the percentage survival of *F. columnare* seeded into mud seems to be higher at 25°C than at 5°C. Temperatures below 5°C are even detrimental to *F. columnare* cells in mud. *F. columnare* also grows well on particulate fish feed. When surviving outside the host, *F. columnare* can change from a virulent to a less virulent form with altered colony morphology, probably to save energy (Kunttu*et al.*, 2012).

2.2.3. Risk factors

Environmental conditions can highly influence morbidity and mortality rates. Columnaris disease is influenced by water quality. Organic matter could concentrate nutrients to feed the bacteria and that degrading enzymes could be kept in close contact with the host tissue. Bacterial titers were furthermore markedly lower in gills placed in an organ bath with distilled water with or without 0.03% NaCl compared to the titers of gills suspended in Ringer´s solution or in formulated water containing divalent ions (magnesium and calcium). The survival of the fish exposed to *F. columnare* significantly increased as unionized ammonia concentrations increased. This suggests that complex interactions can complicate prediction of the responses of fish to concurrent chemical stressors and bacterial pathogens (Morris *et al.*, 2006).

Survival of *F. columnare* for extended periods in water was demonstrated to be influenced by physical and chemical characteristics of the surrounding water. It can survive up to 16 days at 25°C in hard, alkaline water with a high organic load. Soft water with 10 mg/L $CaCO_3$, especially when acid or with a low organic content does not provide a favorable environment for the organism. Calcium, magnesium, potassium and sodium ions all are important for long-term survival of *F. columnare* in water. Survival of *F. columnare* in static, sterile river water was directly related to temperature, with a higher survival percentage at lower temperatures. The bacterium can keep its infectivity in lake water in laboratory conditions for at least five months (Kunttu*et al*., 2012).

Concurrent infections of ectoparasites with *F. columnare* increased the susceptibility of rainbow trout to the bacterial pathogen. Compared with single infections, the mortality was significantly higher and the onset of disease condition occurred earlier in fish which were concomitantly infected by the parasite *Arguluscoregoni* and *F. columnare*(Bandilla*et al.*, 2006).

Furthermore, the fish density at fish farms is also a key in influencing mortality in an outbreak of columnaris disease as mortality rates started earlier and remained higher when fish were stocked at high densities. Water flow is another important factor since it acts as a determining factor with regard to the contact time between the possibly present bacteria and the host tissue. High mortality rates were observed in elvers kept in standing water, while in aquaria with running water, mortality in was reduced by half (Suomalainen*et al*., 2005).

2.2.4. Transmission

Transmission of columnaris disease is more efficient in higher temperatures. Experimentally infected steelhead trout with *F. columnare* were held in water at 12 to 20°C, mortality increased with temperature. Adhesion to gill tissue of a highly virulent *F. columnare*strain is enhanced at increased temperature and the chondroitin AC lyase activity of this pathogen increases along with the temperature. Normal rearing densities with high temperatures (23°C) proved to increase both transmission rate of columnaris disease and mortality in the fish (Suomalainen*et al.,* 2006).

Fish may reside in a clinically healthy carrier status harboring an isolate remaining from a previous outbreak of columnaris disease and in this way act as an infection source for other fish. Rainbow trout surviving a *F. columnare*infection can release up to 5 × 10^3 colony forming units/mL of viable bacteria into tank water. The gills were shown to be the major release site of this pathogen. Dead fish would be able to spread the disease at a higher transmission rate compared to living fish (Shoemaker *et al.,* 2011).

2.3. Clinical Signs and Pathological Findings

F. columnare causes acute to chronic infections and typically affects the gills, the skin and fins. The virulence of the eliciting strain determines the clinical manifestation of columnaris disease. The strains of low virulence induced slow progressive infections at water temperatures above 21°C and caused massive tissue damage before death occurred. Strains of high virulence caused fulminating infections and killed young fish in 12 to 24 h at 20°C and these fish did not show gross tissue damage at the time of death. The gross pathology observed in the fish experimentally infected with strains of *F. columnare*of high virulence was usually very limited. Apparently, death occurred before gross external manifestations of the disease appeared. Age also seems to have an important impact on the severity of the clinical signs besides the virulence of the strain being a determinant factor. In young fish, the disease develops acutely and mostly damages the gills. In adults, the disease may adopt an acute, sub-acute or chronic course. When the disease course is acute or sub-acute in adult fish, yellowish areas of necrotic tissue can appear in the gills ultimately resulting in complete gill destruction (Bernardet and Bowman, 2006).

It takes longer before gill damage appears and skin lesions may develop as well in chronic cases. On the body, small lesions start as areas of pale discolorations of the skin, which usually are surrounded by a zone with a distinct reddish tinge. This mostly begins at the base of the dorsal fin. Fin deterioration then occurs, starting from the lesion at the base of the fin and progressing to the outer edge. The lesions then begin extending laterally from their common location at the base of the dorsal fin to encircle the fish resembling a “saddle-back”. The disease, therefore, is referred to as “saddle-back disease” (Bernardet and Bowman, 2006).

Ulceration of the oral mucosa is common and the disease spreads easily to the mandible and the maxilla. Since the painful oral lesions render the fish anorectic and lead to death due to starvation, mucosal ulceration is more lethal than are the skin lesions. Secondary infections with fungi or other bacteria may deteriorate the situation and can be seen together with the filamentous bacteria. In tropical fish, this clinical sign led to the disease being termed “cotton wool disease” or “mouth fungus”. Lesions can be restricted to local skin discoloration, with or without ulceration, and degeneration of underlying muscle fibers. The skin or gills need to be abraded, for bacteria to enter the bloodstream and cause systemic infections. It is also isolated from internal organs without any external lesions appearing (Michel *et al*., 2002).

Microscopically, the affected gill tissue reveals the disappearance of the normal structure of primary and secondary filaments. In the initial phase, proliferation of epithelial cells of the gill filaments can be accompanied by an increase of mucous cells. The proliferating tissue can occlude the space between adjacent gill lamellae. In more advanced stages, the occlusion can be total causing the gill lamellae to be completely surrounded by the propagating tissue. Congestion of gill lamellae occurs due to accumulation of blood masses and inflammatory cell infiltration can be noticed. Edema causes lifting of the surface epithelium of gill lamellae from the underlying capillary bed. In more advanced stages of the disease, fusion of gill lamellae and/or gill filaments appears. Complete clubbing of gill filaments can finally result in circulatory failure and extensive internal hemorrhage. Moreover, huge clusters of *F. columnare* can be found on the cell surface and/or in between necrotic sites (Declerq, 2013).

Significant changes in blood parameters were observed in the infected koi carp fish. For the hematologic parameters, a significant decrease was noted in Packed Cell Volume (PCV), hemoglobin concentration, red blood cell count, mean corpuscular volume and absolute lymphocyte counts. As for the biochemical parameters, marked hyponatremia, hypochloridemia and hyperglycemia were observed. Calcium and magnesium levels dropped only slightly and total serum protein and albumin-like protein concentrations decreased mildly. Alkaline phosphatase (ALP), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK) secretions were significantly increased (Tripathi*et al.*, 2005).

In other study, blood parameters from brook trout suffering from an acute, natural outbreak of *F. columnare* also revealed anemia, but levels of mean corpuscular volume and mean corpuscular haemoglobin were higher. Total protein levels fell much below physiological parameters in infected fish and the calcium concentrations were reduced significantly. Blood Urea Nitrogen (BUN) measurements were much higher compared to normal levels. The catalytic activity of AST, alanine aminotransferases and LDH reached multiples of normal values. In contrast, alkaline phosphatase concentrations decreased (Řehulka and Minařík, 2007).Similarly, the blood parameter results from naturally infected tilapia showed that there were changes in the erythrocytic series and in organic defense blood cells, in the fish infected with the bacterium, with reductions in erythrocytic variables and significant increases in the numbers of circulating lymphocytes and neutrophils (Sebastião, 2011).

2.4. Diagnosis

A presumptive diagnosis of columnaris disease is based on the characteristic morphologic features of *F. columnare*in cytologic wet mounts or stained smears as well as in histologic preparations. Definitive disease diagnosis requires more extensive or sophisticated laboratory testing. The most important diagnostic tests for the disease are illustrated here below.

2.4.1. Bacterial culture

Bacterial culture is the initial step in identifying a bacterium. Definitive identification of a given bacterium usually relies on biochemical characterization. Culture and identification of *F. columnare*is more problematic because of other contaminating bacteria with subsequent overgrowth. This bacterium does not grow on blood agar, beef-broth agar, fish agar, or fish serum agar. Different media were used to cultivate the bacteria, including Hsu-Shotts or Modified Hsu-Shotts medium, Ordal's medium, Anacker and Ordal's medium also referred to as "Cytophaga medium", *F. columnare* growth medium broth and Shieh or modified Shieh medium (Darwish*et al.*, 2012).

The isolation of *F. columnare*is possible from samples that are taken from the edge of external lesions in recent lesions. Growth does not occur in media that contain NaCl concentrations of over 0.5% or that have a pH lower than six. Depending on the strain, the bacterium grows between 15 and 37°C, with an optimal growth occurring between 25°C and 30°C. Colonies often appear after 24 to 48 h of incubation. Glucose does not improve growth. Considering that *F. columnare* is easily overgrown by contaminating bacteria, selective media have been developed based on inherent resistance of *F. columnare* to different antimicrobial agents, including polymyxin and neomycin (Decostere*et al.*, 2002).

During culture, *F. columnare*exhibits a swarming phenomenon and forms small (1-3 mm diameter), round, flat colonies with rhizoid edges on agar plates**.** Characteristic yellow-pigmented colonies are observed within 48 to 72 hours when appropriate agar plates are incubated at 25°C. These colonies are adherent to the agar plate; therefore, it is difficult to recover a single colony. Usually, the colony is scraped off the agar plate and used to inoculate additional agar plates or liquid media for bacterial culture. Bacterial culture in liquid medium requires constant stirring at 150 to250 rpm. Yellow turbidity will be apparent in the culture medium within 24 to 48 hours when incubated at 25°C (Declerq, 2013).

Gliding movement can be observed in liquid media examined microscopically. Small filamentous aggregates appear in the culture and sediment at the bottom and sides of the culture flask. Bacilli start to disintegrate in 5 to 7 day-old cultures and produce small, spheroid bodies known as microcysts. *F. columnare* largely displays three colony types on solid media; smooth, rhizoid and rough colony morphology variant. The rhizoid colony variants were assigned virulent and moderately adherent, the non-rhizoid rough colony variants non-virulent and highly adherent, and the smooth colony variants non-virulent

and poorly adherent. Colonies of *F. columnare* are notorious for their sometimes strong adherence to the agar. This trait is also exhibited in broth cultures where yellow, filamentous clumps of bacterial cells can form a thick ring at the surface of a glass recipient. Adherence may be lost after several subcultures. Colonies can be recognized by their distinctive yellow pigmentation. The yellow color is due to the production of flexirubin pigments (Bernardet and Bowman, 2006; Kunttu*et al*., 2011).

2.4.2. Cytology

Cytologic wet mount preparations are inexpensive, rapid, and simple to perform. This technique provides the easiest way to obtain a presumptive diagnosis of columnaris disease. *F. columnare*bacteria appear as long, thin bacilli that frequently are arranged in hay-stack-like columns. Occasionally, these bacteria may exhibit gliding movements. Cytologic smears made from skin, gill, or fin lesions may reveal an almost pure population of characteristic bacilli. However, small numbers of other bacteria frequently are mixed with the *F. columnare*. *F. columnare*stain very lightly with Romanowsky-type stains. They have a homogenous appearance but rarely may exhibit a slightly granular texture. Infrequently, microcysts may be present and appear as round spherical bodies (Decostere*et al*., 2002).

2.4.3. Histopathology

Histologic lesions vary according to the presentation of disease. In most cases of columnaris disease, characteristic, long, filamentous bacteria may be observed attached to the surface erosions and ulcerations of the skin, gills, or fin. In some instances, the gills may appear relatively normal. In other cases, the gill lesions vary from dilation of blood vessels to severe necrosis. Clubbing and fusion of secondary lamellae may be seen due to hyperplasia of branchial epithelial cells and goblet cells in early columnaris disease. This results in obliteration of the interlamellar spaces that are essential for gaseous exchange. In advanced columnaris disease, the gill epithelium may detach from blood vessels resulting in accumulation of edema fluid. Large masses of necrotic epithelium, inflammatory cells, and bacterial colonies frequently are observed (Declerq, 2013).

In early skin disease, scattered bacteria are observed in the epidermis and corium from foci of gross discoloration. Inflammatory cell infiltrates are minimal. In scaled fish, bacterial colonies may beobserved in the scale pockets. In advanced skin lesions, epithelium is lost and numerous bacteria are present in the dermis and extending into the underlying skeletal muscle. Necrotic muscle appears pale and muscle fibers are disrupted. Inflammatory infiltrates vary from minimal to severe and consist of neutrophils admixed with monocytes, basophils, and lymphocytes**.** Hemorrhage also maybe apparent. *F. columnare*are better observed in the deeper lesions such as skin ulcers. In many cases, few bacteria may be present because of detachment and loss of organisms during routine tissue processing. The bacteria are difficult to visualize with routine hematoxylin and eosin (H & E) stain or Gram's stain because organisms stain faintly pink with H & E and Gram's stain. Giemsa stain provides better visualization of these bacteria because they stain dark blue in tissue sections. Neither lesions nor bacilli have been reported in histologic specimens of internal organs from fish with columnaris disease. In addition, Geimsa stained tissue sections have failed to reveal bacteria in internal organs (Morrison *et al.*, 1981).

2.4.3. Hematological and clinical chemistry

Hematologic and biochemical parameters are frequently used to detect disease processes in domestic animals and human beings. Although these changes are often nonspecific, they often can detect disease-induced changes in major organ systems. Hematologic and clinical chemistry parameters are not evaluated routinely in aquatic medicine. When such laboratory testing is performed, interpretation of the data is difficult because reference intervals are unavailable for many species of fishes. Piscine hematology and serum chemistry also are complicated by difficulties in blood collection and contamination of blood samples with tissue fluid during venipuncture (La Frentz*et al.*, 2002).

2.4.4. Molecular diagnostic techniques

Recently, more sensitive and less time consuming diagnostic techniques have been developed to accurately diagnose *F. columnare*infections. Polymerase chain reaction (PCR) based techniques utilizing species-specific primers have been used in fish disease diagnostics for the diagnosis and epidemiology of Flavobacterialdiseases. PCR is able to detect very low levels of *F. columnare*. It is specific, sensitive and reproducible for the detection

and quantification of *F. columnare* in tissues of infected fish. PCR has been used for the detection of *F. columnare*nucleic acid in bacterial cultures. This technique used species-specific primers to amplify the 16S rRNA. Combined PCR and restriction fragment length polymorphism (RFLP) allows a more accurate identification of *F. columnare.* The first 500 nucleotides in the 5' terminus of the 16S rDNA contain enough information to allow accurate assignment of bacterial sequences to the main lines of descent, and this terminus has been recommended as the region of interest for molecular analysis (Bader *et al*., 2003).

This technique can differentiate *F. columnare*from other species of yellow pigmented, gliding bacteria, but it does not delineate various isolates of bacteria within the same species. A broad range PCR to the 16S rDNA followed by RFLP analysis and product sequencing was able to differentiate several *Flavobacterium*species including *F. columnare, F. psychrophilum, F. johnsoniae,* and *F. hibernum.* Species-specific primers (Col72F and Col 1260R) have been published for PCR amplification of the ribosomal 16S rRNA gene for identification purposes (Panangala*et al*., 2007).

2.5. Pathogenesis

Bacterial pathogens have numerous ways to cause disease to their hosts. Getting contact with the host and attaching to host tissues are the most important steps in initiating the infection. After this, to utilize the host and escape the immune defense of the host virulent bacteria excrete tissue degrading enzymes and toxins causing the disease. Thus, cell surface structures functioning as adhesion factors or having some other roles in the infection process as well as extracellular products have been studied widely in bacterial fish pathogens. Several virulence factors have been described for bacterial fish pathogens (Arias *et al.*, 2012).

The severity of disease may be affected by certain host factors such as age and species. In experimental infection, young carp were more susceptible to columnaris disease than adults which were generally resistant. Also, certain species of fish, such as buffalo fish and crappie, were more susceptible to spontaneous columnaris infections. Other species of fish, including catfish and carp, were only moderately susceptible to this disease. The difference in disease resistance may be due to compactly arranged layers of scales or tougher skin in adult carp and other resistant fishes. Alternatively, it also is possible that strains of *F. columnare*infecting different species may vary in their virulence. High and low virulence strains of *F. columnare*have been isolated from several species of fish. Occasional natural outbreaks of columnaris disease have occurred in the absence of any obvious stressors and it was assumed that highly virulent strains of the bacterium were involved (Decostere, 2002).

The colonization of the fish tissue is to be regarded as a complex multistep process which can be subdivided into the stages of attraction, adhesion and aggregation. The mucus from the skin and gills of fish promotes chemotaxis of *F. columnare.* The chemotactic response of the more virulent genomovar II isolates suggested that chemotaxis could be associated with virulence. The bacterium has the slowly forward and backward gliding of motility (La Frentz and Klesius, 2009).

The initial step of disease development in most pathogenesis studies is the ability of *F. columnare*to adhere to gill tissue. Adhesion of the bacterium to gill tissues was studied after treatment with different carbohydrates. Three carbohydrate-binding receptors (D-mannose, D-glucose and *N*-acetyl-D-glucosamine) associated with the capsule of *F. columnare* might be involved in chemotactic responses. When pretreated with D-mannose, there was no up regulation of gliding motility gene*sgld*H. The ability to adhere is a prerequisite for the successful colonization of the host tissue. A highly virulent strain adhered more readily to the gill tissue than did the low virulence strain. The adhesion of *F. columnare* to the gill tissue constitutes an important step in the pathogenesis of columnaris disease (Klesius*et al.,* 2010).

F. columnare produces two types of mucus. The first one is an acidic polysaccharide and is made visible by ruthenium red staining. Another type of mucus is a basic, partially acetylated polygalactosamine, which cannot be stained with ruthenium red. A capsular material which coated the surface of the bacterial cell could be stained with ruthenium red. They stated that the ruthenium red-positive material was probably an acid mucopolysaccharide that might be involved in the adhesive properties of the cells. The adhesion to the fish tissue was shown to be impacted by various environmental parameters. The adhesion of a highly virulent strain to the gill tissue was enhanced by a number of factors, including immersion of the gill in

divalent ion water, the presence of nitrite or organic matter, and high temperatures. An aggregative adhesion pattern of a highly virulent *F. columnare* strain onto gill tissue is a distinct feature in both in vivo and organ culture experiments. This results in an irregular gill surface covered by a thick mat consisting of numerous clumps of *F. columnare* bacterial cells, most likely impeding oxygen uptake and causing death of the fish (Declerq, 2013).

The secretion of various extracellular enzymes participates in the destruction of skin, muscle and gill tissue, enhancing pathogenicity. In culture, *F. columnare* produces an enzyme that degrades chondroitin sulfates A and C and hyaluronic acid, the complex polysaccharides of connective tissue. This so-called chondroitin AC lyase acts specifically on a group of acidic mucopolysaccharides found primarily in animal connective tissue. AC lyase is alleged to play a role in the virulence of *F. columnare*. Though high AC lyase activity solely would not be enough to induce virulence in *F. columnare* strains, both high AC lyase activity and gliding motility of the bacteria would be needed for *F. columnare* to be virulent. Proteases also contribute to damaging the tissue or enhancing invasive processes (Kunttu*et al*., 2011).

Some studies reveal that *F. columnare* might be able to avoid parts of the immune system. Antibacterial characteristics of the fish mucus against *F. columnare* have been demonstrated. Skin mucus of different fish species responds differently to the bacteria, or that it is the *F. columnare* strain which is critical in determining the antibacterial capacities of the mucus. Very little or no bactericidal activity was produced against this bacterium. Sialic acid seemed to be the determining factor for the pathogenicity of *F. columnare*. Anti-bactericidal activity of the mucus increased after removal of sialic acid with neuraminidase. Another recurrent feature in *F. columnare* infections is the lack of an inflammatory response as observed upon inspecting affected tissues microscopically. This resulted in the hypothesis that *F. columnare*triggers the endogenous programmed cell death machinery of immune cells to evade the immune system (Olivares-Fuster*et al*., 2011).

2.6. Treatment

Current methods of treatment include antibiotic administration and chemical dips or baths. The use of antibiotics may be governed by whether the fish are intended for human consumption or are being marketed for the tropical fish pet trade. Sodium benzylpenicillin (10,000 IU/L of water), oxytetracycline, and chloramphenicol (to achieve a final concentration of 16 µg/ml of water) have been found to be effective in treating columnaris disease. A complete water change should be done after bath treatment. Systemic infection, if present, can be treated with oral or parenteral antibiotic administration (combined with therapeutic baths if external lesions also are visible).

External treatments are possible only in early stages of the disease, when the infection is still superficial. Drugs which have been used effectively in bath therapies are chloramphenicol, nifurpirinol, nifurprazine and oxolinic acid. If the disease is in an advanced stage and/or signs of septicaemia are observed, it is necessary to administer antimicrobials in the feed. Oxytetracycline given orally for up to 10 days, proved effective in early as well as advanced outbreaks of columnaris disease. Sulfonamides, such as sulfamerazine and sulfamethazine, can be used orally but would be less effective than other drugs.Nitrofuran can also be administered orally for 3 to 5 days. The excessive use of antimicrobial agents to withstand *F. columnare*has its negative attributes. These include possible allergic reactions elicited in the user after food contact. Potential impacts on human health resulting from the emergence of drug-resistant bacteria and the associated risk of transfer of these resistant traits to the environment and human-associated bacteria are also a major concern (Serrano, 2005).

In vitro multiple resistances of *F. columnare* strains originating from ornamental fish toward several clinically important antibiotics has been demonstrated, such as quinolones and tetracyclines. Less practical use of antimicrobials in fish industry and therefore needs to limit their use and to focus on the development of alternative curative and preventive measures against columnaris disease (Declercq, 2013).

Chemicals have also been adopted in the curative treatment of columnaris disease besides resorting to antimicrobial agents. The herbicide Diquat® was shown to significantly reduce channel catfish mortalities to zero percent after challenge with *F. columnare*.It has also proven to be effective in the treatment of columnaris disease in salmonids. Copper sulfate and potassium permanganate are among the older chemicals used for treatment and prevention of columnaris disease in pond fishes. Copper sulfate has

clear therapeutic value against *F. columnare* infections in channel catfish when treated in an ultralow flow-through system during 4 h (Darwish, 2009).

2.7. Control and Prevention

2.6.1. Management

Management plays a key role in the prevention of fish diseases. Columnaris disease is best prevented by good management practices that include minimal handling, attention to water quality and temperature, and prevention of overstocking. However, outbreaks of columnaris disease are difficult to avoid because of the ubiquitous presence of *F. columnare*in water sources. Because infection is less likely at lower temperatures, lowering water temperature by adding cold water or ice may help reduce the severity of disease. When fish are transferred from one aquatic environment to another, adequate time should be allowed for equilibration before release. Production variables such as feed consumption, pond depth, ammonia levels and stocking events were associated with columnaris disease outbreaks. Reduction of fish density could be used in the prevention of columnaris disease especially if water temperature is high. As lower rearing density can also decrease the transmission of ectoparasites and penetrating endoparasites, it could be an efficient tool in ecological disease management as a whole (Suomalainen*et al.,* 2005; Cunningham *et al.,* 2012).

High nitrite levels and organic load can stimulate the adherence capacity of *F. columnare* and therefore it is important to monitor and control these parameters as well. Furthermore, water treatment could aid in averting a bacterial outbreak. Ozone treatment of water significantly reduced the numbers of added *F. columnare*, which could be a practical method of prevention. Salt and acidic bath treatments could be used to disinfect water contaminated by *F. columnare*. An in vivo immersion challenge of *F. columnare*in channel catfish and goldfish (*Carassiusauratus* L.) revealed decreasing mortality as salinity goes up, with significantly lower and no mortalities when salinity reaches values of 1.0‰ and between 3 and 9‰, respectively. If the fish can be adapted to salt levels of at least 1.0‰, this method could be used as a possible preventive measure in columnaris disease. In the absence of natural food, juvenile channel catfish should be fed at least once every other day to apparent satiation in order to maintain normal physiological function and improve resistance to *F. columnare*, since deprivation reduced innate resistance of fish to columnaris disease (Suomalainen*et al.,* 2005).

2.6.2. Use of chemical agents

Chemical agents can also be adopted as a preventive approach besides optimizing and adjusting management practices. The intensification of columnaris disease could be prevented by treating the fish for 20 min in a copper sulfate ($CuSO_4$) bath at 37 mg/L or by adding copper sulfate to pond water at 0.5 mg/L. Prophylactic treatment of channel catfish with 15 mg/L chloramine-T reduced fish mortality from a *F. columnare* infection from 84–100% to 6–14%. The efficacy of prophylactically given oxytetracycline against mortality in channel catfish and also reported zero mortality for the combination of sulphadimethoxine and ormetoprim in feed prior to bacterial challenge with four highly virulent strains of *F. columnare*(Thomas-Jinu and Goodwin, 2004).

2.6.3. Vaccination

Fish can be protected from subsequent *F. columnare* infections by activating the adaptive immune system. Although vaccination trials have not always been successful, success rates have increased as knowledge on fish immunity and its role in the defense against bacterial diseases continues to expand. Bath immunization with a bacterin was shown to protect carp against experimental challenge. Immersion of channel catfish in a bacterin, when performed each year, induced a significant decrease in mortality compared to unvaccinated fish. Oral immunization with heat-killed cells of *F. columnare* incorporated into fish feed protects against columnaris disease in 3-month-old coho salmon. Prolonged feeding of formalin-killed bacteria over three months provided high levels of protection. Immunization with formalin-killed sonicated cells in Freund's complete adjuvant injected intraperitoneally in tilapia resulted in a significant systemic humoral response within two weeks and antibody levels almost tripled following secondary immunization. The mean antibody titer remained significantly elevated at 10 weeks post-immunization. Antibodies were also observed in cutaneous mucus of these fish at six and eight weeks postimmunization (Shoemaker *et al.,* 2011).Commercially available oral and bathing vaccines have been successfully tested in largemouth bass (*Micropterussalmoides*) fry and salmon, respectively (AFS-FCS, 2011).

2.7. Economic Importance

Bacterial disease is the primary cause of mortality in commercially reared channel catfish, accounting for 58% of the total cases examined from 1987 to 1989 in Alabama, Mississippi, and Louisiana diagnostic laboratories. Columnaris disease is the second most prevalent bacterial disease in channel catfish accounting for approximately 23% of the total cases of bacterial etiology. In the channel catfish industry, columnaris disease ranked second only to Enteric Septicemia of Catfish as a cause of economic losses (USDA, 2003).Columnaris disease or mixed infections of columnaris and Enteric Septicemia of Catfish were listed as causing the greatest economic losses on catfish farms by 70% of farmers from the four leading catfish producing states, with losses estimated in the millions of dollars. Columnaris disease can occur as the primary disease in pond or tank raised channel catfish, with mortalities as high as 50%. Mortality rates as high as 34% have been documented in salmonid species. Columnaris outbreaks in the Melvern and Pomona Reservoir in Kansas resulted in the loss of an estimated 54,000 white bass (USDA, 2003).

Conclusion and Recommendations

Columnaris disease is an infectious bacterial disease of freshwater fish leading annually to severe financial and material losses at fish farms worldwide. Infections caused by bacteria belonging to a particular branch of the genus columnare have become increasingly recognized in farmed fish and contains important virulence factors that enhance bacterial survival and pathogenesis in hosts. Columnaris in fish develops in a similar fashion independent of host species with varying associated mortality levels. The disease is highly infectious and often prevalent in affected stocks. Management of fish densities optimization or adjustment and control of water quality parameters are the first critical steps in controlling columnaris disease. Vaccines and chemotherapeutics could possibly have a promising future in the prevention and control of the disease. Use of antibiotics for columnaris treatment is often reported to create antimicrobial resistance.Hence, based on the above conclusion, the following points are recommended:Being highly devastating diseases, prior notifications should be given in cases of localized outbreaks;Control of water quality parameters and fish densities adjustment should be the first critical steps in controlling columnaris disease; the development of efficient curative and effective preventive measures in farmed fish should be of research focus anddevelopment of cost effective and environmentally friendly prevention methods of columnaris disease should be used in the various susceptible farmed fish species.

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