THE BACTERIAL FLORA OF TILAPIA (*OLEOCHROMIS NILOTICUS*) AND CATFISH (*CLARIAS GARIEPINUS*) FROM EARTHEN PONDS IN SAGANA FISH FARM AND MASINGA DAM

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or any other award.

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DEDICATION

I dedicate this thesis to God the almighty for his mercies and strength to persevere to the end of the work. To my loving husband Charles, my children Cynthia, Fiona and Allan for their love, endurance and support.

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LIST OF ABBREVIATIONS AND ACRONYMS

API	Analytical profile index
DAO	Diamine oxidase
DHA	Docosahexaenoic acid
EHEC	Entrohaemorhagic Escherichia
EPA	Eicosapentanoic acid
FAO	Food and Agricultural Organisation
FD	Fisheries Department – Kenya
HMT	Histamine N- methyltransferase
ICMSF	International commission on microbiological specifications for foods
NACA	Network of Aquaculture Centres in Asia-Pacific
NCCLS	National committee for clinical laboratory standards
WHO	World Health organization

DEFINATION OF TERMS

Aquaculture Aquaculture is the farming of aquatic organisms such as fish, shellfish and even plants. The term aquaculture refers to the cultivation of both marine and freshwater species and can range from land-based to open-ocean production.

Capture Fisheries Capture fishery refers to all kinds of harvesting of naturally occurring living

Pathogenic Bacteria These are bacteria that cause bacterial infection such as foodborne illnesses.

Finfish A fin fish is another name for true fish. These are fish that have all the characteristics of fish such as breathing via gills and their body being covered in scales. The other types of fish that are not fin fish are called flat fish.

Shellfish This a culinary and fisheries term for exoskeleton-bearing aquatic invertebrates used as food, including various species of molluscs, crustaceans, and echinoderms. Although most kinds of shellfish are harvested from saltwater environments, some kinds are found only in freshwater

Salmonella roe These are the eggs of the salmon fish cured and used like other roe products. Like other roes, salmon eggs are collected by harvesting female fish shortly before spawning, when they have large and very well developed egg mass.

ABSTRACT

Fish is a worldwide distributed food commodity regarded a cheap source of protein especially in the developing countries like Kenya. It provides a good balance of protein, vitamins and minerals. However, bacteria occur naturally on the skin, in the gut and in the slime of living fish, even though they do no harm to the, the microorganisms may cause harm to consumers. Also, some of the microorganisms associated with fish may carry genes of antibiotic resistance that can be passed to pathogens of clinical importance. Food borne diseases traced to fish consumption have been reported world over including Kenya. In Kenya though aquaculture has been promoted, the aspect of food quality as far as consumption of fish is concerned is underestimated. Sagana fish farm and Masinga dam provide fish for Kenyan markets including Nairobi. No information available when this study was conducted on quality of fish from Sagana fish farm and Masinga dam. The study was designed to determine the bacterial flora of Tilapia and Catfish from earthen ponds at Sagana fish farm and Masinga dam and to determine the anti- microbial response of the pathogenic bacteria. Samples of Tilapia fish and Catfish were collected from Sagana farm and Masinga dam in dry and rainy season. The fish were skinned and gut content taken for laboratory test. Samples of water and water sediments from the two study sites were also collected. The samples were processed and cultured in MacConkey agar and the colonies sub cultured in selective media. The colonies were subjected to morphological examination from cultures and biochemical test carried out using commercially available API kits. The results obtained from this study showed the presence of bacterial species belonging to *Enterobacter* spp. (n=34), *Aeromonas* spp. (n=5), *Vibrio* spp. (n=3), *Pseudomonas* spp. (n=6) and *Acinetobacter* spp. (n=2) were isolated during the dry season while bacterial species belonging to *Enterobacter* spp. (n=31), *Aeromonas* spp. (n=4), *Pseudomonas* spp. (n=6) were isolated during the dry season. Antimicrobial susceptibility showed that the highest rates of resistance was found against Amoxicillin (Aml) (65.9% of isolates), Ampicillin (Amp) (61.5% of isolates), Tetracycline (Te) (31.8% of isolates), and Chlorampenicol (C) (27.5 % of isolates) while the lowest was Nalidixic acid (Na), Streptomycin (S) and Cefuroxine (Cxm) at (4.4% of isolates) each. All isolates were sensitive to Ciprofloxacin (Cip), Gentamycin (Gen) and Cefotaxine (CTX). The presence of the above organisms some of which are potentially pathogenic to humans is an indication that fish improperly handled, undercooked or consumed raw may cause disease to susceptible individuals while the antimicrobial resistance by some of the isolates is an indication that the use of antibiotics in aquaculture for promotion of growth should be studied further with view to policy formulation.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Fish is an important component of diets around the world. An estimated 1 billion people rely on fish as their main source of animal protein (FAO, 2007). The global fish production is estimated to be 148.5 million tonnes per year of which capture fisheries accounts for 88.6 million tonnes and aquaculture 59.9 million tonnes per year (FAO, 2012). Fish provides the much needed protein to people living in developing countries at affordable prices. It feeds millions of people daily and sustains many through employment in services related to fish and fish products. The nutritional attributes of fish are highly praised as it is rich in the essential amino acids, has high quality vitamins and its fatty acids fraction have well established health benefits (anti-thrombotic activity). Therefore, its availability in many developing countries should enable fish to contribute significantly to a healthy and balanced diet in these countries. It is estimated that around 60 percent of the population in many developing countries derive over 30 percent of their animal protein supplies from fish, while almost 80 percent of the population in most developed countries obtain less than 20 percent of their animal protein supplies from fish (FAO, 2000).

The risks of food borne disease associated with products from aquaculture are related to inland or coastal ecosystems, where the potential of environmental contamination is greater when compared with capture fisheries. Most of the food safety hazards associated with products from aquaculture can be controlled by good fish farm management practices and appropriate consumer education regarding such risks as eating raw or partially cooked

products that may contain pathogenic bacteria (Reilly, 1998). The estimated annual mortality of food and water-borne infectious diseases in developing countries amounts to high death rates, mainly of infants and children. In industrialized countries microbiological food borne illnesses affect up to 30 percent of the population. Every year 20 out of each million inhabitants die from food borne disease. Unwholesome fish and fishery products cause up to 30 percent of the food-borne illnesses (WHO, 1999).

Around 40 million people in Asia are affected by fish and water borne parasitic diseases, especially trematodes. These parasitic diseases are widespread mainly in China, Viet Nam, Thailand and Laos, where food habits encourage the consumption of raw fish. In addition to the economic losses incurred because of fish spoilage, fish-borne illnesses can have costly health adverse effects, the loss of productivity, medical expenses and the adverse publicity to the companies. Additional costs in international trade include the cost of rejections, detection of products, recalls and the resulting adverse publicity to the industry and even to the country of origin of the fish (Lahsen, 2003). Financial implications of food borne disease outbreaks shows that the consequences can be grave. An outbreak of cholera in Peru in 1991 cost 770 million dollars, a similar outbreak in Tanzania in 1998, cost 36 million dollars. Simple preventive measures and effective surveillance systems which would cost less might have prevented these outbreaks, or would have definitely reduced the impact of the (Lahsen, 2003). In Kenya there was ban on exports of fish and fishery to the EU due to an outbreak of salmonella in 1996 and outbreak of cholera in 1997 (FD, 1999).

In Kenya the future of aquaculture is bright considering that many people are increasingly turning to fish as a source of their animal protein. With this increase in demand for fish and the decreasing catches from the natural sources aquaculture is

destined to become an important alternative to traditional agricultural practices. The aquaculture production in Kenya in the year 2008 was 1012 metric tonnes (FD, 2009). In 2011 the figures went high to 24,000 metric tonnes due to the government's intervention (FD, 2012). Despite the environmental concerns, aquaculture profitability is so high that money can and should go back into promoting sustainable practices. Furthermore, new methods minimize the risk of biological and chemical pollution through minimizing stress to fish, vaccinating fish, fallowing netpens, and applying Integrated Pest Management (Paul, 2006).

Current knowledge of the health and environmental impact of antibiotics used in aquaculture is poor particularly in developing countries. Drug residues may remain in fish used for human consumption and consequently the antibiotics released into the environment can lead to the development of antibiotic resistant bacteria in the food chain (Cabello, 2006). Due to increase in semi-intensive aquaculture systems there is a potential hazard related to the development of antibiotic resistance in the pond micro flora. Resistance to antibacterial agents is a major global public health problem and one that is increasing as these agents continue to lose their effectiveness (Akinbowale *et al.*, 2006).

1.2 Statement of the Problem

Fish is the most important single source of protein providing 16% of the animal protein consumed by the world's population (FAO, 1997). It is estimated that about one billion people world- wide rely on fish as their primary source of animal protein (FAO, 2000). The emergence of diseases associated with beef and poultry such as the Rift Valley Fever, mad cow disease for beef and bird flu for poultry have all contributed to the increase in consumption of fish. There is increased demand for fish in Kenya which cannot be

met by the capture fisheries with stocks stagnating due to overexploitation. The alternative has been the growth in aquaculture with many people turning to farmed fish which is believed to be of better quality whereas it is documented that aquatic environments harbor many bacteria (Novotny, 2004). The use of organic waste for the fertilization of ponds is also a source of pathogenic organisms that may be transmitted to humans via products of aquaculture. There is little information on the quality of fish from Sagana fish farm and Masinga dam as far as the bacterial microorganisms is concerned. There was therefore a need for this study to bridge this gap.

1.3 Justification of the study

A lot of resources and international support are directed to ensure fish safety and quality of fish for export, from the capture fisheries while aquaculture fish has received very little or no attention. In Kenya a lot of emphasizes has been on the capture fisheries due to the implications on the world trade while the aquaculture fish has received very little if any attention regarding the bacterial flora, water quality and the type of feed used. This is mainly due to the perception that aquaculture fish is assumed to be free of any contamination. Information on the prevalence of bacterial pathogens that may be present in the aquaculture industry in Kenya is unavailable. Additionally there is no data on the status of sensitivity of these bacteria to anti-microbial agents used in the livestock and horticulture industry in the country.

Against the above background, if fish is going to play a major role in both providing the much needed protein and contributing to the national economy current information on such aspect as fish borne diseases is required. And if aquaculture will reduce the gap between supply and demand for fish and fishery products then there is need to establish the bacterial flora of farmed fish, pond water and of sediment in the ponds and dams. This

study was carried out to identify the bacterial flora in the farmed fish and fish in the dams and their anti-microbial response. The findings of this research will be useful in managing aquaculture farms, formulating feeds for aquaculture fish and future choice of antibiotics to use in aquaculture systems.

1.4 Research Questions

1. What is the bacteria flora of fish at Sagana fish ponds and Masinga dam in dry and wet season?
2. Is there any significant difference of bacterial flora of fish between Sagana ponds and Masinga dam, in dry season and wet season?
3. What is the anti microbial response of the micro-organisms from the fish at both sources?

1.5 Objectives of the Study

1.5.1 Main Objective

To determine the bacterial flora of Tilapia, Catfish, water and water sediments and their anti-microbial response from earthen ponds at Sagana fish farm and Masinga dam during dry and wet seasons.

1.5.2 Specific Objectives

i. To isolate and identify bacterial flora species during dry and wet seasons at Sagana fish ponds and Masinga dam
ii. To identify whether there is significant difference on the number of bacteria flora species isolated in fish specimen types from ponds and dams, during dry and wet season
iii. To determine anti- microbial response of the pathogenic bacteria isolates from Sagana fish farm and the Masinga dams.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nutritional and economic value of fish

Fish is a vital source of food for people. It is man's most important single source of high-quality protein, providing -16 % of the animal protein consumed by the world's population (FAO, 1997). Fish oils are the only concentrated source of eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids play a major role in the development and maintenance of brain, and prevention of different pathologies mainly the cardiovascular diseases and also psychiatric disorders such as stress, depression and dementia (Bourne, 2005). It is estimated that about one billion people world- wide rely on fish as their primary source of animal protein (FAO, 2000). FAO estimates the value of fish traded internationally to be US $ 51 billion per annum. Over 36 milion people are employed directly through fishing and aquaculture (FAO, 2000) and as many as 200 million people derive direct and indirect income from fish (Garcia and Newton, 1997).

In Kenya fresh water fish are mainly from Lake Victoria, rivers and the dams. Although aquaculture in Kenya is not fully developed the potential is high and currently the annual production is about 21,487metric tons valued at Kshs. 4, 633, 634, 000 (Fisheries Department, 2012). The fisheries sector provides employment and income to over 500,000 Kenyans engaged in fish production, fish trade, industrial fish processing and related enterprises (Fisheries Department, 2001).

2.2 Bacteria associated with farmed fish

Integrated fish farming combines livestock production with fish farming. In these arrangements, animal manure is shed directly into a fish pond as fertilizer and supports the growth of

photosynthetic organisms. Several bacteria are reported to cause infection and mortality in both fish and humans (Novotny, 2004) and these represent a particular hazard caused either by handling infected fish on fish farms or in grocery stores or by ingestion of raw or inadequately processed infected fish and /or contaminated fish products. Bacterial pathogens are a major cause of infectious diseases and mortality in wild fish stocks and fish reared in confined conditions. Disease problems constitute the largest single cause of economic losses in aquaculture. Concurrent with the rapid growth and intensification of aquaculture, increased use of water bodies, pollution, globalization, and trans-boundary movement of aquatic fauna, the list of new pathogenic bacterial species isolated from fish has been steadily increasing (Ponnerassery *et al.*, 2012).

The level of contamination of aquaculture products by the pathogenic bacteria will depend on the environment and the bacteriological quality of the water in which the fish is cultured. Tilapia, native to Africa and the Middle East is the second most common farm-raised food fish in the world (Fitzsimmons, 1997). Aquatic animals take a large number of bacteria into their gut and gills from water, sediment and food. The intestinal microflora may be significant in fish spoilage (Kaneko, 1971) and may be involved in the spread of fecal contaminants (Geldreich and Clarke, 1966). The microbial populations within the digestive tract of fish are rather dense, the number of micro-organism being much higher than in the surrounding water indicating that the digestive tract provides a favourable ecological niche for these organisms (Horsley, 1977). Some normal bacterial microflora of water, such as *Pseudomonas fluorescens, Aeromonas hydrophilla, Edwardsiella tarda, Vibrio species* and *Myxobacteria* (Sugita *et al.*, 1985), These can be found on the body surface or in the intestinal tracts of fish under normal conditions but due to environmental stress may produce

epizootics diseases. There are two broad groups of bacteria that will contaminate fish. The first group is the indigeneous microflora which occurs naturally in the environment such as *Aeromonas hydrophilla, Vibrio parahaemolyticus, Vibrio cholera, Vibrio vulnificus* and *Llisteria monocytogenes.* The other group is the non- indigenous bacteria that include the members of the family *Enterobacteriaceae* such as *Salmonella* species, *Shigella* species and *Escherichia coli*. A number of pathogenic microorganisms including *Aeromonas, Pseudomonas, Edwardsiella* and *streptococcus* have been implicated in bacterial epidemics in Tilapia (*Oreochromis* species) cultures (Al – Harbi, 1994; Al – Harbi and Uddin, 2003). The frequency of contamination of fishery products by pathogenic microorganisms has been considered a health hazard to consumers (Ingham and Potter, 1991).

2.2.1 *Vibrio paraheamotyticus* and other *vibrios*

Outbreaks of diarrhoea by *Vibrio paraheamolyticus* have been demonstrated in Japan and Taiwan after ingestion of under cooked fish and raw products, *sashimi* and *sushi* (Vuddhakul *et al.*, 2000). *Vibrio parahaemolyticus* has been isolated from sea and estuary waters on all continents with elevated sea water temperatures. It is isolated from fish throughout the year in tropical climates. It causes acute gastroenteritis that is self limiting, however, several cases require hospitalization and on rare occasions septicaemia may occur. In the 1970s *V. parahaemolyticus* was the cause of 14 outbreaks of gastroenteritis in USA (Barker *et al*., 1974), most of which occurred during the warmer months and were attributed to seafood. Cholera is a highly contagious disease caused by infection of the small intestines with *Vibrio cholerae* Ol and O139. It is characterized by massive acute diarrhoea, vomiting and dehydration. It is often transmitted by water but fish or fish products that have been in contact with contaminated water or faeces from infected persons also frequently serve as a source of infection (Kam *et al.*, 1995). In Kenya the European Union banned fish imports from Kenya in 1996 citing poor

sanitary conditions at the beaches and lack of refrigeration /icing facilities (Fisheries Dept, 1999).

2.2.2 *Escherichia coli*

Escherichia coli are enteric bacteria causing gastroenteritis. This bacteria together with other coliforms and bacteria such as Staphylococcus spp. and sometimes Enterococci are commonly used as indices of hazardous conditions during processing of fish. Such organisms should not be present on freshly-caught fish (Chattopadhyay, 2000). An outbreak of diarrhoeal illnesses caused by ingestion of food contaminated with enterotoxigenic E. coli was described in Japan (Mitsuda *et al*., 1998) associated with eating Tuna paste. An outbreak caused by salted salmon roe contaminated, probably during the production process, with enterohaemorrhagic *E. coli* (EHEC) O157 occurred in Japan in 1998 (Asai *et al*., 1999). The salmon roe was stored frozen for nine months but it appears that Enterohaemorrhagic E. coli (EHEC) O157 could survive freezing and a high concentration of NaCI and retained its pathogenicity for humans. The isolation of *E. coli* in fishes grown in sewage-fed farms and also in retail market fishes of Kolkata indicated contamination of fishes with faecal matter of animal and human origin (Manna *et al*., 2008). Food products that show evidence of faecal contamination are generally regarded as a greater risk to human health, as they are likely to contain human-specific enteric pathogens. Some strains of *E. coli* are capable of causing food-borne disease, ranging from mild enteritis to serious illness and death (FAO/NACA/WHO, 1997).

2.2.3 *Salmonella spp*

Fish and shellfish are passive carriers of *salmonella,* which demonstrate no clinical disease and can excrete *Salmonella* species without apparent trouble. Fish may therefore serve as a

vector for *Salmonella* species. In a Canadian outbreak of *Salmonella enterica* serotype Paratyphi B was linked to aquariums (Gaulin *et al.,* 2002). Another outbreak caused by drug resistance *Salmonella enterica* subspecies serotype typhimurium DT104 was described in Singapore (Ling *et al*., 2002). Dried anchovy was found to be the cause of infection. Although most *Salmonella* outbreaks have been linked to poultry, the Hawaii Department of Health studied 35 cases of *Salmonella* that arose from October 2007 to February 2008 and found that 86% of these patients had consumed raw fish in the 7 days before they got sick. In most cases, *ahi*, which is often made from imported frozen tuna, was the reported fish consumed. In April 2008, eight of the nine cases of *Salmonella* infection reported in the mainland United States also involved consumption of raw tuna.

2.2.4 *Staphylococcus aureus*

Enterotoxins produced by *Staphylococcus aureus* are another serious cause of gastroenteritis after consumption of fish and related products. In southern Brazil, *Staphylococcus aureus* was isolated from 20% of 175 examined samples of fresh fish and fish fillets. It was also detected during the process of drying and subsequent smoking of eels in Alaska in 1993 (Eklund *et al*., 2004).

2.2.5 *Listeria monocytogenes*

It is widely distributed in the general environment including fresh water, coastal water and in fish from these areas. Contamination and recontamination may also take place during processing (Huss *et al*., 2000). It is a psychotropic pathogen with the ability to grow at refrigerator temperatures. An outbreak of listeriosis due to vacuum packed gravad and cold smoked fish was described in at least eight human cases for eleven months in Sweden (Tham *et al.,* 2000).

2.2.6 *Clostridium botulinum*

The main habitat of *C. botulinum* is soil but it is also found in sewage, rivers, lakes, sea water, fresh meat and fish (Haagsma, 1991). *Clostridium botulinum* type F caused deaths after consumption of bought herrings without previous heating. Botulism caused by *Clostridium botulinum* type B after eating fish salad was described by (Weber *et al.,* 1993). *Clostridium botulinum* type B which is found in marine and Lake Sediments and in fish intestines does not grow or produce toxins in living fish but is carried passively. The bacterium becomes a hazard when processing practices are insufficient to eliminate botulinal spores from raw fish.

2.2.7 *Pseudomomas aeruginosa*

They are widely found in water and are increasingly recognized as an emerging opportunistic pathogen of clinical relevance. They have an ability to metabolize a variety of diverse nutrients and to form biofilms and hence to survive in a variety of unexpected places. *Pseudomonas aeruginosa* is one of the leading causes of nosocomial infections. The bacteria is intrinsically resistant to many antimicrobial agents, including most β-lactams, the older quinolones, chloramphenicol, tetracycline, macrolides, trimethoprim–sulfamethoxazole and rifampin but a few strains are sensitive to drugs like the ciprofloxacin (Rossolini and Mantengoli, 2005). This state of multi-drug resistance is attributable to a concerted action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelops. Besides intrinsic resistance it easily develops acquired resistance either by mutation in chromosomally encoded genes or by the horizontal gene transfer of antibiotic resistance determinants (Adelaide *et al.,* 2009).

2.2.8 *Aeromonas* species

This bacterium can also be found in fresh, salt, marine estuarine, chlorinated water. It

can survive in aerobic and anaerobic environments. It is very resistant to chorine, refrigeration or cold temperatures making it hard to kill and posing a danger to fish processing. It occurs in contaminated environments and is also ingested through food products that have been infected with the bacterium (Daskalov, 2006). It causes gastroenteritis which occurs mostly in young children and people who have comprised immune systems or growth problems. About 8.1% of cases of acute enteric diseases in 458 patients in Russia were caused by *Aeromonas species* (Pogorelova *et al.,* 1995). This could increase the hazard of food contamination, particularly where there is a possibility of cross- contamination with ready-to-eat food products. Some strains are important fish pathogens in aquaculture (Pillay, 1990), while others have been implicated in food borne disease (Morgan and Wood, 1988). *Aeromonads* can be causative agents not only of human enteritis (Sukroongreung *et al.,* 1983), but also of a fatal septicemia as recorded in a 15- year old healthy girl, where the causative agent was found to be *Aeromonas sobria* (Shiina *et al.,* 2004).

2.2.9 *Citrobacter freundii*

Citrobacter species are found in water, soil and decaying matter and can be isolated from the faeces of man and animals. They are small, Gram-negative, non spore forming rods, and belong to the family *Enterobacteriaceae*. *Citrobacter* species grows best at moderate temperatures but can also grow at low temperatures (7 ºC). *Citrobacter* is one of the major genera of bacteria that are found on fresh meat, minced meat, poultry, plants and plant products (Kleeberger and Busse, 1975; Jay, 2000). Sources of these food contaminants may be the original environment (such as water and soil) of fish, meats and vegetables. Treated wastewater, for example, is reused for irrigation and other purposes in many countries (WHO, 1989). *Citrobacter*

is one of the prevalent species in the influent and effluent of wastewater treatment plants (Abu-Ghazaleh, 2001) therefore; vegetables, fish and other foods in contact with this water may be contaminated. It has strains that have inducible *ampC* genes encoding resistance to ampicillin. The first generation cephalosporins resistance encountered in this organism could also be as a result of plasmid- encoded resistance genes (Abu-Ghazaleh, 2001).

2.2.10 *Edwardsiella tarda*

This is a member of the family *Enterobacteriaceae*. *Edwardsville* spp. has been implicated in gastroenteritis in humans and in bacteremic infections that include wound abscesses and meningitis (Sakazaki *et.al.*, 1971). It has been isolated from a diseased pig, an ostrich and was also implicated as the causative agent of a disease in pond-reared eels (Wakabayashi and Egusa, 1973). Incidence of *E. tarda* in fishes from freshwater aquaculture environment and retail market have been reported (Pankajkumar, 2009), and human liver abscess caused by *E. tarda* bio group in India (Manchanda *et al*., 2006) have also been reported.

2.3 The Analytical Profile Index (API 20E)

The Analytical Profile Index (API) is a miniaturized panel of biochemical tests compiled for identification of groups of closely related bacteria. Different test panels are prepared in dehydrated forms which are reconstituted upon use by addition of bacterial suspensions. After incubation, positive test results are scored as a seven-digit number (profile). Identity of the bacterium is then easily derived from the database with the relevant cumulative profile code book or software.

The API 20E used was biochemical panel for identification and differentiation of

members of the family Enterobacteriaceae. In API 20E for identification of members of the family Enterobacteriaceae, the plastic strip holds twenty mini-test chambers containing dehydrated media having chemically-defined compositions for each test.

2.4 Antimicrobial Resistance

The wide application of antibiotics by humans has led to large-scale dissemination of bacteria resistant to antibiotics in water basins (Schwartz *et al.,* 2003; Dang *et al,*. 2006). For antibiotic resistance to develop, it is necessary that two key elements combine: the presence of an antibiotic capable of inhabiting the majority of bacteria present in a colony and heterogeneous colony of bacteria where at least one of this bacterium carries the genetic determinant capable of expressing resistance to the antibiotic (Levy and Marshall, 2004). Resistance to antibiotics can be natural or acquired and can be transmitted horizontally or vertically. Whereas the natural form of antibiotic resistance is caused by a spontaneous gene mutation in the lack of selective pressure due to presence of antibiotics and is far much less common than the acquired one, it can also play a role in the development of resistance (Alanis, 2005). Sagana fish farm practices integrated fish farming which involves confined units with little exchange of water. Manure from livestock production is administered to fish ponds. It has been shown that such practice supports the growth of mainly photosynthetic organisms (Little and Edwards**,** 1999). This integrated fish farming system produces high yields with low input, with the fish receiving limited, if any, supplementary feed. In contrast, the livestock on the integrated farms, which includes chickens, is reared intensively, and antimicrobial agents are used for prophylactic and therapeutic treatment. Within integrated fish farming systems, antimicrobials, their residues, and antimicrobial-resistant bacteria may enter the fish ponds through

animal manure and/or excess feeding and are potential sources of antimicrobial-resistant bacteria.

Antimicrobial resistance in traditional fish farming systems in temperate waters has been intensively studied (Alderman and Hastings, 1998). A high incidence of bacteria resistant to the antimicrobials used in aquaculture, including multiple resistant bacteria, has been found in fish farms and the surrounding aquatic environments. Residues of antimicrobials have been found in the sediments of marine fish farms (Jacobsen and Berglind, 1988; Björklund *et al.*, 1990). Accumulation of surplus antimicrobials and antimicrobial residues may occur in integrated fish farms when the ponds are only rarely emptied at the time of fish harvest. Such a build up could establish selective pressure favoring selection and growth of antimicrobial-resistant bacteria. Although increased levels of antimicrobial resistance in and around fish farms may only occur transiently, there is a potential risk that antimicrobial resistance genes could be disseminated into a wide range of aquatic environmental bacteria. However, resistance to one antimicrobial within a class of antimicrobials often confers resistance to other members of the same group (cross-resistance). Potential transfer of resistant bacteria and resistance genes from aquaculture environments to humans may occur through direct consumption of antimicrobial-resistant bacteria present in fish and associated products.

2.5 Histamine fish poisoning

Histamine poisoning is one of the most significant causes of illness associated with seafood. It is formed in the fish post mortem by bacteria decarboxylation of the amino acid histidine. The histamine producing bacteria are certain *Enterobacteriaceae,* some *Vibrio* spp few *Clostridium* and *Lactobacillus* sp. The most potent are *Morganella morganii, Klebsiella*

pneumoniae and *Hqfhia alvei* (Stratten and Taylor, 1991). Some are present in the normal micro flora of live fish others are derived from post-catching contamination on fishing vessels, at processing plant or in the distribution system. Once produced in the fish the risk of provoking disease is very high since it is heat resistance. Histamine formation can be prevented by rapid cooling of fish after catching and adequate refrigeration during handling and storage. A total of 22 cases of histamine fish poisoning after the consumption of tuna burgers, tuna salad and fillets were reported in North Carolina from 1998 to 1999 (Barker *et al*., 1974). However, the human body will tolerate a certain amount of histamine without any reaction. The ingested histamine will be detoxified in the intestinal tract by at least 2 enzymes, the diamine oxidase (DAO) and Histamine N-methyltransferase (HMT) (Taylor, 1986). This mechanism can be prevented if the intake of histamine and other biogenic amines such as cadaverine and putrescine is very high. However, the impact of these infections on children, the aging population and the imuno-compromised, cannot be under estimated. The ease of world shipment of fresh and frozen food and the development of new food industries including aquaculture only compounds the problem (Todd, 1997).

2.6 Factors contributing to fish contamination

It is well known that microbiological activity is greatly influenced by temperature changes. In the temperature range from 0°C to 25° C microbiological activity is of great importance. Many bacteria are unable to grow at temperatures below 10°C and even psychrotrophic organisms grow very slowly and sometimes with extended lag phases. When temperatures approach 0° C the growth rate is less than one tenth of the rate at the optimum growth temperature (FAO, 1995). The micro flora responsible for spoilage of fresh fish change with changes in storage temperature. At low temperatures (0°-5°C), *Schewanella putrefaciens, Photobacterium phosporeum, Aeromonas spp* and *Pseudomonas spp* cause

spoilage (Huss, 1994). At high storage temperatures (15° -30°C) different species of *Vibrionaceae, Enterobacteriaceae* and Gram- positive organisms are responsible for spoilage (Gram *et al*., 1990).

The ponds and rivers that harbour the fish may be the source of contaminants due to indiscriminate deposition of human, animal excreta and other environmental wastes into natural water, land and during the rainy season especially, as the faecal matter from various sources are washed from contaminated land into different water bodies. Free roaming animals and pets especially dogs also contribute to faecal contamination of surface water. Run-off from roads, parking lots and yards can carry animal wastes into natural water course and ponds. Birds can also be a significant source of bacteria. Swans; Geese and other water fowl can all elevate bacteria counts in water bodies and ponds (Doyle and Ericson, 2006).

Another factor that affects the microbial load on fish is the accumulation of waste feeds in ponds which stimulates the growth of bacteria including human pathogens which can contaminate products and lead to food-borne diseases. The use of artificial feeds supplemented with antibiotics in the feeds could lead to residues remaining in the fish which in turn will lead to the development of antibiotic-resistant bacteria in the food chain (Doyle and Ericson, 2006). The quality and storage life of many fish decreases once the fish is gutted because it exposes the fish belly and cut surfaces to the air rendering them most susceptible to oxidation and decolourization. Oxidation takes place in the lipid fraction of the fish and involves oxygen and the unsaturated lipid. It is formation of hydrogen peroxides which is degraded to form aldehydes and ketones. It is initiated and accelerated by heat, light and several organic and inorganic substances (Doyle and Ericson, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was carried out at Masinga Dam and Sagana Aquaculture Centre situated on Tana –river and Kirinyaga districts respectively. Masinga dam (Figure 3.1) is situated on Tana River, the main catches are Tilapia and Catfish. Dam fisheries account for about the Kshs. 34million per year with most consumption being in Nairobi City. Sagana Aquaculture Centre,(Figure 3.1) is a Department of Fisheries breeding farm in Sagana town, Kirinyaga District (100 km North East of Nairobi, altitude 1230 m, latitude 0°39'S and longitude 37°12'E). It covers an area of approximately 50 hectares of which 18 hectares are covered by ponds; on average each pond covers an area of about 40 by20M^2 with a depth of 1meter. Water is diverted from river Ragati and delivered by gravity through a canal. It is one of the two main national fish hatcheries of the fisheries department and acts as a training centre for fish farmers in aquaculture.

The farm is involved in the culture of Nile Tilapia and African Catfish as the main species among others. The farm provides quality fish feeds to farmers and demonstrates economic viability of integrated fish farming and it also conducts research. Fish farming is carried out on still- water earthen ponds under semi intensive systems. The ponds are fertilized using artificial fertilizers and organic manure from cattle, chicken and kitchen wastes to enhance growth for the phytoplankton. The other alternative feeds used are rice bran, wheat germ, maize bran. The experimental period started during the dry season in August 2007 and continued to the rainy season of December 2007.

Figure 3.1: Map of Kenya showing Sagana fish farm and Masinga dam

3.2 Sample size

Sample size was determined using the ICMSF sampling standard which relates the stringency of the sampling plan to the degree of hazard of the food. A 3-class plan is used when the health hazard is low. In this plan n = 5 and c= 3, n is the number of samples drawn and c is the maximum allowable number of positive results.

3.3 Study design

The study design adopted was purposive study.

3.4 Sampling procedure

3.4.1 Collection of fish samples

Fish was sampled from 20 organically fertilized ponds at the Sagana fish farm the ponds were selected at interval of 4 ponds and 5 pieces of table size Tilapia were harvested using a scoop net and taken to the laboratory in a cool box. Once in the laboratory; the fish were aseptically skinned to get the skin sample. Up to 25g of fish skin was weighed and mixed with 225ml of buffered peptone water in a sterile blender. It was blend and a portion of the pure mix inoculated into the MacConkey agar culture media. In order to have distinguishable colonies, diluted subcultures were made from the initial culture after overnight incubation at 37 ^{0} C at dilutions of 1:10, 1:100 and 1: 1000 using peptone water and incubated overnight 37^{0}C. Growth of distinct colonies was achieved at the 1: 100 dilutions. The colonies were sub-cultured in different selective media Hektoen enteric (HE) agar, xylose-lysine deoxycholate (XLD) agar and *Salmonella-Shigella* (SS) agar and incubated. The colonies were morphologically identified and then subjected to biochemical tests for further identification. Final identification was done using API 20E method.

For the gut sample the fish was cleaned using 70% alcohol and an incision was made over peritoneal cavity and the fish dissected to get the gut contents. The gut contents were combined and weighed as was done for the skin and the same procedure was followed and again growth of distinct colonies was at 1: 100 dilutions. The same procedure was carried out for the dam fish during the two seasons.

3.4.2 Collection of Water samples

Water was collected from a depth of about 20cm beneath the surface in sterile bottles of 100ml at one end of the pond and at the center of the pond. The water sample was enriched using peptone water and incubated at 37^0C overnight this was inoculated to MacConkey culture media and incubated at 37^0C overnight. Growth of distinct colonies was obtained at the 1: 10 dilution. They were sub cultured in selective media and incubated. The colonies were morphologically identified and further subjected to biochemical tests for further identification. The final identification was by use of API 20E method.

Dam water was collected in the early hours of morning and taken to the laboratory within 2 hours. The sample was collected using sterile bottles away from the bank at a depth of one foot below the surface and the mouth directed towards the current and then it was filled and covered immediately. It was stored in a cooler box during transportation. The same procedure used for the pond water was applied to the dam water.

3.4.3 Collection of water sediments

Sediments samples were collected from the bottom of the pond and dam using a Ekman grab in all the ponds. The sediments were collected at the edge and at the center of the ponds. The sediments were then taken to the Laboratory where they were mixed and enriched using peptone water cultured in MacConkey and incubated at 37 ^{0}C overnight. The colonies were sub-cultured in selective media. The colonies were morphologically identified and then subjected to the biochemical tests. Final identification was done using API 20E method.

3.5 The Analytical Profile Index (API 20E)

All test chambers were rehydrated by inoculation with a saline suspension of a pure culture of the bacterial strain subjected to identification. After incubation in a humidity chamber for 18 to 24 hours at 37°C, the color reactions were read. The results of the test reactions were converted to a seven-digit code. The code was then looked up in the database book for the genus and species identification of the test microorganism.

3.6 Antibiotic susceptibility testing

3.6.1 Antibiotic sensitivity profile

The antibiotic sensitivity testing was performed on all the isolates against commonly used antimicrobial agents' (Table 3.1). Kirby – Bauer disk diffusion test was used as per the recommended standard of the National Committee for Clinical Laboratory Standards (NCCLS, 2009). After incubation at 37^0C for 24 hours the diameters of zones of inhibition were measured and compared with control organism *E. coli* the ATCC 25922.

Table 3.1: Antimicrobial agents used for sensitivity testing

No.	Antimicrobial agent	Concentration
1	Ampicillin (Amp)	10µg/ml
2	Chlorampenicol (C)	30µg/ml
3	Streptomycin (S)	10µg/ml
4	Tetracycline (Te)	30µg/ml
5	Nalidixic acid (Na)	30µg/ml
6	Ciprofloxacin (Cip)	5µg/ml
7	Gentamycin (Gen)	10µg/ml
8	Cefuroxine (Cxm)	30µg/ml
9	Amoxicillin (Aml)	5µg/ml
10	Cefotaxine (CTX)	30µg/ml

CHAPTER FOUR

RESULTS

4.1 Bacteria isolates per specimen type

A total of 91 bacteria were isolated from six specimen types namely; Tilapia Gut, Tilapia skin, Catfish skin, Catfish Gut, water and water sediments. Tilapia gut had the highest proportion (20; 22%) of bacteria isolates compared to the rest. Tilapia skin and catfish skin each had 16 (17.6%) of bacteria isolated. Catfish gut and water had the same number of bacteria isolates 15 (16.5%). Water sediments had the lowest proportion (9; 9.9%) of bacteria isolated compared to the rest of specimens (Figure 4.1).

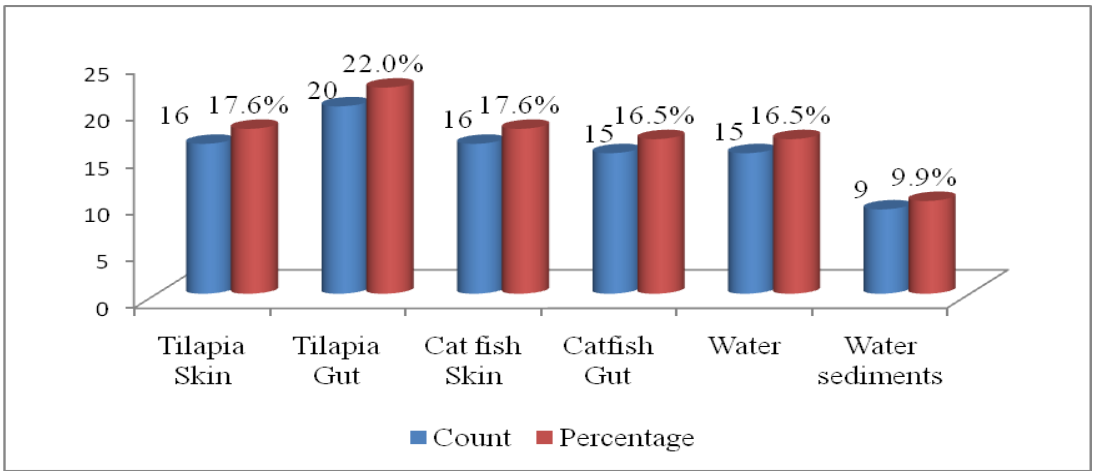

Figure 4.1: Bacteria isolates from various collected samples

4.1.2 Types of bacteria isolates from Tilapia, Catfish, Water, Water sediments.

A total number of 91 (100%) bacterial isolates were indentified of which *Citrobacter freundii* were 16 (17.6%) compared to the rest of bacteria isolated. *Citrobacter freundii* and *E.coli* are the only bacteria that occurred in five specimen type except in water sediments and Tilapia skin respectively (Table 4.1).

In tilapia skin the bacterial isolates were 16 (17.6%) same as the bacterial isolates in Catfish skin. The bacteria isolates that occurred both in Tilapia Skin and Catfish skin were: *Aeromonas sobia, Citrobacter freundii, E.coli, Edwardsiella tarda, Enterobacter cloacae, Enterobacter sakazakii, Proteus mirabilis* and *Pseudomonas aeruginosa* (Table 4.1).

In Tilapia Gut a total number of 20 (22.0%) bacterial isolates were identified. On the other hand, 15 (16.5%) of bacteria were isolated from Catfish Gut. Bacterial isolates that occurred in both Tilapia gut and Catfish skin were; *Aeromonas sobia, Citrobacter freundii, E.coli, Edwardsiella tarda, Enterobacter sakazakii,* .and *Pseudomonas fluorescenes* (Table 4.1)

In water, 15 (16.5%) bacteria were isolated compared 9 (9.9%) bacterial isolates from water sediments. Bacterial isolates that occurred both in water and water sediments were; *E.coli, Enterobacter fergusonii, Pseudomonas fluorescenes* and *Salmonellaspp*.(.(Table4.1)

Table 4.1: Bacteria isolates in Tilapia, Catfish, Water and Water sediments

Isolates	**Tilapia Skin**	**Catfish Skin**	**Tilapia Gut**	**Catfish Gut**	**Water**	**Water sediments**	**Total**
Acinetobacter spp	-	-	-	-	1	-	**1 (1.1%)**
Aeromonas sobia	2	2	1	1	-	-	**6 (6.6%)**
Chromobacterium violaceum	-	-	1	-	-	-	**1 (1.1%)**
Citrobacter freundii	4	2	5	3	2	-	**16 (17.6%)**
E.coli	-	1	2	2	5	4	**14 (15.4%)**
Edwardsiella tarda	1	1	2	2	-	-	**6 (6.6%)**
Enterobacter agglomerans	-	1	-	-	-	1	**2 (2.2%)**
Enterobacter amnigenus	-	-	1	-	-	-	**1 (1.1%)**
Enterobacter cloacae	1	1	-	-	1	-	**3 (3.3%)**
Enterobacter fergusonii	-	-	-	-	1	1	**2 (2.2%)**
Enterobacter sakazakii	1	1	1	3	-	-	**6 (6.6%)**
Klebsiella onithnolytica	1	-	-	-	-	-	**1 (1.1%)**
Klebsiella pneumonia	1	-	1	-	-	-	**2 (2.2%)**
Plesiomonas shigelloides	-	-	-	-	1	-	**1 (1.1%)**
Proteus mirabilis	1	1	-	-	-	1	**3(3.3%)**
Providentia stuartii	-	3	-	2	-	-	**5 (5.5%)**
Pseudomonas aeruginosa	2	3	3	-	1	-	**9 (9.9%)**
Pseudomonas fluorescenes	-	-	1	1	1	1	**4 (4.4%)**
Salmonella spp	1	-	1	-	1	1	**4 (4.4%)**
Shigella boydii	-	-	-	1	-	-	**1 (1.1%)**
Vibrio mechnikovii	1	-	-	-	1	-	**2 (2.2%)**
Vibrio vulnificus	-	-	1	-	-	-	**1 (1.1%)**
Total	**16 (17.6%)**	**16 (17.6%)**	**20 (22.0%)**	**15 (16.5%)**	**15 (16.5%)**	**9 (9.9%)**	**91 (100.0%)**

4.2 Types of bacteria isolates from Sagana pond and Masinga dam

The bacteria isolates from sagan ponds were 54 (59.3%) while isolates from Masinga dam were 37 (40.7%) *Citrobacter freundii* were 16 (17.6%) of which 10 (11.0%) were isolated from Sagana pond while 6 (6.6%) were isolated from Masinga dam. In all types of bacteria isolated, *Aeromonas sobia, Citrobacter freundii, Ecoli, Edwardsiella tarda, Proteus mirabilis, Pseudomonas aeruginosa* and *Pseudomonas fluorescenes* occurred in both Sagana pond and Masinga dam. (Table 4.2)

Table 4.2: Types of bacteria isolated from Sagana pond and Masinga dam

Bacteria isolated	**Sagana Pond**	**Masinga dam**	**Sagana Pond**	**Masinga dam**	**Total**
			n (%)	**n (%)**	**n (%)**
Acinetobacter spp	+	-	1 (1.1)	0 (0.0)	1 (1.1)
*Aeromonas sobia**	+	+	4 (4.4)	2 (2.2)	6 (6.6)
Chromobacterium violaceum	+	-	1(1.1)	0 (0.0)	1 (1.1)
*Citrobacter freundii**	+	+	10 (11.0)	6 (6.6)	16 (17.6)
*Ecoli**	+	+	6 (6.6)	8 (8.8)	14 (15.4)
*Edwardsiella tarda**	+	+	4 (4.4)	2 (2.2)	6 (6.6)
Enterobacter aggromerans	-	+	0 (0.0)	2 (2.2)	2 (2.2)
Enterobacter amnigenus	+	-	1(1.1)	0 (0.0)	1 (1.1)
Enterobacter fergusoni	+	-	2 (2.2)	0 (0.0)	2 (2.2)
Enterobacter sakazakii	-	+	0 (0.0)	6 (6.6)	6 (6.6)
Enterobacter cloace	-	+	0 (0.0)	3 (3.3)	3 (3.3)
Klebsiella onithnolytica	+	-	1 (1.1)	0 (0.0)	1 (1.1)
Klebsiella pneumonia	+	-	2 (2.2)	0 (0.0)	2 (2.2)
Plesiomonas shigelloides	-	+	0 (0.0)	1 (1.1)	1 (1.1)
*Proteus mirabilis**	+	+	1 (1.1)	2 (2.2)	3 (3.3)
Providencia stuartii	+	-	5 (5.5)	0 (0.0)	5 (5.5)
*Pseudomonas aeruginosa**	+	+	7 (7.7)	2 (2.2)	9 (9.9)
*Pseudomonas fluorescenes**	+	+	2 (2.2)	2 (2.2)	4 (4.4)
Salmonella spp	+	-	4 (4.4)	0 (0.0)	4 (4.4)
Shigella boydii	+	-	1 (1.1)	0 (0.0)	1 (1.1)
Vibrio mechnikovii	+	-	2 (2.2)	0 (0.0)	2 (2.2)
Vibrio vulnificus	-	+	0 (0.0)	1 (1.1)	1 (1.1)
Total			**54 (59.3)**	**37 (40.7)**	**91(100)**

+ /- Species occurred/ did not occur, * Bacteria isolated both in Sagana and Masinga dam

4.2.1 Bacteria isolates from Sagana pond and Masinga dam by specimen type

Tilapia gut had a total of 20 (22.0%) bacteria isolates of which 12 (13.2%) were from Sagana ponds and 8 (8.8%) bacteria isolates were from Masinga. Water sediments had the lowest proportion (9; 9.9%) of bacteria isolates both from Sagana ponds (4; 4.4%) and Masinga dam (5; 5.5%) respectively. When the results were subjected to chi-square, there was no significant difference of bacteria flora species isolated between pond and dam water χ^2 =3.853, *df=5,* =0.571) (Table 4.3).

Table 4.3: Number of bacteria isolated from Sagana farm and Masinga dam

Specimen type	Sagana Farm n (%)	Masinga dam n (%)	Total n (%)	χ^2	*Df*	**P-Value**
Tilapia Skin	12 (23.2%)	4 (4.4%)	16 (17.6%)	4.00	1	0.050
Tilapia Gut	12 (23.2%)	8 (8.8%)	20 (22.0%)	0.800	1	0.371
Cat fish Skin	9 (9.9%)	7 (7.7%)	16 (17.6%)	0.250	1	0.617
Catfish Gut	7 (7.7%)	8 (8.8%)	15(16.5%)	0.670	1	0.796
Water	10 (11.0%)	5 (5.5%)	15(16.5%)	1.667	1	0.197
Water sediments	4 (4.4%)	5 (5.5%)	9(9.9%)	0.111	1	0.739
Total	**54 (59.3%)**	**37 (40.7%)**	**91(100%)**	**3.853**	**5**	**0.571**

*Significant at 0.05

4.3 Bacteria isolated from Tilapia, Catfish, Water and Water sediments in dry and wet seasons

Bacteria isolates from tilapia gut were (20; 22%) of which 11 (12.1%) were isolated during dry seasons while 9 (9.9%) of bacteria isolated during wet season. There were more bacteria isolates during dry season as compared to wet season in all specimen types except tilapia skin specimen where (6; 6.6%) bacteria were isolated in dry and

10 (11.0%) in wet season respectively. The reason adduced for higher number of bacteria isolates during dry season compared to wet season according to Wemedo (2002) points out that during the wet seasons, lower temperatures inhibited microbial activity. Another reason given attributed to this phenomenon is that saturation of the soil by rain, limits activity by reducing aeration (Marshall and Devinny, 1998). (Figure 4.2)

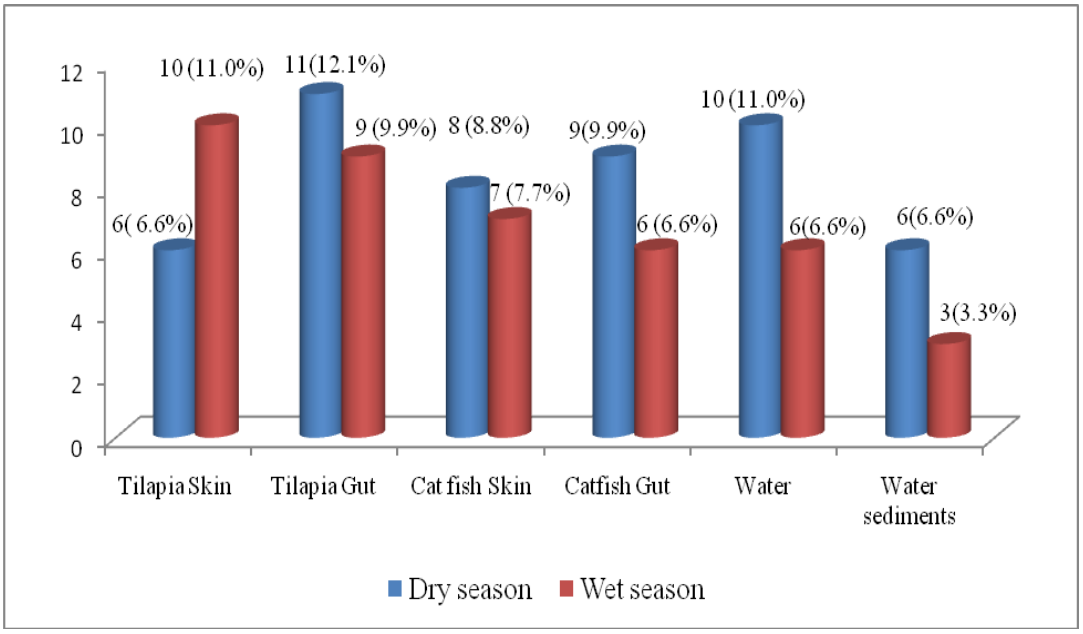

Figure 4.2: Bacteria isolates in dry and wet season by specimen type

4.3.1 Types of bacteria isolated from the dry and wet season

During the dry season 50 (54.9%) bacteria were isolated while 41 (45.1%) bacteria were isolated during the wet season. The number of *Citrobacter freundii* isolated from both dry and wet season were 16 (17.6%) of which 9 (9.9%) were isolated during dry season while 7 (7.7%) isolated during wet season. *Citrobacter freundii, Aeromonas sobia, Acinetobacter spp, Edwardsiella tarda, E.coli, Pseudomonas aeuroginosa, Enterobacter cloacae, Enterobacter sakazakii* and *Providencia stuartii* were isolated in both seasons (Table 4.4).

Table 4.4: Bacteria isolates from dry and wet season

Bacteria isolates	**Dry Season**	**Wet Season**	**Dry Season n (%)**	**Wet Season n (%)**	**Total n (%)**
*Acinetobacter spp**	+	+	1 (1.1)	1 (1.1)	1 (1.1)
*Aeromonas sobia**	+	+	4 (4.4)	2 (2.2)	6 (6.6)
Chromobacterium violaceum	-	+	0 (0.0)	1 (1.1)	1 (1.1)
*Citrobacter freundii**	+	+	9 (9.9)	7 (7.7)	16 (17.6)
*Ecoli**	+	+	6 (6.6)	8 (8.8)	14 (15.4)
*Edwardsiella tarda**	+	+	3 (3.3)	3 (3.3)	6 (6.6)
Enterobacter aggromerans	+	-	2 (2.2)	0 (0.0)	2 (2.2)
Enterobacter amnigenus	+	-	1 (1.1)	0 (0.0)	1 (1.1)
Enterobacter fergusoni	+	-	2 (2.2)	0 (0.0)	2 (2.2)
*Enterobacter sakazakii**	+	+	4 (4.4)	2 (2.2)	6 (6.6)
*Enterobacter cloace**	+	+	2 (2.2)	1 (1.1)	3 (3.3)
Klebsiella onithnolytica	-	+	0 (0.0)	1 (1.1)	1 (1.1)
Klebsiella pneumonia	-	+	0 (0.0)	2 (2.2)	2 (2.2)
Plesiomonas shigelloides	+	-	1 (1.1)	0 (0.0)	1 (1.1)
Proteus mirabilis	-	+	0 (0.0)	3 (3.3)	3 (3.3)
*Providencia stuartii**	+	+	3 (3.3)	2 (2.2)	5 (5.5)
*Pseudomonas aeruginosa**	+	+	4 (4.4)	5 (5.5)	9 (9.9)
Pseudomonas fluorescenes	-	+	0 (0.0)	4 (4.4)	4 (4.4)
Salmonella spp	+	-	4 (4.4)	0 (0.0)	4 (4.4)
Shigella boydii	+	-	1(1.1)	0 (0.0)	1(1.1)
Vibrio mechnikovii	+	-	2 (2.2)	0 (0.0)	2 (2.2)
Vibrio vulnificus	+	-	1(1.1)	0 (0.0)	1(1.1)
Total			**50 (54.9)**	**41 (45.1)**	**91(100)**

+ Species occurred, - Species did not occur, * Bacteria isolated in both dry and wet season

4.3.2 Bacteria isolates during wet and dry season by specimen type

All bacterial isolates were 91 with tilapia fish gut had 20 (22.0%) of which 6 (6.6%) were during dry season while 10 (11.0%) were during wet season. There was no significance difference between the number of bacteria isolated from Tilapia fish gut and the two seasons ($p>0.05$). This was same to other specimen types in spite of more bacteria isolates during the dry season (Table 4.5) including Tilapia fish skin which had 6 (6.6%) and 10 (11.0%) of bacteria isolated from both dry and wet seasons respectively, There was no significance difference to show any association

of bacteria isolates and the source (χ^2 =3.006, *df*=5, P=0.699).

Table 4.5: Bacteria isolates in specimen type by season

Specimen type	Dry Season	Wet Season	Total	χ^2	*df*	P-value
Tilapia Skin	6 (6.6%)	10 (11.0%)	16(17.6%)	1.000	1	0.317
Tilapia Gut	11(12.1%)	9(9.9%)	20(22.0%)	0.200	1	0.655
Cat fish Skin	8 (8.8%)	7(7.7%)	15(16.5%)	0.067	1	0.796
Cat fish Gut	9 (9.9%)	6(6.6%)	15(16.5%)	0.600	1	0.439
Water	10 (11.0%)	6(6.6%)	16(17.6%)	1.000	1	0.317
Water Sediments	6 (6.6%)	3(3.3%)	9(9.9%)	1.000	1	0.317
Total	**50 (54.9%)**	**41(45.05%)**	**91(100%)**	**3.006**	**5**	**0.699**

4.4 Correlation between Bacteria isolates from Specimen types, Site and Season

There was a strong positive correlation between bacteria isolated during dry and wet season from the specimen and the site (Ponds and Dams). The more bacteria isolates from the two sites, the higher significance difference between the bacteria isolated during dry season and wet season (r=0.734, P=0.000) (Table 4.6). Pearson's correlation did not indicate any significant correlation between the bacteria isolated from specimen and the site. Correlation between the two variables was weak and negatively correlated (r=0.734, P=0.000) (Table 4.6). On the other hand, there was a weak positive correlation between the bacteria isolated from specimen types and the site (Sagana ponds and Masinga dam) (r=0.136, P=0.197) (Table 4.6).

Table 4.6: correlation between specimen, site and season

		Season	**Site**
Site	Pearson Correlation	0.734**	1
	Sig. (2-tailed)	**0.000**	
	N	91	91
Specimen	Pearson Correlation	-0.162	0.136
	Sig. (2-tailed)	0.124	0.197
	N		91

**. Correlation is significant at the 0.01 level (2-tailed).

4.5 Overall antibacterial response of isolates

All bacteria isolates were examined for susceptibility to commonly used antimicrobial agents. The zones of inhibition were read after incubation, compared against a standard measurement and recorded as resistant, intermediate or sensitive. CIP antibiotic was susceptible to all (100%) bacterial isolates while on the other hand none of the bacterial isolates that registered resistant to CXT, GEN and CIP antibiotics. The drug with the highest resistance was AML with 60 (65.9%) of bacteria isolated registering resistance followed by AMP at 56 (61.5%) bacterial isolates. On average 64 (70.3%) of bacteria isolated registered susceptibility, 18 (20%) registered resistance to drugs while 9 (9.6%) registered intermediate. (Table 4.7and Figure 4.3).

Table 4.7: Antimicrobial Response of Isolates to various Antibiotics

	Resistant		**Sensitive**		**Intermediate**		**Total**	
Antibiotic	**N**	**%**	**n**	**%**	**n**	**%**	**N**	**%**
AML	60	65.9	27	29.6	4	5.5	**91**	**100**
CXT	0	0	83	91.2	8	7.14	**91**	**100**
CIP	0	0	91	100	0	0	**91**	**100**
S	4	4.4	69	75.8	18	19.7	**91**	**100**
AMP	56	61.5	29	31.8	6	6.6	**91**	**100**
GEN	0	0	90	98.9	1	1.1	**91**	**100**
TE	29	31.8	40	43.9	22	24.2	**91**	**100**
C	25	27.5	47	51.6	19	20.9	**91**	**100**
NA	4	4.4	78	85.7	9	9.8	**91**	**100**
CXM	4	4.4	86	94.5	1	1.1	**91**	**100**
Average	**18**	**20.0**	**64.0**	**70.3**	**9**	**9.6**	**91**	**100**

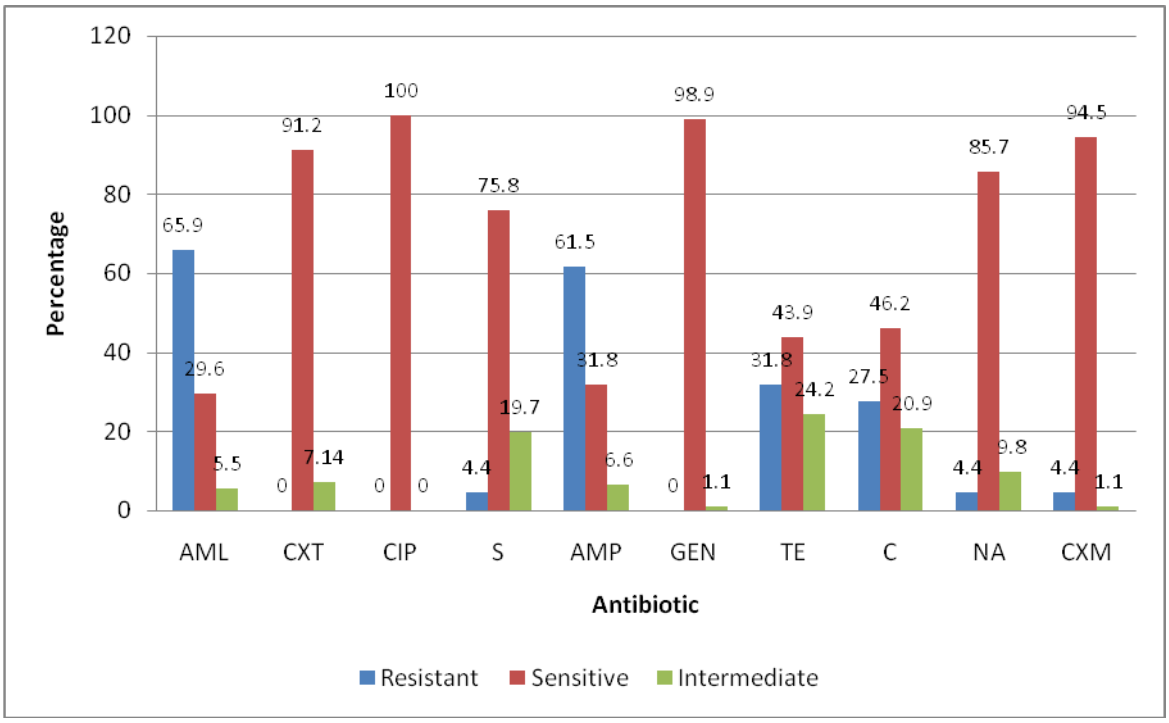

Figure 4.3: Percentage distribution of antimicrobial response of isolates to various antibiotics

4.6 Antibacterial response of isolates from Sagana Ponds

The total isolates from the pond were 54 of which 38 (70.4%) registered resistance to 7 antimicrobial agents. Bacterial isolates from Sagana registered high resistance to AML and AMP drugs, 38 (70%) and 36 (67%) respectively. The rest of isolates resistance registered to antibiotics were as follows; 16 (30%) were resistant to TE, 15 (28%) to C and 5 (10%) to NA and S respectively. There was no resistance registered to CXT, CIP and GEN for bacterial isolates from Sagana ponds. (Figure 4.4)

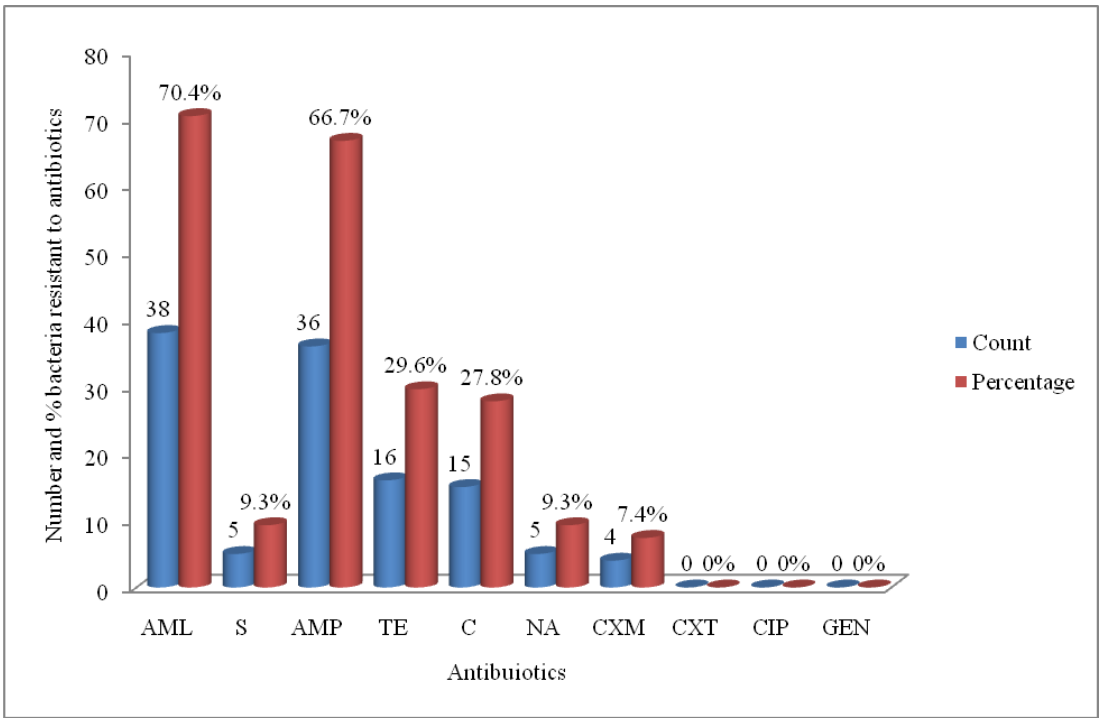

Figure 4.4: Number of Isolates resistant to antibiotics from Sagana

In regards to bacteria types isolated from Sagana pond, *Salmonella spp* showed resistance to AML, AMP and CXM. Most of bacterial isolates from Sagana ponds showed resistance to an average of 2 to 3 drugs with an exception of *klebsiella pneumonia* which registered resistance to four antibiotics namely; AML, S, AMP, TE and C. There was variation in antibacterial response of isolates from Sagana pond (F=8.4, P=0.000) (Table 4.6).

Table 4.6 Antimicrobial Response of Isolates from Sagana

Isolates	No. of isolates	AML			CTX			CIP			S			AMP			GEN			TE			C			NA			CXM		
		R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I
Citrobacter freundii	**10**	9	1	0	0	10	0	0	10	0	0	5	5	9	1	0	0	10	0	2	4	4	3	4	3	0	10	0	0	10	0
Aeromonas sobia	**4**	4	3	0	0	7	0	0	7	0	0	6	1	6	1	0	0	7	0	0	7	0	0	7	0	0	7	0	0	7	0
Vibrio mechnikovii	**2**	2	0	0	0	2	0	0	2	0	0	1	1	2	0	0	0	2	0	0	2	0	1	1	0	0	2	0	0	2	0
Salmonella spp	**4**	1	1	0	0	2	0	0	2	0	0	2	0	2	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0
Edwardsiella tarda	**4**	0	1	1	0	2	0	0	2	0	0	2	0	0	1	1	0	2	0	1	1	0	1	1	0	0	2	0	0	2	0
Shigella boydii	**1**	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0
Ecoli	**6**	0	5	1	0	6	0	0	6	0	0	6	0	0	4	1	0	6	0	3	2	1	2	2	2	0	4	2	0	6	0
Enterobacter amnigenus	**1**	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0
Enterobacter fergusonii	**2**	1	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0
Acinetobacter spp	**1**	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	1	1	0	0	1	0	0	1	0
Pseudomonas aeruginosa	**7**	7	0	0	0	6	1	0	7	0	1	4	2	5	1	1	0	6	1	3	0	4	5	0	2	4	3	0	3	4	0
Klebsiella onithnolytica	**1**	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	1	0
Chromobacterium violaceum	**1**	0	1	0	0	0	1	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	1
Proteus mirabilis	**1**	2	0	0	0	2	0	0	2	0	0	2	0	1	0	1	0	2	0	0	1	1	1	1	0	0	2	0	0	2	0
Pseudomonas Flurosecenes	**2**	1	0	0	0	2	0	0	2	0	0	1	2	0	2	0	0	0	2	0	0	2	0	0	1	1	0	2	0	1	1
Klebsiella pneumonia	**2**	2	2	0	0	4	0	0	4	0	1	3	0	2	2	0	0	4	0	2	1	1	1	3	0	0	4	0	0	4	0
Providencia stuartii	**5**	3	0	0	0	3	0	0	3	0	0	3	0	3	0	0	0	3	0	1	2	0	1	2	0	0	3	0	0	3	0
Total	**54**	**34**	**15**	**2**	**0**	**52**	**2**	**0**	**54**	**0**	**2**	**40**	**11**	**34**	**16**	**4**	**0**	**51**	**2**	**14**	**26**	**14**	**16**	**26**	**9**	**6**	**44**	**5**	**3**	**48**	**2**
%	**100**	**63**	**28**	**4**	**0**	**97**	**4**	**0**	**100**	**0**	**4**	**74**	**20**	**63**	**30**	**7**	**0**	**94**	**4**	**26**	**48**	**26**	**30**	**48**	**17**	**11**	**82**	**9**	**6**	**89**	**4**
F=8.4, df=127, P-value=0.000																															

4.7 Antibacterial Response of Isolates from Masinga

Bacterial isolates from Masinga dam registered resistance to four antibiotics namely; AML, AMP, TE and C. There were 37 isolates of which high resistance was registered in AML where 22 (60%) of isolates displayed resistance followed 20 (54%) of bacterial isolates displayed resistance to AMP. The rest was 13 (35%) displayed resistance to Te and 10 (27%) displayed resistance to C (Figure 4.5).

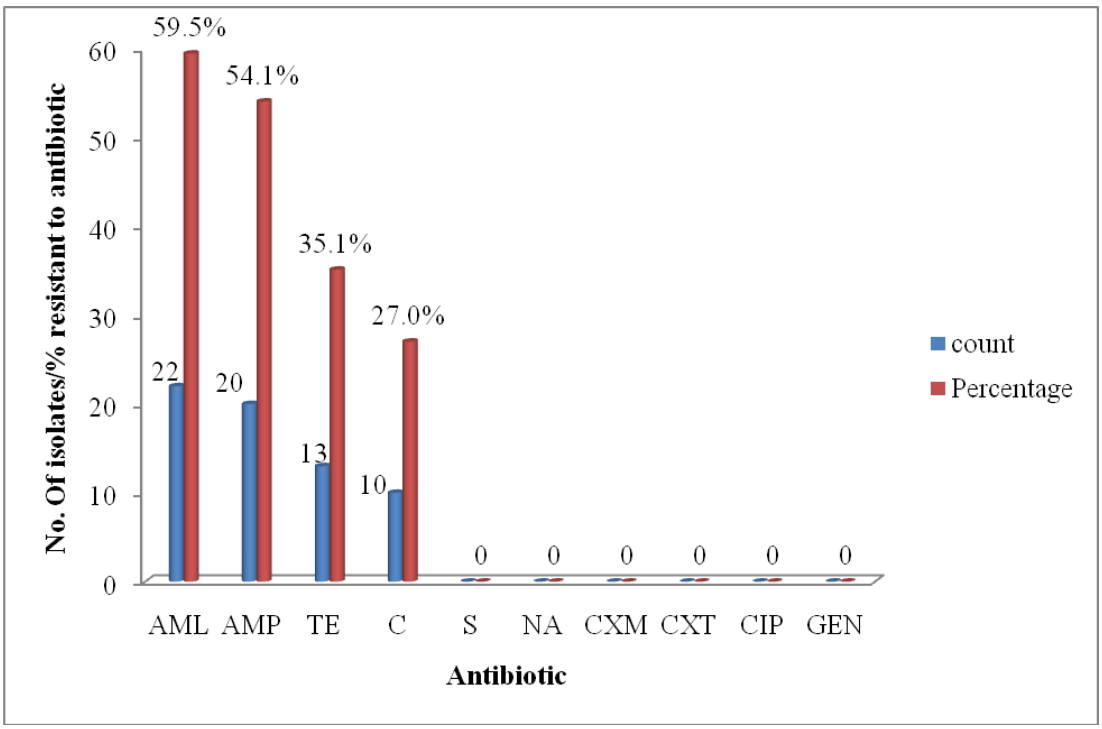

Figure 4.5: Number of bacterial isolates from Masinga dam resistant to antibiotics.

The bacterial isolates from Masinga dam registered resistance to at least one antibiotic *E. coli* registered resistance to AML, AMP and TE while *Pleisiomonas shigelloides* registered resistance to AML and AMP. Like the isolates from the Sagana, the antibacterial response of isolates from dam was more resistant to AML and AMP compared to the rest of antibiotics. However, for bacterial isolates from Masinga dam, there was no significance difference for antibacterial response from dam ($F=1.84$, $P=0.14$). (Table 4.7).

Table 4.7 Antibacterial response of isolates from Masinga dam

Isolates	No	AML			CTX			CIP			S			AMP			GEN			TE			C			NA			CXM		
		R	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**
Citrobacter freundii	**6**	5	1	0	0	6	0	0	6	0	0	3	3	5	1	0	0	6	0	1	3	2	3	3	0	0	6	0	0	6	0
Aeromonas sobia	**2**	1	0	0	0	2	0	0	2	0	0	2	0	1	1	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0
Vibrio vulnificus	**1**	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0
Edwardsiella tarda	**2**	0	1	1	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	2	0	0	0	2	0	0	2	0	0	2	0
Ecoli	**8**	2	6	0	0	6	2	0	5	3	0	6	2	2	3	3	0	6	2	2	4	2	0	4	4	0	4	4	0	8	0
Enterobacter sakazakazii	**6**	3	3	0	0	6	0	0	3	3	0	6	0	2	4	0	0	3	3	2	3	1	1	4	1	0	4	2	0	4	2
Enterobacter cloace	**3**	1	2	0	0	3	0	0	3	0	0	3	0	2	1	0	0	3	0	1	2	0	1	2	0	0	3	0	0	3	0
Pseudomonas fluorescenes	**2**	2	0	0	0	2	1	0	2	0	0	2	0	3	0	1	0	6	1	3	0	4	4	1	2	0	3	4	0	4	0
Proteus mirabilis	**2**	2	2	0	0	4	0	0	4	0	0	4	4	0	0	0	0	4	0	2	2	1	1	3	0	0	4	0	0	4	0
Enterobacter aggromerans	**2**	2	0	0	0	1	1	0	2	0	0	1	1	2	0	0	0	1	1	0	2	0	0	1	1	0	2	0	0	1	1
Plesiomonas shigelloides	**2**	2	1	0	0	2	0	0	1	1	0	1	1	2	0	0	0	1	1	0	2	0	0	1	1	0	2	0	0	2	0
Pseudomonas aureginosa	**1**	1	0	0	0	0	1	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	0	1	0	0	1	0
Total	**37**	**22**	**16**	**1**	**0**	**35**	**5**	**0**	**31**	**8**	**0**	**32**	**11**	**20**	**13**	**4**	**0**	**35**	**9**	**13**	**22**	**10**	**10**	**25**	**9**	**0**	**34**	**10**	**0**	**38**	**3**
%	**100**	**59**	**43**	**3**	**0**	**95**	**14**	**0**	**84**	**22**	**0**	**86**	**30**	**54**	**35**	**11**	**0**	**95**	**24**	**35**	**59**	**27**	**27**	**68**	**24**	**0**	**92**	**27**	**0**	**103**	**8**

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Members of *Enterobacteriacea* are part of the gut flora found in the intestines of humans and other animals, while others are found in water or soil, or are parasites on a variety of different animals and plants. Fish is no exception and in this study most of the baceria isolates were from the family *Enterobacteriacea* ; *Citrobacter freundii, Edwardsiella tarda, Enterobacter sakazakii, Enterobacter cloacae, Enterobacter amnigenus, Enterobacter agglomerans, Escherichia coli, Klebsiella pneumoniae, Klebsiella ornithinolytica, Proteus mirabilis, Providencia stuartii, Shigella boydii, Salmonella spp, and Plesiomonas shigelloides*. This is in line with the findings of Ogbondeminu and Olayemi (1993) who reported that 50% of the microorganisms recovered from both fish and water of earthen pond fertilized with animal faecal waste were members of the family *Enterobacteriaceae*.

The other bacteria isolated include; *Vibrio mechnikovii, Aeromonas sobia, Pseudomonas aeruginosa, Pseudomonas fluorescenes, Chromobacterium violaceum and Acinetobacter spp.* They are widely distributed in aquatic environment and in the soil, and have been implicated as opportunistic pathogens causing diseases in human beings. These bacteria have been isolated in other studies by Nganou *et al.* (2011) who isolated *Aeromonas spp ,Vibrio spp, Pleisomnas spp, Acinetobacter spp, Enterobacteriacea, Pseudomonas spp,* from tilapia fish collected from four lakes in Cameroon. In other studies (Naim, 2012) recovered A. *hydrophila*, *Vibrio* spp., *S. putrefaciens*, *Staphylococcus* sp., *Streptococcus* spp., and *Edwardsiella spp*. *Aeromonas spp, Chromobacterium violaceum, Citrobacter freundii, Escherichia coli,*

and *Plesiomonas shigelloides* were isolated in the gastrointestinal regions of semi-intensively cultured tilapia, *Oreochromis niloticus.* Although the presence of these bacteria is not often associated with fish diseases or enteric diseases in man, the health implications of the introduction of these organisms into natural water via the fish faeces in the aquaculture wastewaters should not be ignored (Naim, 2012).

There were more bacterial species isolated from the ponds than the species isolated from the dams during the two seasons. There was correlation between the sites in which the bacteria were isolated. This can be attributed to the fact that the ponds are fertilized using animal manure to enhance alga bloom and also due to accumulation of feaces of fish and left over feed in the earthen pond (Davis and Goulder, 1993; Makosora and Jazek, 1994). During the wet seasons, lower temperatures inhibit micro bacterial activity, the reason adduced for higher number of bacteria isolates during dry season compared to wet season according to Wemedo (2002). Another reason given attributed to this phenomenon is that saturation of the soil by rain, limits activity by reducing aeration (Marshall and Devinny, 1998).

The results revealed that of all the specimens sampled sediment had the lowest number of bacteria isolates; this is in line with an earlier study by Niemi and Taipalinen (1982) who reported a very low count in sediment. This could be attributed to lack of sunlight as it plays an important role in the bacteria growth (Ferguson *et al*., 1996). In this study there were higher numbers of bacteria in all specimens during the dry season this could be attributed to the fact that bacteria multiply more in high temperatures. (Chowdhury *et al*., 1989) observed similar results in the intestinal bacterial load of tilapia. Research by (Sugita *et al*., 1985; Markosova and Jezek, 1994) reported that

populations of indicator bacteria increased with increasing water temperature as temperature becomes favorable for growth of bacteria during summer months.

The findings revealed different members of *Enterobacteriacea* were isolated from both the dams and the ponds some of which are pathogenic and others are non pathogenic. In other studies there are reports of isolation of different members of *Enterobacteriaceae,* as potential fish and human pathogens from natural manured carps, striped bass, tilapia, eel and its earthen culture environment (Nair and Nair, 1988; Karunasagar *et al*., 1992; Nedoluha and Westhoff, 1997; Muratori *et al*., 2000). The occurrence of these pathogenic *Enterobacteriaceae* was more in the Sagana ponds than the dams which could be due to use of animal manure in the ponds (Ogbondeminu, 1993). In the present study *Salmonella* members of *Enterobacteriaceae,* and *Shigella* were recovered from fish, water and water sediments from ponds at Sagana fish ponds where they use integrated fish culture systems. This is an indication that there exists an inherent risk of contamination by pathogens present in environment as observed in natural manured earthen ponds by Nedoluha and Westhoff (1997). The presence of Salmonella spp. indicates feacal contamination of water from which the fishes were harvested.

Aeromonas sobria was one of the bacteria isolated from both sources during the two seasons. It is a known human pathogen (Mateos *et al*., 1993; Thune *et al*. 1993., Austins and Adams, 1996) and therefore poses a risk of fish-borne *Aeromonas* gastroenteritis in consumers of improperly cooked fish. The finding of *vibrio* spp during the dry season is in line with studies carried out by (Al-Harbi and Uddin, 2003) who found that there were more counts during the summer than during winter.

Pseudomonas aeruginosa was isolated during the two seasons and it is a potential human pathogen. It can persist even after processing posing a health hazard to consumers. Lyhs *et al*. (1998) reported that pseudomonas was responsible for 15.3% of spoilage of preserved fish products, making the organism important in food spoilage with economic losses. It is therefore that the fish from both Sagana and Masinga, be processed and stored well to eliminate contamination.

The *E .coli* has been traditionally recognized as an indicator organism of faecal contamination of water and seafood (Geldreich, 1997). *Escherichia coli* are normal inhabitants of the intestinal tracts of all warm blooded animals. In this study *E. coli* was recovered in all fish samples and in water samples indicating poor hygiene and sanitary condition in Sagana and Masinga dams. Like many of landing beaches in Kenya Masinga dam lacks proper sanitation facilities at the landing sites and this could explain the presence of *E. coli* in all specimens. In other studies Chandraval *et al.* (2010) found that fish and water samples collected from Nadia District of West Bengal in India were contaminated with faecal coliforms like *E. coli.*

Edwardsiella tarda which was isolated from fish samples in both seasons are considered a serious problem in tropical or subtropical areas. Infections associated with this species include gastroenteritis, wound infections, and systemic diseases such as septicemia, meningitis, cholecystitis, and osteomyelitis (Janda and Abbott, 1993). *Edwardsiella tarda* has been isolated in fishes from fresh water aquaculture environment and retail markets in India (Pankajkumar, 2009).

Citrobacter freundii and *Proteus mirabilis* were isolated in the Masinga dam samples and have also been isolated in other studies (Niemi and Taipalinen, 1982; Apun *et al*., 1999).

Chromobacterium violaceum is a Gram-negative rod isolated from soil and water in tropical and subtropical regions. In this study it was isolated in Sagana ponds only. Infections caused by *C. violaceum* are rare among mammals, but Apun *et al.* (1999) reported two cases of human infection caused by both pigmented and non pigmented strains of *C. violaceum*. *Pleisiomonas shigelloides* is a common pathogen in tropical regions associated with diarrhea and occasional opportunistic infections in humans. In this study it was isolated from Masinga dam during the dry season.

The bacterial isolates were highly sensitive to ciprofloxacin (100%), which is gentamycin (98.9%) and this is in line with the findings of (Jawahar, 2011) whose findings were similar with bacterial human pathogens highly sensitive to ciprofloxacin (91%), gentamycin (85%) and chloramphenicol (88%). The relatively high resistance to ampicillin of 61.5 % to most of the isolates is in partial agreement with the findings by (Barat *et al*., 2002) who found a prevalence of 93.4% resistance of gram negative bacteria isolated from fish to ampicillin, also the findings of Newaj-fyzul *et al*. (2006) of predominance resistance to ampicillin of 90.2%. This could be due to the fact that the use of antibiotics in aquaculture in Kenya is very limited. The finding of 31.8 % isolates resistance to tetracycline is comparable with 47% reported by Castro-Escarpulli *et al*. (2003) for isolates recovered from Tilapia (*Oreochromis niloticus niloticus*) intended for human consumption in Mexico.

Cow dung manure serves a potential carrier of pathogenic bacteria which are capable of transmitting zoonotic diseases to humans as a result of contact with the manure, when this untreated manure is used to fertilize fish ponds, it may lead to increase in bacterial infections in the fish and serves as a potential source of food borne infections for the fish consumers. However resistance to the antimicrobial agents may be due to indiscriminate, widespread and lengthy use of tetracycline, chloramphenicol and gentamicin in treatment of cow infections (Omojowo and Omojasola, 2013). Sagana fish ponds are fertilized using cow dung manure and this can explain why in the study bacterial isolates from the ponds showed resistance to more antibiotics than those from the dams. In another study, Andreas *et al*. (2002) concluded that, integrated fish farming seems to favor antimicrobial-resistant bacteria in the pond environment. In another study by Anja *et al.* (2000) found that high levels of individual and multiple antimicrobial resistances were demonstrated within the collected *Flavobacteria* and *Aeromonads*, thus indicating a substantial impact of fish farming on several groups of bacteria associated with aquaculture environments.

5.2 Conclusions

a) Fish from both Sagana ponds and Masinga dam harbors pathogenic bacteria and bacteria which are natural inhabitants of the animal gut flora. In Sagana *E. coli, Salmonella, Shigella boydii,* were isolated among others while in Masinga dam *Pleisiomonas shigelloides, E. coli* were isolated among others.

b) There was no significant difference of bacteria flora species isolated in the two sites or in the two seasons. Some of the bacteria that were isolated in both seasons were *E. coli, Citrobacter freundii* while *Salmonella spp, Shigella boydii* among others. This shows that bacteria species found on the fish skin and in the gut are similar to the ones found in the environments the fish is cultured in.

c) The study showed that there was antibiotic resistance to the isolates with a relatively high resistance to ampicillin of 61.5% and sensitive to ciprofloxacin (100%), gentamycin (98.9%).
d) There was variation in antibacterial response of isolates from Sagana but no significance difference in Masinga dam

5.3 Recommendations

Sanitary conditions under which fish are reared in ponds should be improved, following standard or good practices; such as use of good quality water, use of feeds with high microbial quality, regular draining of pond water after specific period of time, closure of ponds to the public among other things. The public should be enlightened on the inherent danger that may accompany handling of fresh fish or consumption of improperly cooked fish. Therefore, fish must be properly cooked before it is consumed to avoid contact with the microbes that may be associated with it.

The use of antibiotic in fish farming and animal husbandry should be monitored. In addition, the implication of this high level of antibiotic resistance on the choice of antibiotics in relation to zoonotic infections should be noted and efforts should be made to stop indiscriminate use of antibiotics.

Further research on farmed fish species taking into considerations different ponds and dams from different climatology regions to determine relationship between the microbial activities in fish and the different climatic conditions of the regions.

Moreover, the use of antibiotics in aquaculture for promotion of growth in different climatic conditions should be studied further.

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APPENDICES

Appendix 1: Overall data on specimen, isolates and the response to antibiotics

SPECIMEN NO.	ISOLATE	AML	CTX	CIP	S	AMP	CN	TE	C	NA	CXM
ATCC 25922	*E.coli*	S	S	S	S	S	S	S	S	S	S
TS1	*Aeromonas spp.*	R	S	S	I	R	S	S	S	S	S
TG3	*Citrobacter freundii*	R	S	S	S	R	S	R	I	S	S
TS2	*Kleb.ornithnilotyca*	R	S	S	S	R	S	R	I	S	S
TG1	*E.coli*	S	S	S	S	S	S	R	R	S	S
TS3	*Kleb.pneumo*	S	S	S	S	S	S	S	S	S	S
TG1	*Edwardsiella tarda*	R	S	S	I	R	S	S	S	S	S
TG1	*Kleb.pneumo*	R	S	S	R	R	S	R	R	S	S
ST1	*Kleb.pneumo*	R	S	S	S	R	S	R	S	S	S
CS3	*Kleb.pneumo*	S	S	S	S	S	S	I	S	S	S
CS2	*Aeromonas spp.*	R	S	S	S	R	S	S	S	S	S
CS2	*Citrobacter freundii*	R	S	S	I	R	S	S	S	S	S
CS1	*Providencia spp.*	R	S	S	S	R	S	S	S	S	S
CG1	*Citrobacter spp.*	R	S	S	I	R	S	S	S	S	S
CS1	*Pseudomonas spp.*	R	S	S	I	R	S	R	R	R	R
SCG	*Aeromonas spp.*	S	S	S	S	S	S	S	S	S	S
SCG	*Aeromonas spp.*	S	S	S	S	S	S	S	S	S	S
MCS	*E.coli*	S	S	S	S	S	S	R	R	I	S
MTS	*Citrobacter freundii*	R	S	S	I	R	S	R	I	S	S
MTG	*Citrobacter spp.*	R	S	S	S	R	S	I	I	S	S
MCG	*Edwardsiella tarda*	S	S	S	S	S	S	R	I	S	S
MTG	*Citrobacter freundii*	R	S	S	S	R	S	R	R	I	S
STG	*Edwardsiella spp.*	S	S	S	S	S	S	S	S	S	S

STG	*Aeromonas spp.*	S	S	S	S	S	S	S	S	S	S
TG1	*Pseudo.aeruginosa*	R	S	S	I	R	I	R	R	R	R
STS	*Salmonella spp.*	R	S	S	S	R	S	S	S	S	S
TG1	*E.coli*	S	S	S	S	S	S	I	I	I	S
TG2	*Citrobacter spp.*	R	S	S	I	R	S	S	S	S	S
CG1	*Providencia spp.*	R	S	S	S	R	S	S	S	S	S
SCG	*Aeromonas spp.*	R	S	S	S	R	S	S	S	S	S
MCS	*Ent.agglomerans*	R	S	S	S	R	S	S	I	S	S
STS	*Citrobacter spp.*	R	S	S	I	R	S	I	I	S	S
STG	*Edwardsiella tarda*	S	S	S	S	S	S	S	S	S	S
SCS	*Proteus spp.*	R	S	S	S	R	S	R	R	S	S
STS	*Aeromonas spp.*	R	S	S	S	R	S	S	S	S	S
CS3	*Citrobacter spp.*	R	S	S	I	R	S	S	S	S	S
STG	*Proteus mirabilis*	R	S	S	S	R	S	S	S	S	S
STG	*Providencia stuartii*	S	S	S	S	S	S	R	I	S	S
CS1	*Enterobacter spp.*	R	I	S	S	R	S	I	I	S	S
STS	*Vibrio metchnikovii*	R	S	S	I	R	S	S	S	S	S
MTS	*Proteus spp.*	R	S	S	S	R	S	I	I	S	R
MCS	*Citrobacter freundii*	R	S	S	S	R	S	I	R	S	S
STG	*Chromo. Viobceum*	S	I	S	S	S	S	R	R	I	I
MCS	*Aeromonas spp.*	R	S	S	S	R	S	S	S	S	S
CS3	*Enterobacter spp.*	R	S	S	S	R	S	S	S	S	S
STS	*Pseudomonas spp.*	R	I	S	R	R	S	R	R	R	R
SCG	*Providencia spp.*	R	S	S	S	R	S	R	R	S	S
SCS	*Citrobacter spp.*	R	S	S	S	R	S	R	R	S	S
MCS	*Ent.sakazakazii*	S	S	S	S	S	S	S	S	S	S
MTG	*Proteus mirabilis*	R	S	S	S	I	S	I	R	S	S
SCG	*Shigella boydii*	R	S	S	S	R	S	I	S	S	S
SCS	*Pseudo.aeruginosa*	R	S	S	S	R	S	I	I	S	S

SCS	*Pseudomonas spp.*	R	S	S	S	I	S	I	I	S	S
MTG	*Ent.sakazakazii*	R	S	S	S	R	S	I	R	I	S
MTG	*Ent.amnigenus*	R	S	S	S	R	S	S	S	S	S
SCG	*Citrobacter freundii*	S	S	S	S	S	S	S	S	S	S
SCS	*Edwardsiella tarda*	S	S	S	S	S	S	S	S	S	S
MCS	*Enterobacter spp.*	R	S	S	S	R	S	S	S	S	S
ATCC 25922	*E.coli*	S	S	S	S	S	S	S	S	S	S
MCG	*Citrobacter freundii*	S	S	S	S	S	S	R	R	I	S
MTS	*Ente. cloacae*	R	S	S	S	R	S	R	R	S	S
MTG	*Vibrio vulfinificus*	R	S	S	S	R	S	S	R	S	S
Dam water	*Pseudo fluoresc/putida*	R	S	S	S	R	S	R	R	S	S
STS	*Aeromonas sobria*	R	S	S	S	R	S	S	S	S	S
MCG	*E.coli*	I	S	S	S	I	S	R	I	S	S
Sagana water	*Acinetobacter spp*	S	S	S	S	S	S	I	S	S	S
MTG	*Enterobacter spp.*	R	S	S	I	R	S	R	R	I	S
MCG	*Edwardsiella spp.*	I	S	S	S	I	S	R	R	S	S
Sagana water	*Acinetobacter spp*	S	S	S	S	S	S	R	R	S	S
Sagn water	*E. fergusonii*	R	S	S	I	R	S	S	S	S	S
STS	*Pseudo.aeruginosa*	R	S	S	S	R	S	I	R	R	S
STG	*Citrobacter freundii*	R	S	S	S	S	S	I	R	S	S
STG	*Aeromonas sobria*	R	S	S	I	R	S	S	S	S	S
STG	*Salmonella spp.(STM)*	S	S	S	S	S	S	S	S	S	S
MCS	*Enterobacter spp.*	R	I	S	S	R	S	I	I	S	S
Sagana water	*Citrobacter spp.*	R	S	S	S	R	S	I	I	S	S
MTG	*Ent.amnigenus*	R	S	S	S	R	S	R	R	S	S
MCS	*Plesio.shigelloids*	S	S	S	S	S	S	S	S	S	S
STG	*Pseudo.aeruginosa*	R	S	S	S	S	S	I	R	S	S