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Streptococcus: A review article on an emerging pathogen of farmed fishes

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ABSTRACT

Aquaculture is one of the fastest-growing sectors for food production. The development of the aquaculture industry has led to the emergence of various bacterial diseases. One of the leading causes of fish diseases is Streptococcus species. The genus *streptococcus* is large and complex, accommodating a wide range of gram-positive bacteria. *Streptococcus iniae, streptococcus agalactia,* and *streptococcus dysagalactia* are the most pathogenic species. Bacterial diagnosis based on phenotypic and biochemical identification methods has been routinely used. In this review, light is spotted on worldwide distribution, diagnosis, transmission, pathogenesis, and histopathological changes. Antibiotic sensitivity and vaccination are the most effective control methods for the disease.

1.INTRODUCTION

Aquaculture has become a worldwide economically important industry which requires continuing research with scientific and technical developments and innovations **(Bektaş *et al.*, 2017).**To cover the increasing need of food in the World, an intensive production in fisheries has been a must which ended with the occurrence of diseases **(FAO, 2016).** The fact that aquaculture sector is the fastest growing food-production industry in the world is plagued by diseases. The annual economic loss in the aquaculture industry due to diseases is estimated to be billions of US dollars worldwide **(Klesius and Pridgeon, 2011).**

The development of intensive aquaculture has led to the emergence of various bacterial diseases **(Pridgeon and Klesius, 2012).** Infectious disease outbreaks in fish farms might be caused by single or multiple pathogens; however, little is known about the association of different microorganisms causing coinfections and its consequences **(Dong *et al*., 2015a , 2015b).**

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Bacteria can survive well in aquatic environment independently without their hosts. Bacterial diseases have become major impediments to aquaculture, especially in warm temperature. Thus far, bacterial species belonging to at least 13 genera have been reported to be pathogenic to aquatic animals, including either gram-positive bacteria such as *Lactococcus*, *Renibacterium* and *Streptococcus* or gram-negative bacteria such as *Aeromonas*, *Edwardsiella*, *Flavobacterium*, *Francisella*, *Photobacterium*, *Piscirickettsia*, *Pseudomonas*, *Tenacibaculum*, *Vibrio* and *Yersinia* **(Klesius and Pridgeon, 2011).**

Streptococcus spp. is very pathogenic as they can affect many fish species in the world, particularly, *Streptococcos* has been reported to occur in fresh, marine and brackish water fish. It is worth noting that *Streptococcos* has caused millions of economic losses of aquaculture in the world. Moreover,Tilapia is considered a perfect host for *Streptococcus* infection **(Amal and Zamri, 2011).**

Members of the genus *Streptococcus* are widely distributed in the world. Streptococci are common pathogens of fish. It has been reported that dozens of cultured and wild-ranging marine and fresh water fish are susceptible to *Streptococcus* such as salmon, mullet, golden shiner, pinfish, eel, sea trout, tilapia, sturgeon, striped bass, rainbow sharks, red-tailed black sharks, danios, some cichlids and several species of tetras **(Wang *et al*., 2013).**

Streptococcus is a genus of bacteria containing some species that cause serious diseases in a number of different hosts. This disease causes significant economic losses in the aquaculture industry in the United States of America, Japan, South Africa, Iran, Australia, Philippines, Taiwan, Bahrain, Turkey and other countries. In addition, Streptococcal disease in fish was first reported in 1957 **(Bektaş *et al* , 2017).**

Streptococcosis of fish should be regarded as a complex of similar diseases caused by different genera and species capable of inducing a central nervous damage characterized by suppurative exophthalmia and meningo-encephalitis.Warm water streptococcosis (causing mortalities at temperatures above 15˚C) typically involves *L. garvieae, S. iniae, S. agalactiae* and *S. parauberis* whereas cold water streptococcosis (occurring at temperatures below 15 ˚C) is caused by *L. piscium* and *V. salmoninarum*. It is important to report that the etiological agents of warm water streptococcosis are also considered as potential zoonotic agents capable of causing disease in humans **(Bektaş *et al.*, 2017).**

Outbreaks of streptococcal infections in tilapia have been reported in many areas such as Asia, the Middle East, North and South America **(Wongtavatchai and Maisak, 2008).** The genus *Streptococcus* is large and complex, accommodating a wide range of Gram positive bacteria. Only few biotypes have been isolated from fish and the most pathogenic are those belonging to D serogroup, otherwise known as the Enterococci **(Athanassopoulou and Roberts, 2004).** This pathogen is found to cause disease in farmed tilapia in different stages of life, for example, fry, juvenile to brood stocks, hatchery, nursery and grow out phase **(Jantrakajorn *et al.*, 2014).**

The major species of *Streptococcus*, which infect fish are *S. iniae, S. difficile, S. agalactiae, S. parauberis, S. dysgalactiae and S. Shilo* (**Netto *et al.*, 2011**). Pathogenic fish *Streptococcus* species have been associated with *S. agalactiae, S. difficile, S.*

dysgalactiae, S. equi, S. equisimilis, S. (= *E.*) *faecium, S. ictaluri, S. iniae, S. milleri, S. parauberis, S. phocae, S. pyogenes* and *S. zooepidemicus.* In addition, *E. faecalis* NCTC 775 T, *E. faecium* NCTC 7171 T, *L. lactis* NCFB 604, *S. mutans* NCFB 2062 provoke streptococcosis in Atlantic salmon and rainbow trout **(Osman *et al.*, 2017).**

Streptococcus spp., classified as group B streptococci (GBSs), are Gram-positive bacteria known as causative agents for both terrestrial and aquatic animal streptococcosis **(Evans *et al.*, 2008).** *Streptococcus agalactiae*, a gram-positive bacterium that can infect a number of fish species (including freshwater and seawater species), has a serious impact on fish aquaculture. Tilapia is extremely susceptible to infection by *S. agalactiae* **(Chen *et al.*, 2012a).** Since 2009, large scale *S. agalactiae* disease outbreaks have had a devastating effect on the development of tilapia aquaculture in China, resulting in direct economic losses reaching about 0.4 billion dollars in 2011 (**Chen *et al.*, 2012b**).

Outbreaks of *S. agalactiae* infection in tilapia have been reported in several countries around the world **(Asencios *et al.*, 2016).**In fact, *S. agalactiae* is the causative agent of streptococcosis in Nile tilapia **(Buller, 2014).** *Streptococcus iniae* and *S. agalactiae* are the major causes of bacterial infection known to have a devastating effect on survival rate in global tilapia farming **(Amal and Zamri, 2011).**

2. WORLD DISTRIBUTION

Streptococcal disease occurs in all continents (Americas, Asia, Europe, Africa, and Australia). Thousands of *Streptococcus* species (*S. parauberis*, *S. iniae*, *S. agalactiae*, *Lactococcus garvieae*, *S. dysgalactiae*, and *Vagococcus salmoninarum*) have been reported in different parts of the world **(Table 1** and **Fig. 1) (Carson *et al.*, 1993; Chang and Plumb, 1996; Diler *et al.*, 2002; Ruiz-Zarzuela *et al.*, 2005; Baeck *et al.*, 2006; Agnew and Barnes, 2007; Pereira *et al.*, 2010; Abdelsalam *et al.*, 2013; Nho *et al.*, 2013** and **Li *et al.*, 2015).** Streptococcosis is a multifactorial disease in fish, depending on host variety, age, immune status, type of pathogen (species and strain), and environmental conditions **(Ghittino *et al.*, 1999; Ravelo *et al.*, 2001** and **Vendrell *et al.*, 2006).** Epidemiological studies in the major tilapia producing regions of Asia and Latin America from 2001 to 2009 **(Table, 1)** showed that of the nearly 500 streptococcal isolates recovered from tilapia, 82% were identified as *S. agalactiae* and 18% were identified as *S. iniae* **(Liu *et al.*, 2016)**. In China, more than 90% of the clinical bacterial isolates from infected tilapia since 2009 have been *S. agalactiae* **(Chen *et al.*, 2012a).**

Table(1): Streptococcus bacterial agents and detailed information of affected fish species, locations, hosts, and clinical criteria **(Mishra *et al.*,2018)**

Species	Host	Fish species	Clinical Criteria	Geographical Location
Streptococcus iniae	Fish, Human	Hybrid striped bass, Nile tilapia, Hybrid tilapia, Rainbow trout, Red drum, Rabbitfish, Sea bass, Olive flounder, Barramundi, Wild fish	Hemorrhage, exophthalmia, abdominal distension, ascites, lesions (liver, kidney, spleen, and intestine)	Canada, Americas, Bahrain, Israel, Thailand, China, Japan, Singapore, Taiwan, Korea
Streptococcus parauberis	Fish, Cow	Olive flounder, Rainbow trout, Cultured turbot, Hybrid striped bass	Chronic wasting syndrome, hemorrhagic septicemia, exophthalmia, meningitis with abnormal swimming	Israel, Italy, Japan, Spain, USA, China, Iran, Korea, Malaysia, India
Streptococcus agalactiae	Fish, Cow, Human, Chickens, Camels, Dogs, Horses, Cats, Frogs, Hamsters, Monkeys	Nile tilapia, Barcoo grunter, Golden pompano, Giant Queensland grouper, Ya-fish, Silver pomfret	Erratic swimming, appetite, lethargy, uncoordinated movements, exophthalmia (uni- or bi-lateral), intraocular hemorrhage, opaqueness of cornea, ascites	Europe, Turkey, China, Indonesia, Malaysia, Japan, Korea, Vietnam, Philippines, Americas
Lactococcus garvieae	Fish, Cow, Human, Cat, Dog, Water buffalo	Rainbow trout, Yellowtail, Tilapia, Japanese eel, Grey mullet, Black rockfish, Catfish, Wild wrasse, Giant fresh water prawn, Olive flounder, Amberjack, kingfish	Melanosis, lethargy, erratic swimming, disorientation, fins, exophthalmia (uni- or bi-lateral), swollen abdomens, anal prolapses, hemorrhages (periorbital, perianal, buccal regions)	Turkey, Australia, South Africa, England, Portugal France, Balkans, Israel, Korea
Streptococcus dysgalactiae	Fish, Calves, Lamb, Human, Sheep, Dogs, Pig, Lamb, Cats	White spotted snapper, Kingfish, Grey mullet, Cobia, Hybrid red tilapia, Pompano, Basket mullet, Pompano, Golden pomfret, Amur sturgeon, Nile tilapia, Yellow tail, Amber-jack	Abnormal swimming, loss of orientation, exophthalmia	Brazil, Indonesia, Malaysia, Taiwan, China, Japan
Vagococcus salmoninarum	Fish	Rainbow trout, Atlantic salmon, Brown trout	Loss of equilibrium, exophthalmia, melanosis, bleeding (jaw, eye, mouth, abdomen, fins, and anus), necropsy, transparent fluid accumulation, fibrinous deposits (heart, liver, spleen)	France, Italy, Spain

3. DIAGNOSIS

3.1. Clinical signs and postmortem legions

Streptococcosis, known as ‘pop-eye’, is contagious with high mortality and has assumed its importance due to being the most crushing threat as it can bring about huge number of deaths of large size fish causing heavy commercial losses in Australia, Italy, Japan, Korea, South Africa, Colombia, Indonesia and USA. The global commercial losses estimated to reach 250 million USD in 2008. Within 3 to 7 days, acute Streptococcus infections in fish induce > 50% mortality rates, while in chronic infections the mortalities could extend to several weeks, with a daily death of one or two of the fish. In most cases, the clinical symptoms of *Streptococcus* infection, with no species differences is usually in the form of lethargic, erratic swimming (spiraling or spinning swimming), dark skin pigmentation, exophthalmia with opacity and hemorrhage in the

eye, abdominal distension, diffused hemorrhage in the operculum, around the mouth, anus and base of the fins and enlarged blackened spleen **(Osman *et al.*, 2017)**.

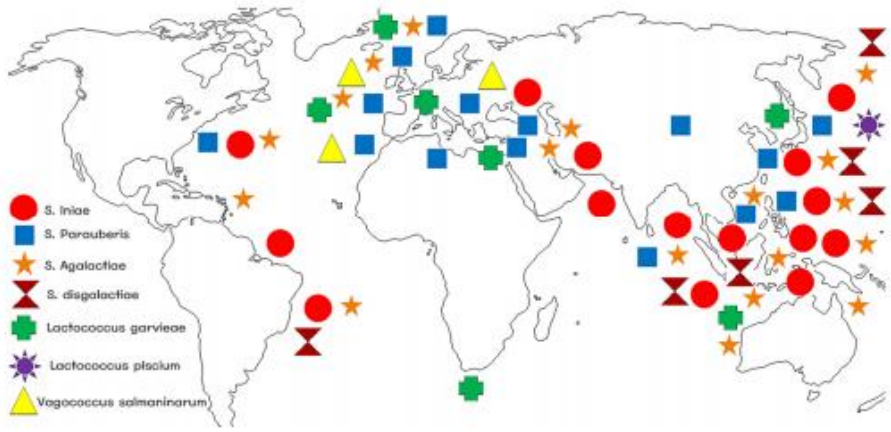

Fig. (1): Phylogeography of major fish pathogens belonging to *Streptococcus* species, distribution pattern shows the presence of these bacterial isolates over the continents **(Mishra *et al.*, 2018)**

The infected fish has clinical signs such as lethargy, anorexia, loss of orientation, exophthalmia, abdominal distension, erratic swimming and scattered hemorrhage around the operculum, mouth, fin, body or sudden death with few signs which are similar to clinical signs of other bacteremia **(Jantrakajorn *et al.*, 2014).**

Common clinical signs of *S. agalactiae* infection reflect damages in diverse tissues, as the affected fish may manifest erratic swimming, lethargy and dorsal rigidity as a result of lesions in the central nervous system. *Streptococcus agalactiae* causes a systemic chronic inflammatory response characterized by the presence of granulomas in different organs. Exophthalmia is a common finding that may develop as a result of retrobulbar infection. Grossly, there are fibrinous pericarditis and peritonitis, haemorrhages around the brain, the retrobulbar region and intestines. Microscopically, the major reported lesions are haemorrhagic or granulomatous meningo-encephalitis, choroiditis, periscleritis, epicarditis, splenitis and nephritis, where abundant Gram-positive cocci are often observed free or within macrophages **(Iregui *et al.*, 2016).**

The clinical signs of streptococcosis, including depression or excitability, anorexia, erratic swimming and whirling, can be observed soon after infection. Pathogenesis in infected fish involves septicaemia and bacterial colonization in numerous organs such as the nares, brain, kidney and intestines **(Kannika *et al.*, 2017).**

3.2. Morphochemical characteristics

The Genus *Streptococcus* contains Gram-positive spherical bacteria less than 2 microns diameters that typically grow in pairs and form chains when grown in liquid media. Most members are facultative anaerobes, as they can grow in absent or limited

oxygen conditions; they are catalase negative with varying nutritional requirements, which reflects adaptation as commensals or parasites. Commonly, *Streptococcus* is grown in culture media supplemented with red blood cells that may allow a preliminary classification based on the production of hemolysis, which can be classified as β-hemolytic, α-hemolytic or non-hemolytic strains **(Edwards and Nizet, 2011).** In Colombia, the entire *Streptococcus agalactiae* strains isolated from tilapia by the Veterinary Pathobiology Group at the Universidad Nacional de Colombia (VPG, UNC) have been found non-hemolytic and belonging to the serotype Ib. The identification scheme followed in the laboratory is based on colony characteristics, hemolytic properties, carbohydrate and protein antigen composition, sugar fermentation and other biochemical reactions. DNA sequencing of 16 and 23S rRNA genes. The majority of pathogenic streptococci have a serologically reactive carbohydrate sheath that is antigenic different from one species or group of species to another. These cell wall associated antigens are designated A-H and K-V, and are the basis of the Lancefield groups **(Iregui *et al.,* 2016).**

S. agalactiae bacterial cells were Gram positive cocci with different characteristics. The diameters of non-haemolytic and β- haemolytic type cell colonies were around 0.591-0.748 μm and 0.787-1.231 μm respectively. Both types of bacteria could grow well at 28°C, and were not significantly different when the growth test was carried out at 37°C **(Suhermanto *et al.,* 2019).** *S. agalactiae* contains the cAMP factor, a pore-forming toxin first identified in this bacterium. The cAMP reaction is based on the co-hemolytic activity of the cAMP factor and is commonly used to identify *S. agalactiae* in the clinic. Although the cAMP toxin has been discovered more than a half century ago, no structure from this toxin family has been reported, and the mechanism of action of this toxin remains unclear **(Jin *et al.,* 2018).** The biochemical characteristics of all isolates with streptococcosis showed non-motile, β-haemolytic/non-haemolytic, catalase and oxidase negative, fermentative positive, did not grow on media sulphide indole motility (SIM), grew on 6.5% NaCl media and bile salt media 40%. The characteristics in the confirmation test using API 20 STREP were different and β-haemolytic bacteria hydrolyzed more sugar than the non-haemolytic type. Test of hemolysis isolate activity in blood agar media showed a difference in hemolysis of blood. β-haemolytic bacteria had different characteristics when cultured on brain heart infusion agar (BHIA) media i.e. they had thick colonies, more transparent, slimy and easily harvested, while non-haemolytic bacteria tended to be rather thin, yellowish, sticky and difficult to harvest **(Suhermanto *et al.,* 2019).**

4. TRANSMISSION AND SOURCE OF INFECTION

Fish depend on water to breathe, feed, excrete wastes, maintain osmolality, and reproduce. In this sense, the physical and chemical quality of an aquatic environment is critical to understand the pathogenesis of fish diseases and to develop effective preventive practices and adequate treatments **(Dang *et al.,* 2012; Soto; Revan, 2012)**. Environmental stress leads to outbreaks of the most common diseases in fish farming **(Martins *et al.,* 2009).** These outbreaks are associated with rapid changes in water temperature, which favors bacterial proliferation **(Marcogliese, 2010)** and lower host resistance **(Hooper *et al.,* 2007)**.

S. agalactiae and *S. iniae*, are the most common bacteria that cause huge economic losses in the tilapia industry. Their prevalence and severity depend on multiple environmental factors, including warm water temperatures (in the summer), increased ammonia levels, and low dissolved oxygen levels (caused by poor husbandry and high stocking density). *S. agalactiae* is more commonly associated with diseases in human and bovine hosts. However, fish-pathogenic *S. agalactiae* were documented as early as 1966, when a non hemolytic Group B *Streptococcus* was identified as the cause of 2 epizootics in golden shiners *Notemigonus crysoleucas* **(Bromage and Owens ,2002).** It is worth mentioning that *S. agalactiae* is more prevalent than *S. iniae* in tilapia **(Liu et al,2016).**

A study by **Xu *et al.* (2007)** showed that the infection by this particular pathogen could occur through wounds and abrasions of the skin. This mechanism usually involved in fish that were cultured in high densities. Furthermore, the transmission of *Streptococcus* between different species of wild and cultured fish, within the same aquatic environment, is likely to occur. This happens due to the fact that wild fish and fish cultured nearby have been found to be infected with the same *S. iniae* strains. Similarly, **Bromage and Owen (2002)** reported that the fish cohabiting barramundi pens had the same *S. iniae* strains as the barramundi. In addition, the transmission among the species of reef fish has also been reported in the Caribbean **(Amal and Zamri, 2011).**

Some stressors that have been associated with the Streptococcal outbreaks include high and low water temperatures, high salinity and alkalinity (pH>8), low dissolved oxygen concentration, poor water quality (such as high ammonia or nitrite concentrations), high stocking densities, as well as harvesting and handling effects (**Yanong & Floyd, 2002).**

Water quality parameters can contribute to the development of disease.The intolerance of tilapias to low temperatures is a well-known fact which causes a serious constraint for commercial culture in temperate regions. Reproduction of tilapia is best in water temperatures above 27℃, but it does not occur when water temperature is below 20℃. It was concluded that the optimal water temperature for the growth of tilapias is between 29℃ and 31℃, but a water temperature of ≥31℃ predisposes tilapias to the outbreaks of *Streptococcus agalactiae* infection. Although tilapia can survive acute low dissolved oxygen (DO) concentrations of less than 0.3 mg/l for several hours, tilapia ponds should be managed to maintain the DO concentrations above 1 mg/l. Furthermore, metabolism, growth, and disease resistance are depressed when DO falls below this level for a prolonged period, predisposing tilapias to streptococcosis **(Amal *et al.*, 2008).** To cause disease, a microorganism must first adhere to the epithelial surfaces of a host. In the case of systemic pathogens such as *S. agalactiae*, the bacterium must first invade the intestinal epithelium before reaching the systemic compartment. Few studies are available where the mechanisms of adhesion and invasion through the epithelia by *S. agalactiae*, both in fish and in mammals, have been addressed **(Barato *et al.*, 2016)**. Using the intragastric route for *S. agalactiae* inoculation, **Iregui *et al.* (2016)** demonstrated the adhesion and invasion of the microorganism through the gastrointestinal epithelium, proposing that the main entry route of the pathogen to the host was oral. The mechanism of adherence and invasion of *S. agalactiae* in tilapias would allow the formulation of ecologically viable prevention and control strategies. The proposed mechanism for this

evasion might be due to the fact that the microorganisms possess sialic acid on their surface, the same that is found on the surface of the host epithelial cells allowing the bacteria to mimic and deceive the host's immune system. It is interesting to note that enterocytes did not show significant mortality even when large numbers of the pathogen were within the cytoplasm of these cells and also were dividing. It might be speculated that affected enterocytes could detach from the epithelial layer and die in the intestinal lumen. Detaching as a natural defense measure against pathogens has been reported **(Williams *et al*., 2015).** In addition, it was confirmed that the gastrointestinal route is the main route of entry and infection and that *S. agalactiae* is deprived of the capsule to adhere to the epithelium **(Vásquez-machado *et al* ., 2019)**.

5. PATHOGEN

The pathogenesis of streptococcosis depends upon several factors that vary with fish species, bacterial species, and isolates. Further details of virulence and pathogenicity of streptococcosis are given below. Genetic virulence depends on several factors; for example, *S. iniae* virulence is associated with a unique genetic profile **(Fuller *et al*., 2001)**. Comparison of 17 geographically different strains of *L. garvieae* based on genetic homogeneity vs. serological data showed that pathogen diversity is related to virulence factors **(Barnes and Ellis, 2004**). Another study showed that the lactococcal bacterial population presented a clonal structure in endemic regions, while in sporadic regions; it displayed a high genetic heterogeneity **(Eyngor *et al*., 2004).** Virulence experiments have shown that capsulated *Lactococcus garvieae* strains are more virulent than non-capsulated strains in rainbow trout **(Barnes *et al*., 2002).** Virulence varies with bacterial isolates within the same species in *S. dysgalactiae* **(Abdelsalam *et al*., 2010).** Currently, the potential for *Streptococcus* species to cross interspecies barriers and cause disease in other hosts is poorly understood. Many streptococcal species are multi-host pathogens. Humans constantly face the risk of infection due to close interactions with the fish industry **(Abdelsalam *et al*., 2010).** *S. iniae*, *S. agalactiae*, *L. garvieae*, and *S. dysgalactiae* are human pathogens, forming a major threat to public health. *S. iniae* can cause bacteremic cellulitis, septic arthritis, meningitis, and endocarditis **(Weinstein *et al*., 1997; Facklam *et al*., 2005; Lau *et al*., 2006; Agnew and Barnes, 2007** and **Al-Harbi, 2011),** while *S. agalactiae* can cause meningitis and pneumonia in humans **(Brimil *et al*., 2006; Johri *et al*., 2006**).

6. HISTOPATHOLOGY

Grossly, there are fibrinous pericarditis and peritonitis, and haemorrhages in the meninges, the retrobulbar region and the intestine are common. Microscopically, the principal lesions of *Streptococcus agalactiae* are haemorrhagic, macrophage rich or granulomatous meningoencephalitis, choroiditis, periscleritis, epicarditis, splenitis and nephritis, where abundant Gram positive cocci are often observed **(Vásquez-Machado *et al*., 2019)**. The most important histopathology findings were: acute or chronic suppurative fibrin type epicarditis, suppurative myocarditis, acute suppurative meningitis and acute suppurative panophthalmitis. Presence of dispersed pigment in epithelial cells ,coagulation necrosis in muscle ,necrosis and mononuclear infiltration in the liver, hyperplasia and fusion of secondary lamella, congestion, increased of melanomacrophages center (MMC) in spleen, acute suppurative perisplenitis, lymphocytolisis, fat and hydropic degeneration **(Ortega Asencios *et al*., 2016)**.

The brain of naturally infected tilapia was found to have signs of meningoencephalitis. It was visible by the presence of hemorrhages, edema, inflamed neurons with margination, hypertrophied nuclei, marginated hemocytes, and hypertrophied hemocytes. Meningoencephalitis and granuloma-like structures were observed in the brain tissue. The kidney of infected tilapia exhibited extensively necrotized tissue, glomerulopathy with dilated Bowman's capsules, and constricted tubular lumen with vacuolation in the epithelial layer .The hematopoietic tissue of the kidney was hemorrhagic and hypoplastic in appearance. At higher magnification, cellular and nuclear hypertrophy, melanin reaction, and eosinophilic ground substances were visible. The liver of naturally infected tilapia showed massive necrosis of hepatocytes as most of them appeared without a nucleus and with marked fatty changes and hemorrhagic areas. There was karyolysis in hepatocytes as well as marginated hepatocytes with nuclear hypertrophy, pyknotic nuclei, hypertrophied nuclei, and eosinophilic deposition .Congestion of liver blood capillaries, necrosis of hepatocytes, melanin reaction, eosinophilic ground substances ,hemosiderin deposition, and a ruptured portal vein were also noted. Changes were also found in pancreatic tissue, which included degradation and vacuolation of exocrine pancreatic cells, inflammation and necrosis of pancreatic cells, fatty changes and necrosis of hepatocytes, and dilation of the pancreatic duct. The spleen of infected tilapia showed melano macrophage aggregates, extensive necrosis, congestion, hemocyte infiltration, hemosiderin deposition, cellular hypertrophy, vasodilation, darkly stained nuclei, and eosinophilic ground substances. Granuloma-like lesions, similar to those found in the brain, were also observed in the spleen.The blood capillaries of the spleen were dilated, and leukocytes were emerging to the site of infection. The bacterial infection also affected the intestine of tilapia and caused degradation of columnar epithelium exposing the enterocytes of the villi and microvilli, inflammation at the tip of the microvilli, a hemorrhagic area at the villi, and disruption and inflammation in the tunica muscularis and submucosa. Karyolysis, pyknotic nuclei, vacuolation, marginated cells, and inclusion-like bodies were also found in the intestine **(Adikesavalu *et al.*, 2017).**

7. CONTROL

7.1. Antibiotic sensitivity

Antimicrobial administration is one type of the streptococcal infection control in fish farming. However, the use of antimicrobials has contributed to the development of drug resistance in fish pathogens **(Smith, 2008).** Control of *Streptococcus* has been carried out using antibiotics with vaccination that has been reported to reduce infection rates. Florfenicol, an antibiotic which is analogous to thiamfenicol, was used in the sun shine bass to control *S. iniae* with 43% difference in mortality between challenged-treated and challenged untreated fish **(Gabriel *et al.*, 2014).**

The susceptibility of 17 *Streptococcus* strains was assessed against different antibiotics. Highest resistant to Tetracycline was 94.1% while the lowest resistance was observed for Nitrofurantoin, Streptomycin, Gentamicin and Trimethoprim/ Sulphamethaxozol with a percentage of 5.9. The 17 isolates showed total susceptibility to Amoxicillin/Clavulanic acid, Piperacillin/Tazobactam, Nalidixic acid, Colistin and Amikacin **(Osman *et al.*, 2017**).

7.2. **Vaccination:**

The health of offspring from the brood stock depends upon the health, as well as the immune status. Transfer of maternal immunity is one of the alternatives to increase immunity in offspring and enhance the survival rate of offspring. Currently, the immunization of brood stock with monovalent vaccines has reported transfer immunity to the offspring such as in turbot, sea bream, zebra fishes, catfish, and tilapia. Immunization of the brood stock with an *S. agalactiae* monovalent vaccine followed by *S. agalactiae* challenge, and immunization with *A. hydrophila* vaccine followed by *A. hydrophila* challenge, could enhance immunity and protection to the offspring produced from immunized brood stock. Several studies have also reported that immune factors can be transferred from brood stock to offspring through antibodies, lysozymes, protease inhibitors, and complement factors **(Pasaribu *et al.*, 2018).**

Vaccination is another effective method of treatment, which has been used against disease outbreak. However, vaccines are often too expensive and unpractical for wide spread use in fish farms in addition to the fact that a single vaccine owes a specific effect against only one type of pathogen **(Awad and Awaad 2017)**. Vaccination is a widely accepted and effective method to control *S. agalactiae* infection and prevent mass tilapia mortalities **(Liu *et al.*,2016).** As vaccines were developed in other fishes, traditional inactivated vaccines were used widely to provide protection for tilapia from *S.agalactiae* infection. During the early days of inactivated vaccine development, most products contained inactivated bacteria mixed with their extracellular products **(Pasnik *et al.*, 2005).**

Because several killed vaccines had been shown to be efficient against piscine bacterial disease caused by *S. iniae* and *Enterococcus spp.* a formalin-killed *S. agalactiae* vaccine was tested successfully on tilapia for the first time in 1995. This formalin-killed *Streptococcus difficile* strain known as non-hemolytic, serotype Ib *S. agalactiae*, was able to protect tilapia against a challenge of 100× the median lethal dose (LD_{50}) when delivered via intra-peritoneal (IP) injection. Since then, several inactivated vaccines have been used to control *S. agalactiae* infection in tilapia **(Liu *et al.*, 2016).** Commercial vaccines for tilapia against streptococcosis are currently available in many countries and are widely used. The most common type of vaccine for streptococcosis is the injectable vaccine, administered via intra-peritoneal route as it provides the best protection against streptococcosis .Injection is the most potent route of vaccination as it produces a stronger immune response compared to other routes of vaccination such as spray and immersion. However, the injection method requires a certain level of manpower, technical prowess and proper equipment. Therefore, a more practical route of vaccination is the oral route as there is no direct contact between handler and fish. Furthermore, no specific technical skill is needed to apply the vaccine to the fish. However, oral vaccination results in lower efficacy and shorter period of protection **(Ismail *et al.*, 2016).**

CONCLUSION

Comprehensive pathophysiological , biochemical and histopathogical research is highly needed to better understand the pathogenic mechanisms of Group B Streptococci (GBS) and to overcome the knowledge gap in epidemiology and control of fish GBS related diseases.

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