POND MANAGEMENT PRACTICES, OCCURRENCE AND ANTIMICROBIAL RESISTANCE OF STREPTOCOCCUS AND LACTOCOCCUS SPECIES IN CULTURED TILAPIA IN MOROGORO, TANZANIA

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ABSTRACT

Streptococcosis is a zoonotic disease of fish reported to cause significant losses in aquaculture, it is caused by bacteria from three major groups; Streptococcus, Lactococcus and Vagococcus. This study aimed to establish the occurrence and antimicrobial resistance of Streptococcus and Lactococcus species in farmed Tilapia in Morogoro. A questionnaire was administered to fish farmers to acquire information about management practices of fish ponds in relation to occurrence of Streptococcus and Lactococcus species. Three hundred and fifty two fish were collected from 22 different ponds to establish occurrence of the bacteria. Fish biodata and water quality parameters were recorded during sampling. Bacterial isolation was done by culturing on blood agar at 37°C. Biochemical tests were performed for preliminary identification of Streptococcus and Lactococcus species, genus specific PCR used to confirm Streptococcus species. A multiplex PCR was set to simultaneously detect *Streptococcus iniae, Streptococcus agalactiae* and *Lactococcus garvieae*. Antimicrobial resistance testing was done by disc diffusion method. Majority of the farmers followed appropriate pond management practices. Water parameters were in a desirable range (6.48 - 11.03, 2.08 - 13.6 mg/L and 21.7 - 36.68 °C for pH, dissolved oxygen and water temperature, respectively) in most ponds. Multiplex PCR detected *L. garvieae* from 6 fish samples. There was no statistically significant association between fish weight and sex with occurrence of *L. garvieae* ($p = 0.09$ and 0.14, respectively). All isolates were sensitive to tetracycline and ciprofloxacin. Fifty percent of the isolates were resistant to chloramphenicol and sulfamethoxazole. All isolates were resistant to ampicillin, penicillin and gentamycin which are common used antibiotics in humans and animals. This study indicated the presence of antimicrobial resistant zoonotic *L. garvieae* among farmed tilapia in Morogoro. Removing dead fish in the ponds, quarantining fish with

abnormal behaviors and exchanging water regularly are some of the means to prevent an outbreak. In an outbreak, the most effective antibiotics are tetracycline and ciprofloxacin.

DECLARATION

I, YUSUPH ARON, do hereby declare to the senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor concurrently being submitted in any other institution.

_____ _____

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Dr. Athumani M Lupindu Date

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DEDICATION

I would like to dedicate this work to my late grandfather, Mzee Palapala Magere for seeing potential in me and always encouraging me to do more.

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m-PCR	Multiplex polymerase chain reaction
DO	Dissolved oxygen
Mg/L	milligram per Litre
SUA	Sokoine University of Agriculture
MJNUAT	Mwalimu Julius K Nyerere University of Agriculture and Technology
DNA	Deoxyribose nucleic acid
FAO	Food and Agriculture Organization of the United Nations
g	Gram
TBE	Tris-borate-EDTA buffer
UV	Ultra Violet
µL	Microlitre
rpm	Revolutions per minute
Taq	*Thermus aquaticus*
Bp	Base pair
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Increasing global demand for fish and shellfish can only be met through intensive aquaculture production. Worldwide, aquaculture produced 59.9 million metric tons (59.9 billion Kg) of fish and shellfish in 2010 at a farm gate value estimated at $119.4 billion (Richards, 2014). Aquaculture in the United Republic of Tanzania has a vast but as yet less used potential. The industry is dominated by freshwater fish farming in which small-scale farmers practice both extensive and semi-intensive fish farming (FAO, 1994). The most farmed species are Tilapia (*Oreochromis spp)* and African catfish (*Clarias gariepinus*) (FAO, 1994). The current per capita fish consumption in the country is estimated at 8 kilogram, lower than the FAO (2006) recommended rate of 11 kilogram leaving a country on 350,000 tons demand annually.

Major challenges facing aquaculture in Tanzania include; poor quality inputs (feeds and seeds), inadequate number of experts, poor technology and diseases. Introduction of pathogens to fish and shellfish can be through contaminated feed, water, contaminated surfaces of equipments used, aerosols, or spread from infected animal to another (Richards, 2014). Intensive farming of fish involves stocking fish in high densities. This leads to low levels of dissolved oxygen and the buildup of metabolic wastes which can be steressful to fish (Schmittou *et al.,* 1998). Many pathogens in aquaculture are opportunistic and may remain undetected until some stress makes the animals susceptible to infection. Stresses commonly include improper temperature, pH, or salinity or rapid shifts in these parameters; poor oxygenation; buildup of toxic

chemicals, like ammonia; overcrowding; over or under feeding; excessive handling; and overall poor water quality (Richards, 2014).

Streptococcosis in fish is a generic term used to designate diseases caused by at least six different species of Gram-positive cocci including *Streptococcus*, *Lactococcus* and *Vagococcus* (Itsaro *et al*., 2012). Streptococcosis/lactococcosis is described as a hyperacute systemic disease that can occur in marine and fresh waters to many species of fish including rainbow, tilapia, sea bass, eel and yellow tail (Karsidani *et al.*, 2010). The disease also known as pop-eye disease, is now one of the most important bacterial diseases in farmed rainbow trout in almost all countries culturing trout (Karsidani *et al.*, 2010).

The entry of new lots of fish in the fish farm is the most frequent method of introduction of the pathogen (Vendrell *et al*., 2006). Asymptomatic carriers are the main infection source. They carry *L. garvieae* in their microbiota and can eliminate microorganisms in feces, infecting the rest of the healthy animals in the pond. Also, some fish that have recovered from *L. garvieae* infection continue disseminating the agent for a certain period (Vendrell *et al*., 2006). Feeds can also infect fish if thermal treatments are insufficient. *L. garvieae* can also remain in frozen fish until 6 months (Vendrell *et al*., 2006). Transmission of the disease is mainly by horizontal mechanisms. Direct transmission between fish that live in the same ponds occurs through the water, especially if fish injuries exist, or by the faecal-oral route (Vendrell *et al*., 2006).

Feeding infected fish with antibiotic-medicated food is a general practice but may lead to antibiotic resistance development in bacterial pathogen, resulting in an inability of first line antibiotics to clear bacterial diseases or higher dose requirement for effective control, a matter of increasing public health concern (Subasinghe *et al*., 2001).

1.2 Problem statement and study justification

There is an increased number of Streptococcosis cases in both farmed and wild fishes (Fortina *et al*., 2007). Streptococcus and Lactococcus are genera of emerging pathogens that cause septicemia and meningoencephalitis with high mortality in wild and cultured species of fish worldwide. These species have been reported to have zoonotic implications to humans, which occur when cleaning fresh whole fish (Aubin *et al*., 2011) or consuming raw fish (Wang *et al*., 2006). Increasing attention has been paid to environmental spread of antibiotic resistance genes due to their potential implications for human health (Wright, 2010). Evidences suggested that the environment may contribute to clinical antibiotic resistance (Wright, 2010). Antibiotic resistance genes in the environment could be acquired by human pathogens via horizontal gene transfer, leading to difficult treatment of infectious disease (Wright, 2010). In Tanzania less is known on Streptococcosis in both wild and cultured species fish and antimicrobial resistance associated with its treatment, previous reports on outbreaks in Mtera dam were associated with Aeromonad infections (Shayo *et al*., 2012). The current study aims to establish occurrence and antimicrobial resistance of Streptococcus and Lactococcus species recovered from cultured Tilapia obtained from fish farms in Morogoro. This pioneer study provides baseline information on the presence of Streptococcus and Lactococcus species in farmed Tilapia in Morogoro. These finds also inform public health personnel to institute appropriate measures as *L. garvieae* is a zoonotic bacteria.

1.3 Objectives of the study

1.3.1 Main objective

Investigation on pond management practices, occurrence and antimicrobial resistance of Streptococcus and Lactococcus species in cultured Tilapia in Morogoro, Tanzania.

1.3.2 Specific objectives

i. To determine the occurrence of Streptococcus and Lactococcus species from cultured Tilapia in Morogoro, Tanzania

ii. To determine the influence of pond management practices on the occurrence of Streptococcus and Lactococcus species in cultured Tilapia in Morogoro, Tanzania

iii. To assess antimicrobial resistance profiles of Streptococcus and Lactococcus species isolated from cultured Tilapia in Morogoro, Tanzania.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General description of Streptococcus and Lactococcus species

Streptococcus species are Gram-positive, cocci shaped, catalase negative bacteria that can be either α-, β- or non-haemolytic (Evans *et al*., 2002). They cause streptococcosis, a septicemic disease affecting both captive and wild fish in freshwater, estuarine and marine environments (Evans *et al*., 2002). The organisms infect more than 50 fish species throughout the world, including the commercially important species of tilapia, rainbow trout, channel catfish, red drum and Yellowtail (Zhou *et al*., 2011).

Lactococcus garvieae is a Gram-positive, facultative anaerobic, non-motile bacterium that does not produce endospores. Growth occurs as cocci in short chains and pairs at temperatures ranging from 4 to 45°C. Optimal growth occurs at 37°C (Eldar *et al*., 1996). The bacterium grows fast in rich media such as trypticase soy broth, bile esculin agar, and brain heart infusion broth, but growth is inhibited on McConkey and Enterococcus agar (Meyburgh *et al*., 2017). It is generally described as α-haemolytic bacterium but has been noted as β-haemolytic (Meyburgh *et al*., 2017).

Lactococcus garvieae (junior synonym *Enterococcus seriolicida*) is an emerging zoonotic agent isolated from economically important fish (rainbow trout and yellowtail), from cattle, and from humans (Zlotkin *et al*., 1998). Lactococcus genus was separated from Streptococcus genus in 1985 on the basis of genetic analysis (Zlotkin *et al*., 1998), but it is still often misidentified as a variant of Enterococcus spp (Zlotkin *et al*., 1998).

2.2 Clinical signs of Streptococcosis

Clinical signs of Streptococcal infections vary among fish species, but the most common signs include the loss of equilibrium, unilateral or bilateral exophthalmia, eye opacity, haemorrhage at the base of the fins, development of necrotic lesions at the caudal peduncle of infected fishes, anorexia, erratic swimming and darkening of the skin (Itsaro *et al.*, 2012; Abdelsalam *et al.*, 2015). Other external signs include swollen abdomen and anal prolapsus (Eldar and Ghittino, 1999). At necropsy, accumulation of ascitic fluid in the peritoneal cavity, congestion of internal organs, enlargement of spleen and liver, and exudate covering the brain are observed (Eldar and Ghittino, 1999).

2.3 Streptococcosis in Aquaculture

In aquaculture, the principal pathogenic species responsible for these streptococcal infections are *S. parauberis, S. iniae S. difficilis, S. agalactiae, L. garvieae and S. pneumoniae* (Suanyuk *et al*., 2010). Streptococcus and Lactococcus species are responsible for streptococcal disease outbreaks in different species of commercially raised and wild fish causing up to 50% mortality in stocks and economic losses in aquaculture worldwide are estimated to be over US$100 million annually (Xu *et al*., 2007).

In 1986, a streptococcal infection causing meningoencephalitis was found to be spreading through various cultured fish stocks, especially tilapia, *O. niloticus*, and rainbow trout, *Oncorhynchus mykiss*, resulting in 30 ± 50% mortality. *Streptococcus iniae* has been identified by the American Tilapia Association as the most important

pathogen affecting the tilapia culture industry. The bacteria are prevalent in healthy as well as diseased tilapia (Dodson *et al*., 1999).

2.4 Zoonotic Streptococcus species

Streptococcus agalactiae, S. iniae, L. garvieae and *S. pneumoniae* can also infect human beings, and most of the infected people are confirmed to have handled fish shortly before infection (Dodson *et al*., 1999; Zhou *et al*., 2011). The cases include cellutis, meningitis endocarditis and pneumonia. In Taiwan, Prospective and retrospective epidemiologic surveillance of four patients with *L. garvieae* infection between 2000 and 2003 and their relations to the aquaculture outbreaks of *L. garvieae* were conducted. The four patients with *L. garvieae* infection were associated with gastrointestinal disorders. Three of the four patients gave a history of consuming raw fish and in three of the four patients, the infection occurred in summer between June and August while there is a decrease of fisheries production and an increase in *L. garvieae* infection in aquaculture farms. There was a 100% identity of 16S rDNA sequence of *L. garvieae* isolates from patient 1 and from the squid muscle obtained from the restaurant where patient 1 consumed the raw fish. Sporadic occurrence of *L. garvieae* infection in human appears to correlate with the seasonal outbreaks of *L.garvieae* infection in aquaculture. Presence of gastro-intestinal disorder may facilitate *L. garvieae* infection (Wang *et al*., 2007).

2.5 *Streptococcus iniae*

Streptococcus iniae is a non-groupable beta-hemolytic streptococcus which was first isolated from Amazon river dolphins (Lin, 2011). It is a zoonotic pathogen that can

cause infection in human when people get injured by contaminated fish during preparation. Cellulitis is the most common type of infection caused by *S. iniae*. *Streptococcus iniae* is thought to colonize on the fish skin surface. Once fish are infected, abnormal behavior and clinical signs might be seen such as spiraling swimming behavior, bilateral exophthalmia or ulcerations (Lin, 2011). Outbreaks of *S.* iniae in aquaculture farms have been reported intermittently with a relatively high mortality rate. One of the virulence factors includes the capsule of the bacteria which can resist the phagocytosis by macrophages (Lin, 2011).

2.6 *Streptococcus agalactiae*

Streptococcus agalactiae has been reported to be the most frequent cause of neonatal infection (Facklam, 2002). It also causes infection in immunocompromised patients with alcoholism or diabetes (Facklam, 2002). Only *S. agalactiae* has group B antigen. The variety of infections caused by Group B Streptococcus is broad and includes pneumonia, endocarditis, urinary tract infection, skin and soft tissue infection (Facklam, 2002).

2.7 *Streptococcus pneumoniae*

Streptococcus pneumoniae, which belongs to *S. mitis* group in the viridans Streptococci, is responsible for the community-acquired pneumoniae (Lin, 2011). It is also the major cause of meningitis to humans (Lin, 2011). Asymptomatic colonization of *S. pneumoniae* in the upper respiratory tract of human is common and it is frequently reported in children (Spellerberg *et al*., 2007). Infections caused by *S. pneumoniae* can be endocarditis and sinusitis (Lin, 2011).

2.8 Antimicrobial resistance in Streptococcus and Lactococcus species

Antimicrobial agents are used to inhibit growth or kill microbes. Antibiotics have been widely used to control streptococcal infections in various fish. Several drugs have been tested for the treatment of streptococcosis. Among other, Darwish and Griffin (2002) found that oxytetracycline was effective in controlling *S. iniae* in blue tilapias (*O. aureus*). Oxytetracycline was incorporated into the feed at 25, 50, 75, and 100 mg/kg body weight. The 75 and 100 mg doses significantly increased the survival of the infected fish from 7% to 85 and 98%, respectively (Darwish and Griffin, 2002).

Some reports concluded that erythromycin is effective against streptococcal infections in cultured Yellowtails (Shiomitsu *et al.*, 1980) and rainbow trout (Kitao *et al.*, 1979) at doses of 25-50 mg/kg/day for 4 to 7 days. Doxycycline, oxytetracycline, kitasamycin, oleandomycin, josamycin, and lincomycin have also been used to control streptococcosis in the cultured yellowtail in Japan (Kitao *et al.*, 1979). Doxycycline, at 20 mg/kg/day for permanent treatment, has also been advocated (Nakamura, 1982). Similarly, a novel fisheries therapeutant, i.e. sodium nifurstyrenate, dosed at 50 mg/kg/day (Nakamura, 1982).

Management of streptococcal infections is potentially complicated by the emergence of resistance of the pathogens to many of the commonly used first line antimicrobial agents. Antibiotic resistance is emerging in these microorganisms and effects all the various classes of drugs, including the beta-lactams, the macrolides, and the fluoroquinolones. Even multidrug resistance is occurring (Feldman *et al.*, 2004). Resistance to penicillin and other beta-lactam agents has been the most discussed

resistance problem, but is arguably the least important clinically, since it can usually be overcome by appropriate dosing. Administration occurs generally via the oral route by combining antibiotics with specially formulated feed. Antimicrobial agents show strong invitro activity against *L. garvieae*, but perform poorly under field conditions due to low drug intake of infected fish and possibly the ineffective metabolism of antibiotics in fish (Bercovier *et al*., 1997). In rainbow trout, erythromycin, oxytetracycline, amoxicillin and low-level doxycycline are used to treat outbreaks of lactococcosis (Vendrell *et al*, 2006). Increasing attention has been paid to environmental spread of antibiotic resistance genes due to their potential implications for human health. Evidences suggested that the environment may contribute to clinical antibiotic resistance (Wright, 2010).

Antibiotic resistance genes in the environment could be acquired by human pathogens via horizontal gene transfer, leading to difficult treatment of infectious disease. Although antibiotic resistance is ancient and naturally occurring phenomenon widespread in the environment (D'Costa *et al.*, 2011), it is accelerated by antibiotic use in medicine and veterinary. The spread of antbiotic resistance genes in the environment is promoted by anthropogenic activities, such as animal farm (Zhu *et al.*, 2013), waste/wastewater treatment (Yang *et al.*, 2014), and aquaculture. Aquaculture ponds have been proved as reservoirs for antibiotic resistance (Heuer *et al.*, 2009). Researchers have paid increasing attention to antibiotic resistance genes in aquaculture environment due to the rapid development of global aquaculture industry. Animal manure used as fertilizer are used directly into fish ponds this practice leads to the spread of antimicrobial resistance genes and antibiotic resistance bacteria (Xiong *et al.*,

2015) since manure often contains antimicrobials used as growth promoters for terrestrial animals including swine and chicken. Aquaculture could accelerate the occurrence of antibiotic resistance (Xiong *et al.*, 2015). Human might acquire antibiotic resistance genes via ingestion of contaminated aquaculture food (fish and shrimp) and water. Multiple resistances is frequently encountered, referring to the occurrence of resistance to more than one chemotherapeutic agent in one isolate (Vendrell *et al.*, 2006).

2.9 Streptococcosis and water quality parameters

The quality of water in which fish live is very important to their livelihood. However different fish species can tolerate different levels of contamination and deterioration of water quality parameters. For example salmonids will grow only in conditions of good water quality, whereas species such as Channel catfish can live in conditions where salmonids would not (Brown, 1993). Critical water parameters which require to be monitored consistently are water temperature, dissolved oxygen, water pH, ammonia, heavy metals and dissolved solids (Brown, 1993).

Stress often plays a significant role in the outbreaks of infectious disease in fish populations. 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, high ammonia or nitrite concentrations, high stocking densities, as well as harvesting and handling effects (Chang and Plumb, 1996).

The presence of the pathogen in the environment of the fish is inadequate to cause a disease. Oxygen is the first limiting factor for growth and well-being of fish. Fish

require oxygen for respiration, which physiologists express as the mg of oxygen consumed per kilogram of fish per hour (mgO_2/kg/h) (Vendrell *et al.*, 2006). Although tilapia can survive acute low DO concentrations of less than 0.30 mg/L for several hours, tilapia ponds should be managed to maintain the DO concentrations above 1 mg/L although the optimal DO level for Tilapia growth is 5mg/L (Vendrell *et al.*, 2006). Metabolism, growth, and disease resistance are depressed when DO falls below this level for a prolonged period predisposing tilapias to streptococcosis (Popma and Masser, 1999).

Moreover, it is a well-known fact that increasing water temperature will reduce the rate of DO in the water (El-Sayed, 2006). The high water temperature also leads to increased respiration rate and oxygen consumption by tilapias because of the high metabolism rate. This further increases the demand for oxygen by tissues. Therefore, dissolved oxygen concentration greater than 5 ppm is required for a good growth of tilapias (El-Sayed, 2006).

In general, tilapias can survive in pH ranging from 5 to 10, but they do best in a pH range of 6 to 9 (Popma and Masser, 1999). On the contrary, low water pH leads to behavioral changes, damage of gill epithelial cells, reduction in efficiency of the nitrogenous excretion and increased mortality (Popma and Masser, 1999). Wangead *et al*. (1998) reported that fingerlings and adult tilapias exposed to pH 2-3 showed rapid swimming and opercula movement, surfacing and gulping of air, as well as lack of body position and mass mortality within 1-3 days. A study by Chen *et al*. (2001), on the other hand, showed that tilapias exposed to high water pH for 7 days decreased ammonia

excretion, but increased urea nitrogen excretion which is less toxic to fish compared to ammonia.

Lactococosis regularly occur during the warm summer months due to increase in water temperature which is stressful to fish and expose them to opportunistic pathogens present in the environment (Didinen *et al*., 2014). The disease outbreak occurred in rainbow trout (15-107 g) at an average water temperature of 13.50 °C during September-December 2012 (Didinen *et al*., 2014). In rainbow trout farms, losses due to lactococcosis can exceed 50–80% of the total production (Didinen *et al*., 2014).

2.10 Cultured Nile tilapia (*Oreochromis niloticus)*

Tilapia (*Oreochromis niloticus*) is the most cultured freshwater fish in the world (Vendrell *et al.*, 2006). It has been contributing to the world aquaculture since the ancient Egyptian days and remains a major freshwater fish species to be cultured. Although tilapias are more resistant to unfavourable water quality than other freshwater fish, tilapias have been reported to succumb to infection by Streptococcus species.

In the world where captured wild fisheries are becoming increasingly depleted, tilapias offer a possibility of commercialization because of their superior culture adaptability. In fact, the production of tilapias made the fish one of the most important species for the 21st century aquaculture (Fitzsimmons, 2000) which also rose commercially in more than 100 countries (Shelton and Popma, 2006).

Initially, tilapias were considered to be more resistant to bacterial, parasites, fungal, and viral diseases compared to other cultured fish species. In more recent times, however,

tilapias have been found to be susceptible to both bacterial and parasitic diseases. Common tilapia pathogens include *Streptococcus* sp., *Flavobacterium columnare, Aeromonas hydrophila, Edwarsiella tarda, Ichthyophitirius multifillis, Tricodhina* sp., and *Gyrodactylus niloticus* (Klesius *et al*., 2008). It is important to note that streptococcal infections have become a major problem in tilapias farming and contributed to severe economic losses in the United States (Shoemaker and Klesius, 1997). *Streptococcus iniae* and *S. agalactiae* are the major bacterial species that affect the production of tilapias in the world (Evan *et al*., 2006).

2.11 Transmission of Streptococcosis

Nguyen *et al*. (2002) reported that, the newly introduced infected fish was the most important factor that introduced Streptococcus species into the farm. The bacteria were excreted in faeces of infected fishes, survive in the water and can infect other healthy fish (Nguyen *et al.*, 2002). Besides, using infected thrashed fish as feed is considered to be responsible for the outbreaks of streptococcosis among flounder fish occured in Korea (Kim *et al.*, 2007). An experimental study revealed that cohabitation of dead or infected fish with healthy fish resulted in the infection of the healthy fish (Kim *et al.*, 2007). Horizontal transmission of the pathogens between fish is believed to be the most common mechanism of dissemination. 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 (Evans *et al.*, 2002).

2.12 Biochemical identification of streptococcus species

Hemolysis is one of the phenotypic characteristics that can be used for identification of *Streptococcus* species. There are 3 types of hemolysis: beta, alpha and gamma. Beta hemolysis is shown on the blood agar with a complete lysis of red blood cells surrounding the colony whereas alpha hemolysis refers to a partial hemolysis around the colonies causing green coloration surrounding the colonies. This is due to the reduction of red blood cells close to the colonies. Colonies that cannot hemolyse the red cells are regarded as gamma hemolytic (Spellerberg *et al*., 2007).

Lancefield grouping is another biochemical test that can be used for identification of *Streptococcus* (Vendrell *et al.*, 2006). It is a kind of serology test that used to identify cell wall antigens and capsular antigens, particular in group B streptococci. Latex particles with group-specific antibodies are used to agglutinate with the corresponding group of streptococci. Twenty serogroups can be classified by Lancefield grouping with denotation A to H and K to V. *S. iniae* and *S. agalactiae* belongs to lancefield group B whereas *Streptococcus pneumoniae* belongs to Lancefield group A (Vendrell *et al.*, 2006).

2.13 Identification of streptococci by molecular methods

Apart from biochemical identification of streptococci, molecular techniques have been advancing rapidly (Vendrell *et al.*, 2006). One of the commonly used molecular techniques is the comparison of 16S rRNA gene sequences of different bacterial species. 16S rRNA is used due to its high degree of conservation within a species and among species of the same genus (Clarridge, 2004). As 16S rRNA gene is considerably

long with about 1550 bp, adequate interspecific polymorphisms can give a definitive and statistically significant bacterial identification (Clarridge, 2004). As 16S rRNA gene exists in all bacteria, relationships among different species and differentiation at the genus level can be achieved. This molecular technique can identify unusual bacteria which have conventional biochemical tests profiles that do not fit into any bacterial species (Facklam, 2002). In addition, this technique can be used to identify non-cultivable bacteria or slow growing bacteria such as mycobacteria. Therefore, it becomes the new gold standard for determining the bacterial species (Clarridge, 2004).

2.14 Prevention of Streptococcosis

It is important to note that, the keys to disease prevention in fish includes maintaining good water quality, providing proper nutrition and keeping the environment clean (Yanong and Francis-floyd, 2016). Knowing which species are susceptible to Streptococcosis and seeking assistance for rapid diagnosis and proper therapy if a disease outbreak occurs are important ways to decrease losses. Most infectious diseases of fish are opportunistic, this means that the simple presence of the pathogen in the environment of the fish is inadequate to cause a disease outbreak (Yanong and Francis-floyd, 2016). Other factors usually come into play such that either the pathogen has an advantage over the host or the immune system of the host is compromised in some way, increasing its susceptibility to the pathogen. This phenomenon is often precipitated by stress, Stress often plays a significant role in outbreaks of infectious disease in fish populations (Yanong and Francis-floyd, 2016).

Some stressors that have been associated with Streptococcosis outbreaks include high water temperatures, high stocking densities, harvesting or handling, and poor water quality, such as high ammonia or nitrite concentrations (Yanong and Francis-floyd, 2016). A tentative diagnosis of Streptococcus and Lactococcus species can be made from the history and clinical signs, necropsy findings, and identification of Gram-positive bacteria from stains of impressions (produced by blotting sections of fresh tissues onto a glass slide) from the brain, spleen, kidney, or liver (Yanong and Francis-floyd, 2016). Streptococcus and Lactococcus species should be highly suspected if fish exhibit abnormal swimming behavior, pop-eye, hemorrhages, and rapid and severe mortalities, and Gram-positive cocci are found in brain, kidney, and/or other organs (Yanong and Francis-floyd, 2016).

Vaccines may be useful for facilities that have continual or cyclic outbreaks of Streptococcosis. Autogenous vaccines (vaccines developed for a specific facility, targeting a specific bacteria isolated from a disease outbreak at that facility) have been shown to be effective under certain conditions (Vendrell *et al.*, 2006). Commercial vaccines may also be available for use within the next few years. Prevention of disease is always preferable and more profitable than treatment of disease outbreaks. Preventive medicine programs should be designed to minimize stress, maintain the best water quality possible and minimize exposure to infectious agents by following appropriate disinfection and sanitation protocols (Yanong and Francis-floyd, 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

This study was done conducted in Morogoro region, Tanzania, geographical coordinates are 6°49′S 37°40′E (see figure 1 below) from November, 2016 to May, 2017 to determine the influence of pond management practices, occurrence and antimicrobial resistance of Streptococcus and Lactococcus species in cultured Tilapia. Tilapia is the second most cultured fish worldwide. Initial reports shows in freshwater, Streptococcosis affects Tilapia and less in other fish species. Morogoro region was chosen due to the presence of a significant number of fish farms owned by the government, institutions and individual farmers. Also convenience in accessibility of the Laboratory. Samples were collected from fish ponds involved in fish farming (at the time of sampling) in three districts (Mvomero, Morogoro rural and Morogoro Municipal).

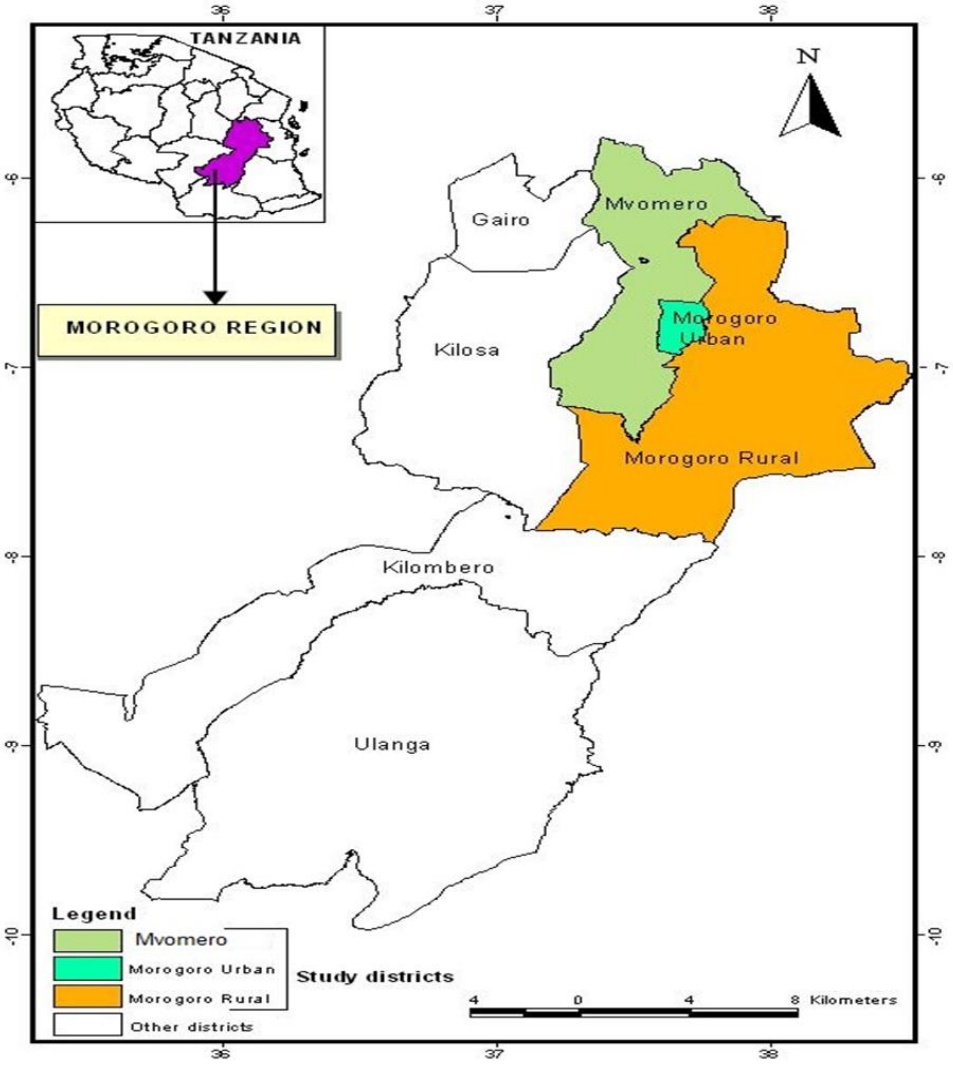

Figure 1: Map of Morogoro region showing the study sites

3.2 Study design

This was a cross sectional study, and purposive sampling was done from districts, wards, villages which were involved in fish farming at the time of sampling. A total of 10 villages were involved in the study, 5 villages from Morogoro Municipal (Bigwa, Mazimbu, Mbuyuni, Kingorwira and Magadu), 3 villages from Mvomero (Tangeni, Mgeta and Mkindo) and 2 villages from Morogoro rural (Mtamba and Lugeni). From each village 2 to 3 ponds were sampled and a total of 16 fish were collected from each

pond. Collected fish were put into separate plastic covered containers and transported alive to the Microbiology laboratory at SUA for analysis within 8 hours of collection. Before dissection, fish were humanly killed by stunning (sharp blow on the cranium), dissected and aseptically the kidney, eye and gill swab samples were taken for bacteriology. Swabs were temporarily put in nutrient broth to maintain the bacteria before culture as the broth supports growth of most bacteria. Bacteria culture was done after three hours Analysis of collected samples was done in Microbiology Laboratory at the Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture.

3.3 Sample size estimation

Number of subjects to be involved in the study was determined by the formula; $n=Z^2 P (1-P)/d^2$ (Naing *et al*., 2006) where n = sample size, Z = Z statistic for a level of confidence (1.96), P = (According to Aquatic animal health code of 2010, a suitable design prevalence value at the animal level may be between 1% and 5% for infections that are present in a small part of the population or are transmitted slowly or are at the early stages of an outbreak of disease. For this study 1% prevalence was used), and d = precision (0.05). A total of 352 fish were collected from 22 ponds in Mvomero, Morogoro rural and Morogoro Municipal.

3.4 Water quality assessment

Core water quality parameters (dissolved oxygen, pH and water temperature) were recorded during the study using Multiparameter meter (HI98194, USA) Parameters were recorded on site, at the pond inlet and outlet.

3.5 Administering a questionnaire

A semi-structured questionnaire was administered to every fish farmer by face to face interview in order to get information about pond management practices. Questions covered in the questionnaire included; general pond description, stocking density, use of water, feeds, manure application, fingerling sources and diseases management.

3.6 Live fish for bacteriological assessment

Before euthanizing, each fish was clinically examined, weighed using digital weighing balance, and their lengths (girth, standard and total length) were measured.

3.7 Bacteria isolation

Surface swabs of kidney, eyes and gills were inoculated onto 5% HBA plates and incubated at 37 ℃ for 18 to 24 hours. *Lactococcus garvieae* colonies are small, white, raised colonies with alpha hemolysis on 5% horse blood agar. Single colonies from plates with dense, virtually pure culture growth were re-streaked onto Brain heart infusion agar to obtain pure isolates. The hemolytic reaction is particularly useful in the differentiation of the Streptococci. The hemolytic reaction is determined on agar media containing 5% animal blood. A beta-hemolytic reaction is interpreted as complete clearing around the colony. An alpha-hemolyic reaction is interpreted as greening around the colony and gamma hemolysis is interpreted as no change in the media surrounding the colony. Targeted Streptococcus and Lactococcus species are β hemolytic and α hemolytic, respectively (Murray *et al.,* 2003). Growth of Streptococci and Lactococci species is inhibited in salt enriched media (Murray *et al.*, 2003).

3.8 Identification of the isolates

Preliminary identification of the isolates involved Gram staining technique, catalase test, Vancomycin, Optochine and Bacitracin sensitivity test, growth on mannitol salt agar and hemolysis on 5% blood agar plates.

3.9 Genomic DNA Isolation

The isolates were grown on Nutrient agar (NA) at 37 °C for 24 hours. Genomic DNA extraction was performed by boiling method as described by Gun Wook *et al.* (2006). Briefly, the colonies were picked and suspended in 300 µl of DNA free water. The mixtures was then boiled in a water bath (Julabo SW23, Germany) at 100 °C for 10 minutes and then centrifuged (MIKRO 220R, Hettich zentrifugen, Germany) at 5000 rpm for five minutes. Bacterial DNA was collected on the upper aqueous phase of the supernatant and stored at -20 °C.

3.10 PCR amplification

A genus specific conversional PCR was performed according to previous published protocol by Zhang *et al.* (2014). Briefly, the PCR was set to amplify 197 bp tuf gene in genus Streptococcus. The optimum PCR reaction in a final volume made up to 25 µl contained 1.25 U of Taq polymerase (Inqaba), 1 x PCR buffer, 2.0 mM of $MgCl_2$, 200 µM of each dNTP, 0.2 µM each of Strep 1 and strep 2 primers and 50 ng of DNA template. Amplification of target DNA was performed with the following parameters: An initial denaturation 95 °C for 15 min, denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min extension at 72 °C for 1.5 min and final extension at 72 °C for 10 min. A species specific multiplex PCR assay was performed according to previous published

protocol by Itsaro *et al.* (2012). Briefly, PCR was set to simultaneously amplify *S. iniae, S. agalactiae* and *L. garvieae.* Targeted genes in the Multiplex PCR assay were 870 bp *lctO* gene, 220 bp *16SrRNA* and 1100 bp *16SrDNA* for *S. iniae, S. agalactiae* and *L. garvieae* respectively. The optimum PCR reaction in a final volume made up to 25 µl contained 1.25 U of Taq polymerase (Inqaba), 1 x PCR buffer, 2.0 mM of MgCl2, 200 µM of each dNTP, 0.2 µM each of F1/IMOD and LOX-1/LOX-2 primers and 0.4 µM of each of pLG-1/pLG-2 and 50 ng of DNA template. Amplification of target DNA was performed with the following parameters: an initial denaturation step of 94 °C for 4 min; 35 serial cycles of a denaturation step at 94 °C for 1 min, annealing at 58 ºC for 1 min, and extension at 72ºC for 2 min; and final extension step of 72 ºC for 10 min. The amplification of target gene in the m-PCR assay permitted an appropriate identification of *L. garvieae*. A negative control (no template DNA) and a positive control of *L. garvieae* were used. The PCR products were analysed by 1.50% agarose gel electrophoresis in 1% Tris-borate-EDTA buffer. Gels were stained with Gel red, visualized and photographed under UV illumination.

Table 1: Primer sequences used in the study

Pathogen	Sequence	bp size	Targeted gene	Primer pair	Reference
S. iniae	AAGGGGAAATCGCAAGTGCC ATATCTGATTGGGCCGTCTAA	870	*lctO*	LOX-1 LOX-2	Itsaro *et al*. (2013)
S. agalactiae	CGAGTTTGATCATGGCTCAG ACCAACATGTGTTAATTACTC	220	*16S rRNA*	F1 IMOD	Itsaro *et al*. (2013)
L. garvieae	CATAACAATGAGAATCGC GCACCCTCGCGGGTTG	1,100	*16S rDNA*	pLG-1 pLG-2	Itsaro *et al.* (2013)
S. species	GTACAGTTGCTTCAGGACGTATC ACGTTCGATTTCATCACGTTG	197	*Tuf gene*	Str1 Str2	Zhang, (2014)

3.11 Antimicrobial Resistance testing

In this study antimicrobial resistance testing of *L. garvieae* isolates was performed by disc diffusion method on Muller Hinton (MH) Agar (Pronadisa, Spain) as described by Bauer and Kirby (1966), using strain ATCC 51286 for quality control purposes. Briefly, *L. garvieae* were prepared in sterile normal saline and adjusted to a turbidity equivalent to a 0.50 McFarland standard. Sterile cotton-tipped swabs were inserted into the standardized inoculums, drained of and then used to transfer the inoculums onto well dried Mueller-Hinton plates. Inoculated plates were dried in an incubator for five minutes and antibiotic discs were distributed over them using a Sensi-disc dispenser (Oxoid Ltd, UK). The plates were then incubated at 37°C for 48 hours under microaerobic conditions. After 48 hours of incubation, the diameters of inhibition zones were measured. Interpretation of results were guided by both standardized tables supplied by the National Committee on Clinical Laboratory Standards (currently

known as Clinical and Laboratory Standards Institute) (NCCLS, 2002) and manufacturer's instructions. In this study the most commonly used antimicrobial agents in livestock and humans in the study area and others that have been used and tested elsewhere (Pezzoti *et al*., 2003) were tested for resistance. They included seven (7) different antimicrobial agents; Oxytetracycline (TE 30), Gentamycin (CN 10), Chloramphenicol (C 30), Sulfamethoxazole (SMX 5), Ampicilin (AMP 10), Penicillin G (10 IU) and Ciprofloxacin (CIP 5) (Liofilchem, Italy). An isolate that was resistant more than two classes of antimicrobials was referred to as multi-drug resistant.

3.12 Data analysis

Collected data were cleaned in Microsoft Excel and then analyzed by SPSS software version 16. Descriptive statistics were computed to determine proportions of positive samples for *L. garvieae*. The Fisher's Exact test was used to determine the significance of the risk factors in occurrence of Lactococcus species. Statistical tests were done at $p \leq 0.05$

3.13 Ethical approval

Before commencing a research an ethical approval was requested from research and publication committee at the College of Veterinary and Medical science. Reference number for this study is SUA/VET/016/12. Biosafety procedures as described in biorisk management; laboratory biosecurity guidance (WHO, 2006) were followed when analyzing sample in the Veterinary Medicine and Public Health laboratory at SUA.

CHAPTER FOUR

4.0 RESULTS

4.1 Questionnaire survey results

Twenty two (22) fish farmers were interviewed in this study, 8% were women and 92% were men. The minimum stocking density was 200 fish per pond while the maximum stocking density was 1500 fish per pond. Average pond size was 126 m^2. Table 2 below shows variation in pond management practices among the farmers in the study sites and their association with occurrence of *L. garvieae*.

Table 2: Pond management practices and their association with *L. garvieae* occurrence

Practice	Category	Frequency of ponds	Percentage of ponds	Frequency of *L. garvieae*	p value
Pond Location	Residential area	22	91.70	3	0.20
	Rice field	2	8.30	3	
Source of water	Wells	6	25	3	0.68
	Streams/river	18	75	3	
Water exchange	Exchange	12	50	3	0.41
	No exchange	12	50	3	
Cleanliness before stocking	Cleaned before stocking	13	54.20	0	0.01
	Not cleaned before stocking	11	45.80	6	
Number of species farmed	More than one fish species	5	20.80	0	0.59
	Only one species	19	79.20	6	
Quality of feeds used	Balanced feed	8	33.30	0	0.17
	Imbalanced feed	16	66.70	6	
Feeding frequency	Once	7	29.20	2	0.92
	Twice	17	70.80	4	
Fertilizer used	Chicken manure	11	45.80	5	0.35
	Cow dung	13	54.20	1	
Amount of fertilizer used	Amount used is appropriate	9	37.50	0	0.09
	Amount used is inappropriate	15	62.50	6	

4.2 Water quality parameters

Water parameters ranged from 6.48 - 11.03, 2.08 - 13.60 mg/L and 21.70 - 36.68 ℃ for pH, dissolved oxygen and water temperature respectively. These parameters were categorized based on desirability of their level for Tilapia culture and their association with *L. garvieae* occurrence was assessed. All core water quality parameters had no association with occurrence of *L. garvieae* (Table 3).

Table 3: Association of water quality parameters and *L. garvieae* occurrence

Water parameter	Category	Frequency of ponds	Percentage of ponds	Frequency of *L. garvieae*	p value
pH	Desirable	13	54.20	3	
	Undesirable	11	45.80	3	0.41
Dissolved Oxygen	Desirable	19	79.20	6	
	Undesirable	5	20.80	0	0.58
Temperature	Desirable	15	62.50	4	
	Undesirable	9	37.50	2	0.47

4.3 Fish biodata

Overall mean weight in all sampled fish was 77.79 ± 54.26 g. *L. garvieae* were detected from fish weighing 45.02 g to 117.20 g (mean weight 77.18 ± 29.89 g). Fifty seven percent (57%) of all sampled fish were male and forty three percent (43%) were female. There was no significant association between fish sex and weight with occurrence of *L. garvieae* (Table 4). However all *L. garvieae* positive fish were males.

Table 4: Association between fish biodata and occurrence of *L. garvieae*

Observation	**Category**	**Frequency**	**Percentage**	**Frequency of *L. garvieae***	**p value**
Fish Weight	Above 90 gram	63	28.90	6	0.09
	Below 90 gram	155	71.10	0	
Fish sex	Male	124	56.90	6	0.14
	Female	94	43.10	0	

4.4 Occurrence of *L. garvieae*

On gram staining suspected isolates were gram positive, coccal shaped which occurred in pair or chains. Isolates were catalase negative, no growth on mannitol and macconkey agar and they were α and β hemolytic. Isolates were resistant to Vancomycin, Optochine and sensitive to Bacitracin. On primary isolation twenty (20) isolates from 17 fish had characteristics similar to *L. garvieae*. Confirmation by multiplex PCR detected six isolates from six fish (6) out of three hundred and fifty two (352) to be *L. garvieae*. Three (3) isolates were from one pond in Morogoro Municipal, other two (2) isolates were from Morogoro rural and one isolate was from Mvomero. Therefore 3 out of 22 (13.6%) sampled ponds were positive.

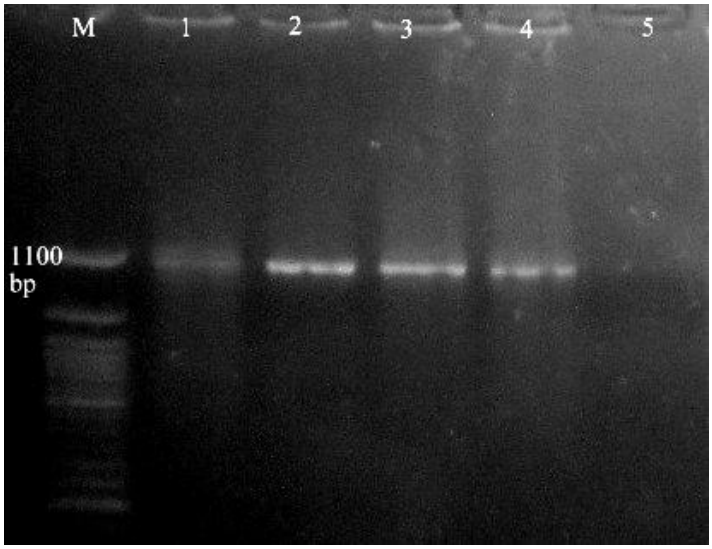

Figure 2: Agarose gel showing 1,100 bp m-PCR amplification products for detection of *L. garvieae*. Lane M = 100 bp DNA Ladder; Lane 1= TOTF9K; Lane 2= MTPF5K; Lane 3= MPF2K, Lane 4 = Positive control and Lane 5 =Negative control

4.5 Antimicrobial resistance pattern of isolated *L. garvieae*

Table 5: Sensitivity test of isolated *L garvieae* to commonly used antibiotics

Antibiotic	**Concentration µg**	**Susceptible (%)**	**Intermediate (%)**	**Resistant (%)**
Tetracycline	30	6 (100)	0 (0)	0 (0)
Gentamycin	10	0 (0)	0 (0)	6 (100)
Chloramphenicol	30	3 (50)	0 (0)	3 (50)
Sulfamethoxazole	5	3 (50)	0 (0)	3 (50)
Ampicillin	10	0 (0)	0 (0)	6 (100)
Penicillin	10 IU	0 (0)	0 (0)	6 (100)
Ciprofloxacin	5	6 (100)	0 (0)	0 (0)

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

In this study, six fish (6) out of three hundred and fifty two (352) were positive to *L. garvieae*, accounting for 1.7% of the sampled fish. These were obtained from 3 out of 22 (13.6%) sampled ponds. At farm level, these findings agree with results by Arabaci and Önalan (2016) who reported an occurrence of *L. garvieae* in 4 farms out of 19 rainbow trout farms in Van Province, Iran. However, Soltani *et al*. (2013) reported higher occurrence of *L. garvieae* in Charmahal–va-Bakhteyari and Kohgiloyeh-va-Boyerahmad Provinces, Iran in which among 25 fish farms examined, 13 (52%) were infected with *L. garvieae*.

At individual fish level, many other studies have reported higher frequencies of occurrence of *L. garvieae* than what the current study reports (Sharifiyazdi *et al*., 2010; Adel *et al*., 2014). In these studies the clinically sick and dead fish were sampled, which could have increased the chances of detecting the bacterium as opposed to this study in which the sampled fish were asymptomatic and healthy ones. In a streptococcal disease outbreak which occurred in Iran, Sharifiyazdi *et al*. (2010) reported to have sampled 200 sick rainbow trouts with clinical signs suggestive of the bacterial infection and *L. garvieae* was detected from 32 fish (16%) using conventional biochemical tests. In another study conducted by Adel *et al*. (2014) a total of 200 sick rainbow trouts, suspected of lactococcosis from 10 rainbow trout farms in Mazandaran, Iran, were sampled and out of which 38 fish (19%) were confirmed by molecular test to be *L. garvieae* positive.

L. garvieae were detected from fish weighing 45.02 g to 117.2 g (mean weight 77.18 ± 29.89 g). Similar observations were made earlier by other authors (Didinen *et al*., 2014; Vendrell *et al*., 2006) who observed that clinical signs and mortalities do occur in fish weighing under 80 g. Occurrence of the disease in under 80 g fish may be explained by poorly developed acquired immune response of fish which develops as fish grows (Magnadottir, 2006). In their study Didinen *et al.* (2014) reported Lactococcosis outbreak in rainbow trout weighing 53.75 ± 40.77g whereas; Karsidani (2010) reported a Lactococcus outbreak in rainbow trout in which most cases involved fish that weighed 100 g. In Malaysia, farmers are advised to manage the cultured fish so that harvesting of fish of 100-200 g can be done before the coming of critical period of April-June where temperature increases and Lactococcosis occurs (Amal and Zamri-Saad, 2011).

The current study did not find statistically significant association between different management practices and occurrence of the bacterium (Table 2). The majority of the sampled ponds (91.75%) were in residential areas, 3 *L. garvieae* isolates were detected in these ponds. These isolates may have been introduced into the ponds by people through sewages or other household activities. However a less number of isolates in these ponds may be partly explained by easiness of farmers to attend their ponds when required to and therefore reducing stress to fish which could have resulted into an outbreak. Amal and Zamri-Saad (2011) suggested that reducing overcrowding, avoiding overfeeding, minimizing unnecessary handling, removing dead fish, feeding pathogen free ration and keeping excellent sanitary conditions as well as cleaning and disinfecting all production units and equipments reduces the risks of Streptococcosis.

Seventy five percent (75%) of the respondents reported to use stream water, 3 *L. garvieae* isolates were detected in ponds using streams directly. Contaminated stream water may save as a potential source of bacterial infections, if prior treatment of water by UV is involved surface water is recommended for its less metals concentration and adequate amount of dissolved oxygen required for fish farming, ground waters are not saturated with oxygen, but are supersaturated with nitrogen and may have high levels of carbon dioxide (Robert, 2001). Only 50% of respondents (Table 2) reported to exchange their pond water after every two weeks hence reducing ammonia concentration which may be toxic to farmed fish if left to accumulate, ammonia may affect fish feeding causing stress and reduced immunity of fish. Fifty four percent (54%) of respondents reported to thoroughly clean their ponds before stocking aiming to significantly reduce microbial load present in the pond. Six isolates were detected in the other 46% of ponds which were not cleaned before stocking.

Fifty four percent (54%) of respondents used cow dung as the sole organic manure. Cow dung is mostly used as it has less ammonia compared to chicken, poultry manure contains high levels of ammonia and organic nitrogen due to the high content of protein and amino acids (Krylova *et al.*, 1997). Five *L. garvieae* isolates were detected in ponds fertilized with chicken as opposed to only one isolate detected in ponds fertilized with cow dung. Good husbandry and management practices at farm level shift the balance in favor of cultured organisms versus opportunistic or real pathogens is in all cases the cornerstone of any successful health strategy (Christofilogiannis, 2004). Reducing stress factors, such as poor water quality and insufficient nutrition, is generally known to be important in controlling Streptococcal infection (Park *et al.*, 1997). Correct feeding

strategy and good hygiene standards are among factors that could play significant role in the improvement of farm health status (Christofilogiannis, 2004).

Fish immunity is weakened by stress conditions including increased fish density and poor water quality, injury during handling, inadequate nutrition, poor sanitation and increased water temperatures (Bekker *et al*., 2011).

Karsidani *et al*. (2010) reported streptococcosis outbreaks in trout farms during the warm seasons when water increases up to 20 °C. Increase in water temperature together with impact of polluted water sources cause significant decline in water quality parameters resulting in outbreaks of streptococcosis (Karsidani *et al*., 2010). Dissolved oxygen, water pH and water temperature are critical parameters in Tilapia farming and they were all in desirable range. Recommended water pH level for Tilapia farming ranges from 6.6 - 8.6, while dissolved oxygen levels have to be greater than 3 mg/L and desirable water temperature ranges from 22 °C to 28 °C. Having desirable water quality parameters may have contributed to low occurrence of *L. garvieae*. Streptococcosis becomes more pronounced when the aquatic environment is poor, and oxygen deficiency increases virulence and distribution of the agent. Also, excessive ammonium concentration causes an increase in mortality (Vendrell *et al*., 2006).

Environmental pollution and water quality deterioration in the aquatic environment might be incriminated as primary stress factors that promote the invasion of bacteria (Abu-elala *et al*., 2016). Decrease in water pH as well as elevated pH has been reported to be stressful to fish (Boyd and Tucker, 2016). High proportion of the ammonia nitrogen is in the toxic form that is, ammonia (NH_3) as opposed to the less toxic

ammonium (NH^+) form (Boyd and Tucker, 2016). This study found pH to be ranging from 6.48-11.03, however desirable levels of pH in Tilapia farming are 6.5-8.5 (Brown, 1993). pH values reported in this study were desirable in majority of the samled ponds (54%). However pH levels had no significant association with occurrence of *L. garvieae*. Appropriate water quality and stocking densities play significant role in the improvement of fish farm health status (Christofilogiannis, 2004).

Different studies have been conducted on antimicrobial resistance of *L. garvieae* and reported different proportions of resistant isolates to several antimicrobial agents (Ture and Boran, 2015; Kav and Erganis, 2008; Li *et al.*, 2015; Maki *et al*., 2008; Kawanishi *et al.*, 2004). This study found all six (100%) isolates to be sensitive to tetracycline and ciprofloxacin. Three of them (50%) were moderately resistant to chloramphenicol and sulfamethaxazole while 100% resistance was observed to ampicillin, penicillin and gentamycin. Low resistance of *L. garvieae* to tetracycline has been reported earlier by different authors in Tukey (Ture and Boran, 2015; Kav and Erganis, 2008). The authors reported resistance to tetracycline of 0% and 26.6% respectively in *L. garvieae* isolates obtained from Rainbow trouts, many other authors have reported higher resistance of *L. garvieae* to tetracycline, ranging from 31% to 44% (Gibello *et al*., 2016; Vendrell *et al*., 2006; Fortina *et al*., 2007; Kawanishi *et al*., 2004; Maki *et al*., 2008).

Other researchers have reported moderate resistance to tetracycline among *L. garvieae* isolates obtained from dairy products (Raissy and Shahrani, 2015, 38%; and Alrabadi, 2012, 58%). However, majority of studies reported low resistance of *L. garvieae* to ciprofloxaxin (Li *et al.*, 2015; Kav and Erganis, 2008 and Maki *et al*., 2008). As it is a

third generation cephalosporin, it is highly effective in wide range of bacteria. On the other hand, Penicillin, Ampicillin and Gentamycin have been widely used in treatment of bacterial infections to cattle and humans. However, this study found all *L. garvieae* isolates to be resistant to these drugs. Higher resistance to penicillin G (Vendrell *et al*., 2006; Perez-Sanchez *et al.,* 2011), Ampicillin (Li *et al.,* 2015; Perez-Sanchez *et al.,* 2011) and Gentamycin (Ture and Boran, 2015) has also been reported previously. However, sensitivity to penicillin G (Wang *et al.*, 2007; Alrabadi, 2012; Ture and Boran 2015; Kav and Erganis, 2008), Ampicillin (Wang *et al.,* 2007; Vendrell *et al*., 2006; Ture and Boran, 2015; Kav and Erganis, 2008) and Gentamycin (Wang *et al.*, 2007; Li *et al* 2015; Perez-Sanchez *et al.,* 2011) among *L. garvieae* isolates has been observed by authors in several other studies.

In this study, *L. garvieae* isolates showed moderate resistant to chloramphenicol and sulfamethaxazole. Similar observations were reported by (Raissy and Shahrani, 2015; Maki *et al*., 2008) for chloramphenicol and (Raissy and Shahrani, 2015) for sulfamethaxazole. Contrary to findings of this study and other mentioned studies (Sharifiyazdi *et al*., 2010; Vendrell *et al*., 2006; Li *et al.,* 2015) found chloramphenicol to be highly effective; whereas others (Alrabadi, 2012) found sulfamethaxazole to be highly effective. Resistance of *L. garvieae* isolates to sulfamethaxazole has been reported elsewhere (Li *et al.,* 2015; Perez-Sanchez *et al.,* 2011). Antibiotic resistance of *L. garvieae* isolates among antimicrobial agents can be attributed by pond management practices. Animal manure were used continuously directly into fish ponds, this practice may lead to the spread of antibiotic resistance genes and antibiotic resistance bacteria (Xiong *et al.,* 2015).

5.2 CONCLUSIONS

This study indicates the presence of zoonotic *L. garvieae* in farmed Tilapia in Morogoro. Isolates were resistant to some of commonly used antimicrobial agents (ampicillin, penicillin and gentamycin) and sensitive to tetracycline and ciprofloxacin. When disease occurs the most effective antibiotics are tetracycline and ciprofloxacin. The current study did not find statistically significant association between different management practices and occurrence of the bacterium.

5.3 RECOMMENDATIONS

To prevent possible occurrence of clinical form of the disease, proper fish farm management practices are insisted, these include;

- Removal of dead fish from ponds,
- Quarantining the ones which show abnormal behaviors,
- Regular water exchange,
- Supply balanced feeds in a proper ration and
- Ensuring water quality is constantly monitored.

5.4 STUDY LIMITATIONS

The current study was faced with two major limitations (i) Fish used in the study were asymptomatic and healthy reducing the chances of occurrence of *L. garvieae* and (ii) there were few fish farmers who were actively involved in Tilapia farming during the study period, majority of the farmers had harvested their fish due to water scarcity.

REFERENCES

Abdelsalam, M., Eissa, A. E. and Chen, S. (2015). Genetic diversity of geographically distinct *Streptococcus dysgalactiae* isolates from fish. *Journal of Advanced Research* 6: 233–238.

Abu-elala, N. M., Abd-elsalam, R. M. and Marouf, S. (2016). Eutrophication, Ammonia Intoxication and Infectious Diseases: Interdisciplinary Factors of Mass Mortalities in Cultured Nile Tilapia Eutrophication , Ammonia Intoxication, and Infectious Diseases. *Journal of Aquatic Animal Health* 145: 187–198.

Adel, M., Esmailian, A. D., Yaghoubzadeh, Z., Khalili, E. S. and Babaalian, A. (2014). Isolation and Characterization of *Lactococcus garvieae* from Diseased Rainbow Trout (*Oncorhynchus mykiss*, Walbaum) Cultured in Northern Iran Based on the Nucleotide Sequences of the 16s rRNA Gene. *Walaikak journal of science and Technology* 12: 533–538.

Alrabad, N. I. (2012). The effect of several antibiotics on *Lactococcus garvieae* isolated from jordanian dairy products. *American Journal of Agricultural and Biological Sciences* 7: 468-472.

Amal, M. N. A. and Zamri-Saad, M. (2011). Streptococcosis in Tilapia (*Oreochromis niloticus*). *Pertanika Journal of Tropical Agricultural Science* 34: 195 – 206.

Aoki, T., Park, C. I., Yamashita, H. and Hirono, I. (2000). Species-specific polymerase

chain reaction primers for *Lactococcus garvieae*. *Journal of Fish Diseases* 23: 1–6.

Arabaci, M. and Önalan, Ş. (2016). Determination of Bacterial Disease Map for Rainbow Trout Farms in Van Province. In: *International Conference on Advances in Natural and Applied Sciences* 1726: 020030-020032.

Aubin, G. G., Bémer, P., Guillouzouic, A., Crémet, L., Touchais, S., Fraquet, N., Boutoille, D., Reynaud, A., Lepelletier, D. and Corvec, S. (2011). First report of a hip prosthetic and joint infection caused by *Lactococcus garvieae* in a woman fishmonger. *Journal of Clinical Microbiology* 49: 2074-2076.

Austin, B. and Austin, D. A (2007). Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish. *Clinical Microbiology* 49: 2074-2076.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *The American Journal of clinical pathology* 45:493-496.

Bekker, A., Hugo, C., Albertyn, J., Boucher, C. E. and Bragg, R. R. (2011). *Lactococcus garvieae* in fish. *Journal of Fish Diseases* 34: 483–487.

Bercovier, H., Ghittino, C. and Eldar, A. (1997). Immunization with bacterial antigens: infection with streptococci and related organisms. *Developmental Biology Standardization journal* 90: 153–160.

Beveridge, M. C. M. and Baird, D. J. (1998). Feeding mechanism and feeding ecology.

In *Tilapias: Their biology and exploitation;* Edited by Beverigde, M. C. M. and McAndrew, B. J. Chapman and Hall, London: pp 12-21.

Boyd, C. E., Somridhivej, B. and Tucker, C. S. (2016). Alkalinity and Hardness: Critical but Elusive Concepts in Aquaculture. *Journal of the world aquaculture society* 47: 6–41.

Bragg, R. R. and Broere, J. S. E. (1986). Streptococcosis in Rainbow trout in South Africa. *Bullettin of the Europian Association of Fish pathologists* 6: 89-91.

Brown, L. (Ed). (1993). Aquaculture for Veterinarians: Fish husbandry and medicine, First edition. Pergamon press, Oslo. 297 pp

Chang, P. H. and Plumb, J. A. (1996). Histopathology of experimental *Streptococcus* sp. infection in tilapia, *O. niloticus* and channel catfish, *Ictalarus punctatus*. *Journal of Fish Diseases* 19: 235-241.

Christofilogiannis P. (2006). Permanent network to strengthen expertise on infectious diseases of aquaculture species and scientific advice to EU policy Deliverable 10 - Environmentally safe control strategies. 154 pp.

Clarridge, J. E. (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious disease. *Clinical Microbiological Review* 17: 840-862.

D'Costa, V. M., King, C. E., Kalan, L., Morar, M., Sung, W. W., Schwarz, C., Froese,

D., Zazula, G., Calmels, F. and Debruyne, R. (2011). Antibiotic resistance is ancient. *Nature* 477: 457–461.

Darwish, A. M. and Griffin, B. R. (2002). Study of oxyetracycline controls *Streptococcus* in tilapia. *Global Aquaculture Advocate* 5: 34-35.

Didinen, B. I., Yardimci, B., Onuk, E. E., Metin, S. and Yildirim, P. (2014). Naturally *Lactococcus garvieae* infection in rainbow trout (*Oncorhyncus mykiss* Walbaum , 1792): new histopathological observations, phenotypic and molecular identification. *Revue de medecine Veterinaire* 165: 12-19.

Dodson, S. V., Maurer, J. J. and Shotts, E. B. (1999). Biochemical and molecular typing of *Streptococcus iniae* isolated from fish and human cases. *Journal of Fish Diseases* 22: 331–336.

Eldar, A., Horovitcz, A. and Bercovier H. (1996). Development and efficacy of a vaccine against *Streptococcus iniae* infection in farmed rainbow trout. *Veterinary Immunology and Immunopathology* 56: 175-183.

El-Sayed, A. F. M. (2006). Oceanography Department, Faculty of Science, Alexandria University. *Tilapia culture*. CABI Publishing Egypt. 275pp.

Evans, J. J., Klesius, P. H., Gilbert, P. M., Shoemaker, C. A., Al Sarawi, M. A.,

Landsberg, J., Duremdez, R., Al Marzouk, A. and Al Zenki, S. (2002). Characterization of b -haemolytic Group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus* L., and wild mullet, *Liza klunzingeri* (Day), in Kuwait. *Journal of Fish Diseases* 25: 505–513.

Facklam, R., Elliott, J., Shewmaker, L. and Reingold, A. (2005). Identification and characterization of sporadic isolates of *Streptococcus iniae* isolated from humans. *Journal of Clinical Microbiology* 43: 933-937.

FAO (2004). Year book. Food and Agriculture Organization of the United Nations. FAO. Rome. Italy. 140 pp.

FAO (2006). Year book. The State of World Fisheries and Aquaculture. 180 pp.

FAO (2014). Year book. The State of World Fisheries and Aquaculture. 243 pp.

Feldman, C., Bch, M. B. and Anderson, R. (2009). Bacteremic Pneumococccal Pneumonia Current Therapeutic Options; Tshwane Academic Division, Johanessburg. 200 pp.

Fitzsimmons, K. and Carvalho, J. F. (2000). Tilapia Aquaculture in the 21st Century Fifth *International Symposium on Tilapia Aquaculture* 6: 3-8.

Fortina, M. G., Ricci, G., Foschino, R., Picozzi, C., Dolci, P., Zeppa, G., Cocolin, L. and Manachini, P. L. (2007). Phenotypic typing, technological properties and safety aspects of *Lactococcus garvieae* strains from dairy environments. *Journal of Applied Microbiology* 103: 445–453.

Gibello, A., Galán-Sánchez F., Mar Blanco M., Rodríguez-Iglesias M., Domínguez L. and Fernández-Garayzábal, J. F. (2016). The zoonotic potential of *Lactococcus garvieae*: An overview on microbiology, epidemiology, virulence factors and relationship with its presence in foods. *Research in Veterinary Science* 109: 59–70.

Heuer, O. E., Kruse, H., Grave, K., Collignon, P., Karunasagar, I. and Angulo, F. J. (2009). Human health consequences of use of antimicrobial agents in aquaculture. *Clinical Infectious Diseases* 49: 1248–1253.

Itsaro, A., Suanyuk, N. and Tantikitti, C. (2012). Multiplex PCR for simultaneous detection of *Streptococcus agalactiae* , *Streptococcus iniae* and *Lactococcus garvieae*: a case of *S* . *agalactiae* infection in cultured Nile tilapia (*Oreochromis niloticus*) and red tilapia (*Oreochromis niloticus x Oreochromis mossambicus*). *Songklanakarin Journal of Science and Technology* 34: 495–500.

Karsidani, H. S., Soltani, M., Nikbakhat-Brojeni, G., Ghasemi, M. and Skall, H. F. (2010). Molecular epidemiology of zoonotic streptococcosis / lactococcosis in rainbow trout (*Oncorhynchus mykiss*) aquaculture in Iran. *Iranian journal*

of microbiology 2: 198-200.

Kav, K. and Erganis, O. (2008). Antibiotic susceptibility of *lactococcus garvieae* in rainbow trout (oncorhyncus mykiss) farms. *Bulletin of the Veterinary institute in Pulawy* 52: 223–226.

Kawanishi, M., Kojima, A., Ishihara, K., Esaki, H., Kijima, M., Takahashi, T., Suzuki, S. and Tamura, Y. (2004). *Letters in Applied Microbiology* 40: 322–328.

Kim, J. H., Gomez, D. K., Choresca, C. H. and Park, S.C. (2007). Detection of major bacterial and viral pathogens in trash fish used to feed cultured flounder in Korea. *Aquaculture* 272: 105-110.

Kitao, T., Aoki, T. and Iwata, K. (1979). Epidemiological study on streptococcosis of cultured yellowtail. Distribution of Streptococcus spp. In sea water and muds around yellowtails farms. *Bulletin of the Japanese Society of Science of Fish* 45: 567–572.

Klesius, P. H., Shoemaker, C. A. and Evans, J. J. (2008). Streptococcus: A worldwide fish health problem: In: *8th International Symposium on Tilapia in Aquaculture*. Cairo, Egypt. pp 83-107.

Krylova, N. I., Khabiboulline, R. E., Naumova, R. P. and Nagel, M. A. (1997). The Influence of Ammonium and Methods for Removal during the Anaerobic Treatment of Poultry Manure. *Journal of Chemical Technology and*

Biotechnology 70: 99-105.

Li, L., Olsen, H. R., Ye, L., Yan, H., Nie, Q., Meng, H. and Shi, L. (2015). Antimicrobial Resistance and Resistance Genes in Aerobic Bacteria Isolated from Pork at Slaughter. *Journal of Food Protection* 79: 589–597.

Lin, C. N. (2011). Characterization of a novel Streptococcus species associated with marine environment, University of Hong Kong, 67 pp.

Magnadottir, B. (2006). Innate immunity of fish (overview). *Fish and Shellfish Immunology* 20: 137-151.

Maki, T., Hirono, I., Kondo, H. and Aoki, T. (2008). Drug resistance mechanism of the fish-pathogenic bacterium *Lactococcus garvieae*. *Journal of Fish Diseases* 31: 461–468.

Merrifield, D. L. and Ruiz-Zarzuela, I. (2011). *Journal of Fish Diseases* 34: 499–507.

Meyburgh, C. M., Bragg, R. R. and Boucher, C.E. (2017). *Lactococcus garvieae* : an emerging bacterial pathogen of fish. *Diseases of aquatic organisms* 123: 67–79.

Murray, P. R., Baron, E. J., Jorgensen, J. J., Pfaller, M. A. and Yolken, R. H. (2003). Manual of Clinical Microbiology, ASM Press: Washington, DC 1760 pp.

Naing, L., Winn, T. and Rusli B. N. (2006). Practical Issues in Calculating the Sample Size for Prevalence Studies. *Archives of Orofacial Sciences* 1: 9-14.

Nakamura, Y. (1982). Doxycycline. *Fish Pathology* 17: 67-76.

Nguyen, H. T., Kanai, K. and Yoshikoshi, K. (2002). Ecological investigation of *S. iniae* isolated in cultured Japanese Flouder, *Paralicthys olivaceus* using selective isolation procedure. *Aquaculture* 205: 7-17.

Park, K. H., Matsuoka, S., Nakail, T. and Muroga, K. (1997). A virulent bacteriophage of *Lactococcus garvieae* (formerly *Enterococcus seriolicida*) isolated from yellowtail *Seriola quinqueradiata. Diseases of aquatic organisms* 29: 145-149.

Perez-Sanchez, T., Balcazar, J. L., Garcia, Y., Halaihel, N., Vendrell, D., de Blas, I., Merrifield, D. L. and Ruiz-Zarzuela, I. (2011). Identification and characterization of lactic acid bacteria isolated from rainbow trout, *Oncorhynchus mykiss* (Walbaum), with inhibitory activity against *Lactococcus garvieae Journal of Fish Diseases* 34: 499–507.

Pillay, T.V.R. (1990). *Aquaculture principles and practices*. Fishing News Books, Blackwell Science, Oxford, UK. 575 pp.

Popma, T. and Masser, M. (1999). *Tilapia life story and biology*. Southern Regional Aquaculture Center, Johannesberg. 283 pp.

Pullin, R. S. V., and Lowe-McConnel, R. H. (1982). The biology and culture of tilapias: In: *ICLRAM Conference Proceeding*. Manila. 433-438 pp.

Raissy, M. and Shahrani M. (2015). Detection of Tetracycline Resistance Genes in *Lactococcus garvieae* Strains Isolated from Rainbow Trout. *International Journal of Biological, Food, Veterinary and Agricultural Engineering* 9: 2-8.

Richards, G. P. (2014). Aquaculture: a review of the technology Bacteriophage remediation of bacterial pathogens in aquaculture, review of the technology, [http://dx.doi.org/10.4161/21597081.2014.975540]. Site visited in December 2016.

Robert, R. J. (Ed). (2001). Fish Pathology, fourth edition. Black well publishing, London. 571 pp.

Schmittou, H. R., Cramer, M.C. and Jian, Z. (1998). Principles and Practices of High Density Fish Culture in Low Volume Cages. American Soybean Association, USA. 88. pp.

Sharifiyazdi, H., Akhlaghi, M., Tabatabaei, M. and Mostafavi, Z. S. M. (2010). Isolation and characterization of *Lactococcus garvieae* from diseased rainbow trout (*Oncorhynchus mykiss*, Walbaum) cultured in Iran. *Iranian Journal of Veterinary Research* 11: 342-351

Shayo, S. D., Mwita, C. J. and Hosea, K. (2012). Ulcerative Aeromonas Infections in Tilapia (Cichlidae: Tilapiini) from Mtera Hydropower Dam, Tanzania. *Open Access Scientific Reports* 1: 115-119.

Shelton, W. L. and Popma, T. J. (2006). *Biology: Tilapia biology, culture and nutrition*. Food Production Press New York, USA. 49 pp.

Shiomitsu, K., Kusuda, R., Osuga, H. and Munekiyo, M. (1980). Studies on chemotherapy of fish disease with erythromycin. *Fish Pathology* 15: 17-23.

Shoemaker, C. A. and Klesius, P. H. (1997). *Streptococcal disease problem and control: A review.* Tilapia Aquaculture Ithaca, New York, USA. 200 pp.

Soltani, M., Pirali, K. A., Taherimirkahead, E., Shafie, S., Mohamadian, S. and Roholahi, S. (2013). Molecular Study of Streptococcosis/Lactococcosis Distribution in Farmed Rainbow Trout in Charmahal–va-Bakhteyari and Kohgiloyeh-va-Boyerahmad Provinces, Iranian Journal of Epidemiology 9: 59-68.

Spellerberg, B. and Brandt, C. (2007). Streptococcus Manual of Clinical Microbiology. *American Society for Microbiology* 9: 412-429.

Suanyuk, N., Sukkasame, N., Tanmark, N., Yoshida, T., Itami, T., Thune, R. L., Tantikitti, C. and Supamattaya, K. (2010). *Streptococcus iniae* infection in cultured Asian sea bass *(Lates calcarifer)* and red tilapia (Oreochromis sp.) in southern Thailand. *Songklanakarin Journal of Science and Technology* 32: 341-348.

Subasinghe, R. P., Bondad-Reantaso, M. G. and McGladdery, S. E. (2001). Aquaculture development, health and wealth. In: *Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third*

Millennium; (Subasinghe, R.P., Bueno, P., Phillips, M. J., Hough, C., McGladdery, S.E. and Arthur, J. R.); Bangkok; Thailand; 20-25 February; pp 167-191.

Ture, M. and Boran, H. (2015). Phenotypic and genotypic antimicrobial resistance of Lactococcus sp . strains isolated from rainbow trout (*Oncorhynchus mykiss*). *Bulletin of the Veterinary institute in Pulawy* 17: 37–42.

Vendrell, D., Balcazar J. L., Ruiz-Zarzuela, I., de Blas, I., Girones, O. and Muzquiz J. L. (2006). *Lactococcus garvieae* in fish. A review *Comparative Immunology, Microbiology Infectious Diseases* 29: 177–198.

Wang, C. Y. C., Shie, H. S., Chen, S. C., Huang, J. P., Hsieh, I. C., Wen, M. S., Lin, F. C. and Wu, D. (2007). *Lactococcus garvieae* infections in humans: possible association with aquaculture outbreaks. *International Journal of Clinical Practice* 61: 68–67.

Wangead, C., Greater, A. and Tansakul, R. (1988). Effect of acid water on survival and growth rate of Nile tilapia (O. niloticus): In: *Proceeding of the Second International Symposium on Tilapia in Aquaculture* (Pullin, R.S.V. Bhukaswan, T. Tonguthai K. and Maclean J.L).. Bangkok, Thailand. pp 433-438.

WHO (2006). Biorisk management Laboratory biosecurity guidance. 41 pp.

Wright, G. D. (2010). Antibiotic resistance in the environment: a link to the clinic?. *Current Opinion in Microbiology* 13: 589–594.

Xiong, W., Sun, Y., Zhang T., Ding, X., Li, Y., Wang, M. and Zeng, Z. (2015). Antibiotics, Antibiotic Resistance Genes, and Bacterial Community Composition in Fresh Water Aquaculture Environment in China. *Microbial ecology* 70: 425–432.

Xu, D. H., Shoemaker, C. A. and Klesius, P. H. (2007). Evaluation of the link between grodactylosis and *Streptococcus* of Nile tilapia (*O. niloticus*). *Fish Diseases* 30: 230-238.

Yang, Y., Li, B., Zou, S., Fang, H. H. and Zhang, T. (2014). Fate of antibiotic resistance genes in sewage treatment plant revealed by metagenomics approach. *Water Research* 62: 97–106.

Yanong, R. P. E. and Francis-floyd, R. (2016). Streptococcal Infections of Fish. University of Florida, Circular 57: 1–5.

Zhang, D. F., Zhang, Q. Q. and Li A. H. (2014). Development of a multiplex PCR assay for rapid and simultaneous detection of four genera of fish pathogenic bacteria. *Letters in Applied Microbiology* 59: 471-478.

Zhou, S. M., Fan, Y., Zhu, X. Q,, Xie, M. Q. and Li, A. X. (2011). Rapid identification of *Streptococcus iniae* by specific PCR assay utilizing genetic markers in ITS rDNA. *Journal of Fish Diseases* 34: 265–327.

Zhu, Y, G., Johnson, T. A., Su, J. Q., Qiao, M., Guo, G. X., Stedtfeld, R. D., Hashsham, S. A. and Tiedje, J. M. (2013). Diverse and abundant antibiotic resistance

genes in Chinese swine farms. *Proceedings in the National Academy of Scsience* 110: 3435–3440.

Zlotkin, A., Eldar, A. V. I. and Ghittino, C. (1998). Identification of *Lactococcus garvieae* by PCR. *Journal of clinical microbiology* 36: 983–985.

APPENDICES

Appendix 1: Questionnaire for fish farmers

Date and time of interview: ____/____/2016/17 ____:____ a.m. /p.m.

Name of respondent: _____

Age and sex Age: _____ years male ☐ female ☐

Village: _____

I. GENERAL POND DESCRIPTION AND USE OF WATER

1. How many ponds do you own? _____ ponds.

2. How big are the respective ponds (in m^2)? _____

3. Please describe the location of your pond. _____

(1 = residential area, 2 = paddy field area)

4. What are the respective ponds used for? _____

(1 = grow-out, 2 = nursery)_____)

5. When did you construct the respective pond (year)? _____

6. Is the respective pond used during the whole year or only during the rainy season?

_____

(1 = whole year, 2 = only during rainy season

8. Where does the water come from in the respective pond? _____

(1 = Underground 2 = stream _____)

9. Is there water flowing through your pond? Yes ☐ No ☐ _____

10. Does the water flow through other ponds, paddy fields or other places before entering the respective pond? _____

(1 = paddy fields, 2 = ponds_____)

11. Where does the pond water flow out to? _____

1 = other ponds, 2 = canal _____

12. How often do you exchange water _____ per month

(1 = once, 2 = twice, 3 = never)

13. Do you use antibiotics? Yes ☐ No ☐

14. Have you had to cope with flooding in the past several production cycles? Yes ☐ No ☐

15. Do you dry-out the pond before stocking? _____ (1 = yes, 2 = no,)

16. Do you remove the pond mud? : _____ (1 = yes, 2 = no,)

III. STOCKING OF POND

1. When did you stock the respective pond? ____

2. How were the fish transported from the place of purchase to your farm?

_____

3. Do you also stock non-fish aquatic products (e.g. shrimps, snails etc.) in your pond?

Yes ☐ No ☐

4. How many fish did ypo stock?

IV. FEED MANAGEMENT

1. Which ingredients do you use to make your fish feed?

_____

2. How often do you feed per day?

(1 = once, 2 = twice)

V. MANURE MANAGEMENT

1. Do you use manure in your ponds? Yes ☐ No ☐

2. What kinds of manure do you usually use? ____

(1= cattle, 2 = chicken)

3. Please estimate the amount and frequency of manure application. ____

VII. POND INPUTS

1. Do you have access to lime? Yes ☐ No ☐

VIII. FISH DISEASES

1. Have you ever had to cope with fish diseases in the respective pond? : _____:

_____ (1 = often, 2 = sometimes, 3 = never)

2. Do you know the name of the disease?

_____ Please describe the symptoms:

3. How many fish have died? _____ What was the approximate weight of dead fish (g)?

_____

4. Have you taken any action toward curing your fish? Yes ☐ No ☐

Appendix 2: Water quality parameters record sheet

Name of the owner ………………. Date…………………. Pond number………………… District………………….

Pond Number	Water quality parameters					Time
	pH	T^0c	DO	DO %	Transparency	

Appendix 3: Sampled fish biodata sheet

S/N	Weight	Total Length	Standard	Sex

Appendix 4: Client consent form

DEPARTMENT OF PUBLIC HEALTH AND VETERINARY MEDICINE

Study Title: Occurrence and Antimicrobial resistance of *Streptococcus* and *Lactococcus species* in cultured Tilapia in Morogoro

You are invited to participate in a research study aiming to contribute towards reduction of fish mortalities due to bacterial diseases. Because the study requires obtaining samples in fish ponds, after consulting the District fisheries officer we were directed to you. This study is being conducted by Mr. Aron Yusuph, Masters Student in Health of Aquatic Animal Resources. Please read this form carefully and ask any questions you may have before agreeing to participate in this study.

We ask that you read this form and ask any questions prior to agreeing to participate in this study.

Background and Purpose:

There is an increased number of Streptococcosis cases in both farmed and wild fishes. Streptococcus is a genus of emerging pathogens that cause septicemia and meningoencephalitis with high mortality in wild and cultured species of fish worldwide. *Streptococcus iniae* and *Streptococcus pneumoniae* are zoonotic bacteria, attracting attention as a result of their zoonotic implication to human which occur during the cleaning of fresh whole fish (Facklam, 1996). In Tanzania less is known on streptococcosis in both wild and cultured species of fish. The current study aims to identify Streptococcus species in cultured Tilapia in Morogoro, determine the risk factors for occurrence of diseases and assess antimicrobial resistance profile of

Streptococcus species. Finding may help public health professionals to institute appropriate measures and/or being used in streptococcus vaccine establishment.

There will be no treatment applied during the study.

Study Procedures:

The samples will be collected in 30 days and it will involve, seining a maximum of 7 fish per pond, recording water quality parameters and request you to fill a questionnaire which may help us determine the risk factors for occurrence of bacterial diseases in you ponds. Fish sampled will be put into separate covered containers and transported to the Microbiology laboratory at Sokoine University of Agriculture for analysis. Organs including kidney, eyes and liver will be used for bacteriology.

Risks of study participation.

During sampling, pond floors may be destructed, and people involved in sampling may be pierced by fish spines. Stress caused by sampling may also cause mortalities on remaining stock.

Benefits of study participation:

The benefits of study participation are direct payments of all sampled fish. People involved in seining during sampling will also be paid.

Study costs/compensation:

We do not anticipate any risks to you participating in this study other than those encountered in day to day life. However if you experience unusual mortalities associated with sampling, the study will cover for the losses.

Confidentiality:

All results will be confidential. Information about your animal may be used in scientific presentations and/or publication. However, no personal or identifying information about you or your animal will be released. Your animals' records for the study may, however, be reviewed by One Health Central and Eastern Africa donors and by departments at the Sokoine University of Agriculture with appropriate regulatory oversight. To these extents, confidentiality is not absolute.

Voluntary Participation:

Participation in this study is voluntary. Your decision whether or not to participate in this study will not affect your current or future relations with the University, your veterinarian or the community. If you decide to participate, you are free to withdraw at any time without affecting those relationships.

Please do not hesitate to contact us if you have any questions or concerns about this study.

You will be given a copy of this form to keep for your records.

Investigator; Mr. Aron Yusuph

Phone number; 0758 594 500

Email: kidole1962@gmail.com

I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

Client name……

Signature ……

Date……