ENSIGN COLLEGE OF PUBLIC HEALTH, KPONG, EASTERN REGION, GHANA

ANTIBIOTIC USE IN FISH FARMING IN ASUOGYAMAN DISTRICT OF THE EASTERN REGION, GHANA

by
Mary Nkansa

A Thesis submitted to the Department of Community Health in the Faculty of Public Health in partial fulfilment of the requirements for the award of the degree

MASTERS IN PUBLIC HEALTH

APRIL 2017

DECLARATION

I hereby certify that except for reference to other people work, which I have duly cited, this Project submitted to the Department of Community Health, Ensign College of Public Health, Kpong is the result of my own investigation, and has not been presented for any other degree elsewhere.

Mary Nkansa (ID -157100069) (Student)	________________ Signature	________________ Date
(Certified by) Dr. Frank Baiden (Supervisor)	________________ Signature	________________ Date
(Certified by) Dr Stephen Manortey (Ag. Head of Academic Programme)	________________ Signature	________________ Date

Dedication

This work is dedicated to my beloved husband, Ing. Agyaaku Kani Nkansa and my children: Joel, Maame Abena and Kwabena Nkansa. You are such a blessing to me.

Acknowledgement

I appreciate God for the uncommon favour granted me throughout this project work.

I specially acknowledge my supervisor, Dr. Frank Baiden for the guidance, counsel and intellectual input into the completion of this research.

I am grateful to my lecturers and other faculty members of Ensign College of Public Health for their support and encouragement.

I also want to appreciate Mum, siblings and in-laws for their continuous prayers and support throughout my programme.

I also want to acknowledge Mr. Samuel Quaatey and Dr. Peter Ziddah for their role in obtaining financial assistance for this programme.

I will also commend the staff of Fisheries Commission (Akosombo zone) and Veterinary Services Division laboratory (La) for their immense contribution to this work.

My sincere gratitude goes to colleagues at the Fish Health Unit and also to Nana Fredua - Agyeman and Micheal Dawutey for your efforts.

Finally, to all my wonderful colleagues and friends at Ensign College of Public Health, God bless you for creating that enabling environment for me to realise this dream.

ABSTRACT

Introduction

Rapid growth in aquaculture has led to increased fish production. There is however global concern about the unregulated use of antibiotics. Antibiotics use in aquaculture is undertaken to make up for unhygienic fish breeding practices. Very little is known about antibiotics use in aquaculture in Ghana. This study was carried to investigate antibiotics use in fish farms located along the Volta River Basin.

Methods

Three approaches were use in data collection. A questionnaire was administered to farm managers at their farms. Fish samples procured at site were analysed for the presence of antibiotics in the laboratory at the Veterinary Services Division. In-depth interviews were conducted with officials of the Fisheries Commission. Descriptive analysis was conducted, with triangulation of information obtained from the three sources.

Results

Seventy (70) farms were surveyed and fifty (50) fish samples bought for analysis. The majority of farms were commercial and offered fish for sale at farm sites. Most (95.7%) of the owners were males. About 67.1% of farmers admitted to the use of antibiotics. Antibiotics use was largely for treatment of suspected wounds and without authority or supervision from veterinary officers. Farmers lacked sufficient knowledge on the long term effects of unregulated antibiotic use. Seventy-six percent of the fish samples tested positive for antibiotic residues; these includes fish bought from farms where farmers denied antibiotics use during the interviews. Although officials of the Fisheries Commission

acknowledge the unregulated practice, they point to the lack of laboratory capacity to quantify the levels of antibiotics in fish as a major challenge.

Conclusion

Unregulated use of antibiotics is common in the farms surveyed, and likely on other farms as well. Farmers need to be educated about the potential harm this poses to human health, the environment and growth of the industry. There is the need to urgently establish the capacity to detect levels of antibiotics residues in fish at the Fisheries Commission.

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

AAHS	Aquatic Animal Health Specialist
AMR	Antimicrobial Resistance
ARGs	Antibiotic Resistant Genes
CA	Codex Alimentarius
DNA	Deoxyribonucleic acid
EU	European Union
FAO	Food and Agriculture Organization
FC	Fisheries Commission
HPLC	High Performance Liquid Chromatography
IM	Intramuscular
IP	Intraperitoneal
MH	Mueller Hinton
MOFA	Ministry of Food and Agriculture
MOFAD	Ministry of Fisheries and Aquaculture
MRL	Maximum Residue Limit
MT	Metric Tonnes
OIE	World Organization for Animal Health
SDGs	Sustainable Development Goals
SPS	Sanitary and Pytosanitary Agreement
SRAP	Student Research Award Program
UV	Ultra Violet
VA	Veterinary Antibiotics
VSD	Veterinary Services Division
WAHIS	World Animal Health Information System

WARFP	West African Regional Fisheries Programme
WHO	World Health Organization
WTO	World Trade Organization

Chapter 1

Introduction

Fish farming or aquaculture is the rearing of aquatic animals (fishes, molluscs, crustaceans and plants) for food and/ or recreation (FAO, 2010). It involves interventions to improve production, such as regular stocking, feeding and protection from predators. Depending on the species being reared, aquaculture may be carried out in freshwater, brackish water or marine water (Troell *et al.*, 2017). Globally, the most important species used in fish farming are carp, tilapia, catfish, sturgeon, salmon, striped bass, and trout (Gupta and Acosta, 2004). Fish farming can be done under intensive, semi-intensive or extensive systems based on the level of inputs (feed, broodstocks etc.) supplied.

Fish farming plays an important role in food security and the broader economy, with far reaching impact on rural development, water and environmental management, poverty alleviation, livelihood, trade, gender and household nutrition (Kwadwo Frimpong and Adwani, 2015). Globally aquaculture has developed far more rapidly than all the other animal food production sectors. This is largely due to decline in catch in ocean fisheries and the need to satisfy the high demand in aquatic products (FAO, 2010). Evidence from various research show that the fish from fish farms contains nutrients that are very healthy (Picklo, 2016; Lundebye *et al.,* 2017). It has been estimated that aquaculture production contributes about 47% of the world's total fish supply. These massive volumes of fish are produced from highly intensified farms and hence the need to invest in research and development of sustainable management practices to protect biodiversity and human health (Naylor *et al.*, 2000).

In Ghana, fish is a preferred source of animal protein. The per capita consumption of fish is estimated at 20 - 25kg per annum, higher than the world average of 13kg per annum (MOFAD, 2016) Fish contributes about 60% of animal protein intake of Ghanaians, since fish is less expensive than the various types of meat. Currently, Ghana's fishery resources can supply 40 metric tons (MT) of fish against a national demand of 80 MT. Products from fish farming make up a substantial part of the deficit (MOFAD, 2016) .

Since fish farming started in Ghana in the 1950's on subsistence basis in Northern Ghana, successive governments have put in a lot of resources and policies to create the enabling environment for aquaculture development. Several incentives which range from loan facilities, equipments and machinery, fish seeds and feed have been provided to individuals and corporate entities to boost the growth of the aquaculture industry and make it sustainable (Rigby et al., 2017)

Just as fish farming has evolved in other parts of the world, Ghana is also experiencing a similar occurrence with the production of huge volumes of fish by the farms usually through the intensive system of culture. In this culture of production, fishes are kept under strict containment, where by their nutrition and other environmental conditions are monitored to ensure optimum production (Cole *et al.*, 2009; Bosma and Verdegem, 2011). As fishes share and compete for all the resources, any unfavourable alterations in these conditions resulting from poor husbandry practices may predispose the fish stocks to unpredictable morbidities and mortalities. Infections and disease occurrence are therefore very common among fishes kept under intensive culture as compared to their counter parts in the other culture systems. There is therefore heavy dependence on the use of drugs and chemicals such as disinfectants, antibiotics and antihelmintics to prevent and control infections in industry to reduce huge losses of stocks (Caruso, 2016). In recent times, antibiotics usage has become common, since it serves as an easier alternative to good management practices in the farms (Cabello, 2006). Researches have shown that the use of antibiotics in fish farming poses enormous public health threats which include the development and spread of antibiotic resistant strains and genes within the food chain, the presence of antimicrobial residues in the aquatic products and the environment, the risk of unhealthy occupational exposure, high cost of production and the destruction that may be associated with aquatic biodiversity.

Antibiotics are substances that have the capacity to inhibit or treat infections / diseases caused by microorganisms (bacteria and certain parasites). They can be produced naturally or synthetically in the laboratory and are used in both human health care and veterinary practice (Cabello, 2006). They are used as prophylactic, therapeutic or metaphylactic. Prophylaxis refers to the preventive use of antibiotics in the development of infections in groups or individuals. Many developed nations have banned the use of antibiotics as prophylaxis in fish farming. Therapeutic use refers to the treatment of established infections with antibiotics over a short period. Metaphylaxis refers to administration of group - medication that seeks to treat sick animals while at the same time medicating other animals in the group from getting infected (Benbrook, 2002; Gyssens, 2017). Metaphylaxis is common practice in aquaculture establishments.

There are regulations that govern the administration of antibiotics with regards dosage, permissible route of delivery and withdrawal time to minimise exposure to antibiotic residues in the fish (Cabello, 2006). Non-adherence to these rules leads to drug toxicity, resistance and possible deaths. In many developed countries, the use of antibiotics is strictly regulated. For instance, in Norway, drugs used in aquaculture are sold in licenced pharmacies and can be assessed with a prescription from a licenced veterinarian and also administered under the supervision of the veterinarian. Fish farmers are mandated by law to report and keep records on diseases and antibiotic usage on their farm as part of the good farm management practices (Meek *et al.*, 2015). In developing countries, the regulations on drug use (antibiotics) are documented but with very little enforcement, which results in its abuse in the sector.

1.0 Background

In Ghana, the growth of the aquaculture industry is moving rapidly as seen in the shift from the subsistence level to a more sophisticated commercial level of farming, and the volumes of fish harvested has increased, the use of drugs (antibiotics) in disease prevention and treatment practices are far from regulated and standardised. Currently, 80 % of fish outputs from aquaculture establishments are derived from the cages on the Volta Lake. High production implies that there is increased dependence on formulated feeds, antibiotics and other aqua related drugs to enhance production outcomes. Similar to most human food-production activities, there are bound to be husbandry and disease - related challenges which will require the use of veterinary drugs in their management. Contrast to the developed countries, where there is availability of vaccines and adherence to better management practices (biosecurity measures) on farms, so the dependence on drugs (antibiotics) in aquaculture to manage diseases/infections is minimal and thus fewer residues in the produce and products (Burridge *et al.*, 2010; Subramani and Michael, 2017).

Disease problems are the largest single cause of economic losses in aquaculture. Bacterial, fungal, parasitic and viral diseases are important sources of disease problems in all the various types of fish culture system (Moriarty, 1999). This is one of the main reasons why farmers resort to the use of antibiotics. Veterinary antibiotics (VAs) such as fluoroquinolones, tetracyclines, penicillins, sulphonamides and other antibiotics are widely used for the treatment and prevention of fish diseases. As probiotics, they are incorporated into animal feed to improve growth rate and feed efficiency. The use of large amounts of antibiotics may lead to the presence of residual antibiotics in fish tissues and fish products. Many antibiotics used in animal husbandry are poorly adsorbed in the gut of the animal. It is estimated that about 25-75% of the antibiotic administered to the animals may be excreted unchanged in faeces and urine and this can persist in the environment for a long time. Evidence has shown that unrestricted use of antibiotics is known to result in the emergence, persistence and spread of antibiotic resistant bacteria in fish pathogens in aquaculture environments and also transfer of these resistance determinants to human pathogens (Gyansa-Lutterodt, 2013).

Antibiotic resistance refers to the ability of bacteria to resist the effects of an antibiotic. It occurs when bacteria change in a way that reduces the effectiveness of drugs, chemicals or other agents designed to cure or prevent infections (Bieranye et al., 2013). Globally, it is a huge public health issue as antibiotic resistance increases the cost of health care with lengthier stays in hospitals and more intensive care required. At the 71st session of the United Nations General Assembly held in September 2016, member states agreed that antimicrobial resistance threatens the achievement of the Sustainable Development Goals and thus requires a global response. Members reaffirmed their commitment to develop National Action Plans (in order to strengthen regulation of antimicrobials, improve knowledge and awareness, and promote best practices - as well as foster innovative approaches using alternatives to antimicrobials and new technologies for diagnosis and vaccines) based on the Global Action Plan on Antimicrobial Resistance developed in 2015 by the World Health Organisation (WHO), in collaboration with Food and Agriculture Organisation (FAO) and World Organisation for Animal Health (OIE) (OIE, 2016; Singer *et al.*, 2016). In Ghana, there is growing public concern about the use of antibiotic and growth enhancers in fish farms, and how residues may enter the food chain. Evidence is however anecdotal as no systematic enquiry has been conducted. The Volta Lake and its tributaries are one of the places in Ghana with the highest concentration of fish farmers. Currently, very little is known about antibiotic use in fish farming and there is virtually no enforced regulation of its possible use. Compliance to existing regulations is very little on antibiotic use in fish farming to protect public health and food safety (Gyansa-Lutterodt, 2013).

1.1 Problem statement

Preserving the effectiveness of antibiotic drugs is vital to protecting human and animal health. In Ghana, tetracyclines are commonly used in aquaculture production. The unavailability of sufficient data on antibiotic use in fish farming in Ghana is a major reason for the lack of effective regulation on the practice. As the country aims to promote fish farming and expand yield for both domestic and the international market, there is the need to explore how the possible use of antibiotics could impact on the health of Ghanaian consumers. To make for well-informed management (institutional level) and policy (national level) decisions on regulations of antibiotic use in fish farming, evidence from systematic enquiry is needed.

1.2 Rationale of the study

A recent preliminary report from monitoring visits to Asuogyaman, Shai-Osuduku districts, Kpeve and some districts in the Ashanti region during the month of January 2015 by a team from the Fish Health Unit of the Fisheries Commission revealed that fish farmers used antibiotics either without technical supervision or adherence to any standard guidelines with regards to the type of antibiotics, levels of the dosage and the withdrawal periods to be observed as stated in the Fisheries Regulations (Huchzermeyer, 2016).

In solidarity with the global community to prevent emerging antimicrobial resistance in humans by ending the imprudent use of antimicrobials in food production, an exploratory study to determine antibiotic use in Ghana's rapidly growing aquaculture industry was timely to address a knowledge gap upon which further studies could be carried out to highlight the required interventions to make the industry sustainable.

This research is expected to contribute to the current knowledge about antimicrobial use in aquaculture with particular reference to Ghana and with possible recommendations to the on-going Ghana Aquatic Animal Health Policy being developed to serve as a guide for sustainable aquaculture production.

1.3 Objectives of the study

Main objective

To explore the use of antibiotics in Ghana – A case study of Asuogyaman District of the Eastern Region.

Specific objectives

- To describe the use of antibiotic in fish-farming in terms of

    - Types of antibiotics used

  - Forms of fish farming in which antibiotics are used
  - Reasons (indications) for the use of antibiotics
  - The sources of antibiotics used
  - Fish farmers' knowledge of the adverse effects of antibiotic use
- To test for the presence antibiotic residues in fish samples
- To assess the challenges faced by regulators of antibiotics use in the industry

1.4 Research Questions

- Are antibiotic residues present in fish samples?
- What is the extent of use of antibiotics in fish farms?
- What are farmers' knowledge on the adverse effects of antibiotic usage on fish farms?

1.5 Organisation of report

The proposed study would be in six chapters with appropriate sub-titles. Chapter one comprise of the introduction, background, problem statement, research objectives and questions and rationale for the study. Chapter two review a wide source of literature (articles, conference reports, book sections etc.) on the subject matter from domestic and international sources which would be cited as references and used in discussing the findings from the study. Chapter three is the methodology which include research methods and design to be used for the study. It also includes the data collection techniques and tools, information on study population and variables. The sampling, pretesting of questionnaires as well as data handling and analysis will be discussed. The chapter finally outline the ethical considerations, limitations and assumptions of the study.

Chapter four entails the results, a summary presentation of the key variables from the survey and analysed data. Chapter five involves the discussion of the findings. The linkage between research questions and objectives as well as the findings, citing relevant references in support or contrast of results. Chapter six concludes the discussions on the findings of the study and the necessary recommendations for the regulatory agency and the various stakeholders and interested parties.

Chapter 2

Literature Review

2.0 Occurrence of antibiotics residues in fish

The discovery of antibiotics by Fleming in 1928 has contributed immensely to the improvement in the health of populations due to the significant reduction in morbidity and mortality(Paterson *et al.*, 2016). Antibiotics have been used to treat infectious diseases, mainly bacterial infections in humans and sometimes prescribed for viral infections which don't even response to treatment (Cabello, 2006; Nayak, 2010). Antibiotics have also become an essential drug in animal health and welfare.

In veterinary medicine, antibiotics have also been used for treatment and prevention of diseases and also used as growth promoters. Antibiotics have been administered to treat and prevent diseases such as respiratory diseases, mastitis, gastrointestinal infections, arthritis and other bacterial diseases in rearing animals (Masood et al., 2015). There has been an increased use of prophylactic antibiotics due to the fish's inability to clear up bacterial infections as a result of hygienic shortfalls in fish raising methods in industrial aquaculture which includes increased population densities, absence of biosecurity measures and many more (Cabello, 2006).

As growth promoters, antibiotics are included in the diet of fishes and served daily to enhance growth of the fish (Nayak, 2010). The unconsumed feed and fish faeces containing antibiotics fall to the bottom of the aquatic environment. The antibiotics diffuse into the environment for wild fishes and shell fishes ingest while the microflora and microfauna within the ecosystem is altered. The dosages are increased from time to time to achieve the expected results and thereby increase the levels of antibiotic residues in fish.

2.1 Incidence of antibiotics in food

As production surges and the demand for food fish heightens, many aquaculture facilities resort to antibiotics to combat diseases in an environment that creates ample opportunities for bacterial pathogens to thrive. It is estimated that about 80% of antibiotics used occurs in food production industry in the United States of America. These antibiotics finally end up in humans as we consume the food products. Studies have shown that consumption of antibiotic contaminated fish meat has a potential of altering the human digestive organs, thus favouring infections such as Salmonella (Benbrook, 2002; Aarestrup, 2005) .

2.1.1 Types and quantity of antibiotics used

Globally it is estimated that 50% of all antimicrobials serve veterinary purposes (Hernandez Serrano, 2005). Fish are always susceptible to a variety of lethal diseases caused by different types of bacterial, fungal, viral and parasitic agents as poor husbandry practices such as over feeding, high stocking densities and destructive fishing techniques increase the probability of disease outbreaks in aquaculture establishments. Large quantities of antimicrobials specifically antibiotics are used to control the disease outbreak and to ensure adequate fish production as the aquaculture industry plays a significant role in global food security.

Veterinary antibiotics (VAs) are widely used in many countries worldwide to treat disease and protect the health of animals. Fluoroquinolones, tetracyclines, penicillins, sulphonamides and other antibiotics are widely used for the treatment and prevention of fish diseases (Cabello, 2006). Several findings have linked antibiotic resistance development and spread with animal production and Aquaculture which is now the fastest growing animal production sector may also promote similar or new resistance mechanisms (Done et al., 2015).

Among the antibiotics used in aquaculture, several are classified by the World Health Organization as critically important for use in humans (Heuer *et al.*, 2009) and occurrence of resistance to these antibiotics in human pathogens severely limits the therapeutic options in human infections (Cole *et al.*, 2009). Some studies have shown that out of 51 antibiotics commonly used in aquaculture and agriculture, 39 (76%) are also of importance in human medicine and six (6) classes of these antibiotics can found among the WHO's list of critically important antimicrobials as shown in figure1. Various zoonotic pathogens isolated from meat and seafood were observed to feature resistance to multiple antibiotics on the WHO list regardless of their origin. The resistant bacteria isolated also shared the same resistance mechanisms which gave an indication that aquaculture is contributing the same resistance issues just like in agriculture (Done et al., 2015). Despite the extensive use of antibiotics in aquaculture facilities, limited data is available on the specific types and amounts of antibiotic used (Heuer et al., 2009) as there are large variations with the distribution and registration systems between different countries in terms of antibiotic use ranging from 1 g per metric ton of production in Norway to 700 g per metric ton in Vietnam (Defoirdt et al., 2011).

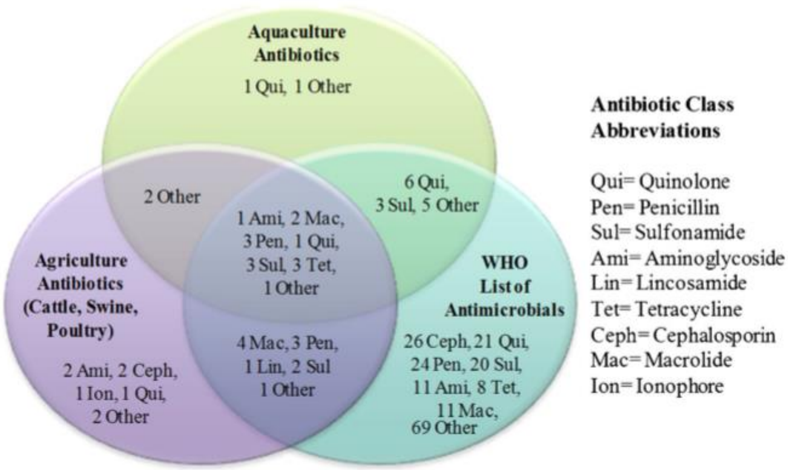

Figure 2.1: Common antibiotic classes for aquaculture and agriculture (WHO antimicrobial list, 2011)

2.1.2 Forms of fish farming in which antibiotics are used

Aquaculture can be carried out successfully in both freshwater and marine environments. In both types of farming, fishes may be reared on intensive, semi-intensive or extensive basis depending on the scale of production which could be small, medium or large. Several findings have indicated that intensification of aquaculture has led to the promotion of conditions that favor the development of a number of diseases and problems that warrant the use of heavy quantities of drugs and chemicals to enhance production (Sekkin & Kum, 2011).

It is believed that the husbandry measures on the industrial farms generally stress the fish, impairing their immunity and leaving them more vulnerable to infections. In addressing the challenge, poor health management measures employed on these large scale farms which results in drug toxicity, development and spread of resistance pathogens within the food chain and aquatic environment that threatens global food security and public health concern (Holmström *et al.*, 2003; Lupin, 2009).

In small scale aquaculture, there is minimum dependence on antibiotic use as the cost of antibiotics may increase their total production cost. Besides ponds and cages may not be densely populated to create favourable conditions for the buildup of harmful microbial load that require chemotherapy with antibiotics. Usually the presence of chemicals and drugs (antibiotics) in small scale farms can be traced to sources outside the facility and therefore the need to invest enough control efforts to manage the agricultural activities around the aquaculture facilities (Braykov et al., 2016) .

The type of drug for the treatment of diseases or infections will also be dependent on the type of aquaculture as in freshwater or mariculture. Whereas similar types of pathogens affect freshwater and marine fish, relatively few pathogens are transmissible from freshwater fish to marine fish and viceversa, thus most pathogens affect either marine or freshwater fish but not both (Sekkin and Kum, 2011)

Aquaculture may also be classified into monoculture, polyculture and integrated culture. In monoculture system, only one type of species of fish is reared or kept for production whereas in the polyculture two or more species of fish are reared together in the same cage or pond and they compete for all available resources.

In the integrated fish culture which is not so popular in practice in industrial sector, fishes are combined with ducks or poultry within the same compound and the waste from the poultry serve as manure and or feed for the fishes. A study conducted in China using these two culture systems revealed that monoculture ponds showed higher relative abundance of antibiotic resistance genes (ARGs) both in water and sediment but in the integrated culture system showed the lowest absolute abundance of ARGs in water and the highest in sediments (Xiong *et al.*, 2015; Huang *et al.*, 2017). In certain countries with an important aquaculture industry, it is mandatory to report the amount of antibiotics used and retain records of prescription (Larson, 2006; Gyssens, 2017).

Ornamental aquaculture facilities also depend heavily on antibiotics in order to prevent huge losses to the stock as species are of high economic importance due to their value and aesthetic nature. Antibiotics used in these facilities do not raise a major public health concern as fishes are not edible but the concern only arises when untreated effluents are discharged from the facilities into the environments (Hollis and Ahmed, 2014).

Various approaches could be instituted in industrial farms as alternatives to administration of antibiotics which may include vaccination and other innovative methods such as phage therapy, probiotics etc in addition to creating biosecurity measures which includes keeping less crowded pens, better sanitary conditions, and isolation of infected / diseased fish to minimize the risk of transmission of infections and diseases in ponds and cages (Meek *et al.*, 2015).

2.1.3 Reasons (indications) for the use of antibiotics

The rapid expansion of the aquaculture industry is having a significant impact on the aquatic ecosystems. As the industry expands, there is also an increase in microorganisms that inhabit the environment. Microorganisms can be of great importance in the aquatic ecosystem as they play different roles in nutrient recycling, degrade organic matter and occasionally they infect and kill the fish, their larvae or the live feed when there is microbial imbalance in the aquatic ecosystem. Some may also protect the fish against disease. Thus adequate knowledge on the microbial interractions and the overall ecosystem is required in the management of the microbial communities in any aquaculture environment besides the potential in assessing and improving water quality and controlling the development of microbial infections (Marshall and Levy, 2011)

Antibiotics are used in livestock, pets, aquaculture and crops and have great variation in value (Hollis and Ahmed, 2014). Veterinary Antibiotics (VAs) are used globally to treat diseases, protect the health of animals, prevent infections and for tranquilization (Sekkin & Kum 2011; Benbrook 2002; Meek et al. 2015; Romero et al. 2010). . They are also added to animal feed to improve the growth rate and feed efficiency. Several concerns have arisen due to increased resistance to microorganisms with regards to veterinary antibiotic on chemical nature, fate processes, occurrence, and effects on plants, soil organisms and bacterial community (Marshall and Levy, 2011)**.**

Antibiotics have become important drug for both human and animal health and welfare. Antibiotics are defined as substances that have the capacity to kill or inhibit growth of microorganism. They can be derived from natural sources or have artificial origins. They are safe to the host, allowing their chemotherapeutic agents for the treatment of bacterial infectious diseases. Antibiotics can be categorized as therapeutic, prophylactic or metaprophylactic (Sekkin and Kum, 2011).

2.2 Mechanisms of action

2.2.1 Anti-infectious agent

Generally, antibiotics have different types of chemical structures and act on different parts of bacterial machinery. First, they may have a bactericidal effect where by the antibiotics will kill the bacteria by interfering with either the formation of the bacterium's cell walls or its cell contents. Examples of such antibiotics include penicillin, fluoroquinolones and metronidazole.

Secondly, antibiotics may exhibit a bacteriostatic effect in which the antibiotic stops bacteria from multiplying by interfering with bacterial protein production, DNA replication, or other aspects of bacterial cellular metabolism. Some examples are tetracyclines, sulphonamides, chloramphenicol and macrolides.

The therapeutic use of antibiotics involves the treatment, prevention and control of infections. In treatment, specific doses of antibiotics are administered to clinically sick animals to cure specific infectious diseases. Antibiotics can also be given to control infections from spreading and also offer protection to animals that have been exposed to infected animals (Aminov, 2013; Gyansa-Lutterodt, 2013; Paterson *et al.*, 2016). The preventive uses of antibiotics involve giving antibiotics to healthy animals who are at risk of getting infected due to their exposure to the specific infectious agents either as a result of stress from transportation, overcrowding usually in intensive systems of farming. In intensive fish farming, antibiotics are administered generally by either water-borne or oral means or by injections.

2.2.2 Water medication

It is the common and easiest method of administering treatment to fish. The medication in the water will dissolve in the water, treat the fish, and also reduce the rate of transmission by killing parasites in the water. There is evidence that as the marine fish consumes large volumes of water, significant quantities of drugs will be absorbed in the gastrointestinal tract in the process (Edward J. Noga, 2010). Treatment by water is encouraged as most sick fishes would not want to eat any feed and also for surface dwelling fishes.

Active agents that are intended to treat infection/ disease must reach therapeutic levels in target tissues and adequate plans for detoxification and the removal and disposal of used drugs must be in place before treatment is began (Aarestrup, 2005)**.**

2.2.3 Oral medication

This method of treatment is preferably used for aquarium and food fish aquaculture using medicated feeds. Sick fish may not eat immediately, therefore it is also advisable to withhold feed for 12-24 hours to increase the acceptance of medicated feed (Rogers and Basurco, 2009; Cabello *et al.*, 2013). Feeds are incorporated with medication through a powdered premix which could be fish or vegetable oil to make it palatable to the fish. The dosage rates of the medicated feed may depend on the antibiotic used. Another important factor to note when using medicated feed is the bioavailability of the antibiotic. Studies have shown that treatment of marine species with some antibiotics have been less effective due to reduced bioavailability of the drug.

2.2.4 Injections

The injection of antibiotics is considered as the most effective treatment for bacterial infections than medicated feed especially for advanced infections and the best way to ensure that the right dose is administered (Lupin, 2009) . This method is only practicable for valuable fishes in a pond or in an aquarium and not for large scale production facilities. Sick fish is usually anaesthetized before treatment. The typical injection sites include the intraperitoneal (IP) cavity and the intramuscular (IM) route.

The disadvantages associated with this method is the damage to the carcass quality and also the potential to form sterile abscesses (Edward J. Noga, 2010). The volume required for the injection of antibiotics is dependent on the weight of fish to be treated, the recommended dosage for the antibiotic being used and its supplied concentration (Lupin, 2009).

2.2.5 Growth promotion

Antibiotics are administered in feed or water in some countries to promote the growth and size of animals bred for food. Unregulated use of antibiotics as growth promoters has led to the selection of resistant bacteria, which subsequently contaminate the environment. The European Union (EU) regulation has banned the use of specific antibiotics of relevance to human health as animal growth promoters (Gyssens, 2017).

The feeding of antibiotics to animals eliminates the pathogenic organism populations and alters the non-pathogenic intestinal flora, producing beneficial effects on the digestive process by reduction of the animal gut mass to increase the intestinal absorption of nutrients to be used for growth and production (Cabello, 2006).

Studies by (Bagal et al., 2016) showed that growth performance of birds in terms of body weight, body weight gain and feed conversion efficiency improved significantly in 1% citric acid than antibiotic (chlortetracycline) supplemented feed as growth promoter. Findings have revealed that non-therapeutic doses of antibiotics affect a variety of biological systems within the host, including potentially detrimental sequelae for the health of the animal fed as well as consumers who are exposure to the antimicrobial agents (Marshall and Levy, 2011; Brown *et al.*, 2016). Evidence from studies using antibiotics as growth promoters in pigs revealed higher serum levels of an insulin-like growth factor. The effect of the antibiotic is seen not only in the digestion in the intestine but also stimulation of metabolic processes (Cole *et al.*, 2009)

2.3 The sources of antibiotics used

Current techniques to measure antibiotic consumption are often inadequate and may miss important sources of antibiotics (Lee *et al.*, 2007). A survey conducted in some countries in Europe, the USA, Canada and Australia concluded that existing (old) and useful antibiotics are less and less available to clinicians and the situation keeps worsening over the years and the global efforts have a conservation measure to ensure sustainable and responsible use of antibiotics (Pulcini *et al.*, 2017). The various sources of antibiotics may include fish feed, sediments, water, and culture organisms (fish and shrimp) aquaculture farms (Cabello, 2006; Kathleen *et al.*, 2016).

The continuous and widespread use of antibiotics in feed as growth promoters, the uneaten feeds that are absorbed into the environment remains for years depending on the chemical nature of the antibiotic used although others decompose very fast. In a similar manner, the antibiotics in the medicated feeds when consumed by the fish may be biodegradable and in others non-biodegradable in nature.

Land animals may also constitute another important source of antibiotic in the integrated system of fish culture when poultry are treated or given antibiotics as growth enhancers, their fecal matter may end up contaminating the water source (aquatic environment) and also in the fish products (Cabello, 2006; Caruso, 2016). A study conducted in Jiulongjiang River in China traced major antibiotics to effluents from swine waste water (Jiang *et al.*, 2013).

Floods and drainage from aquaculture facilities (ponds and hatcheries) were identified in a study as a major source of antibiotic pollution in the Tiaoxi watershed (Zhang *et al.*, 2014; Li *et al.*, 2016). There is the need to regulate effluent discharges into open environment through effective monitoring mechanisms to prevent the release of antibiotics into the environment and reduce environmental degradation.

2.4 Fish farmers' knowledge of the adverse effects of antibiotic use

Generally, farmers have a perception that administration of antibiotics in their facilities would help to cure infections / diseases regardless of the prevailing sanitary conditions and also increase their profitability (farm output).

Results from a study conducted in Vietnam to monitor antibiotic residues revealed that farmer's decision-making processes about antimicrobial use are influenced by biased sources of information, such as drug manufacturers and sellers and by financial incentives (Pham *et al.*, 2015) and that there is a general lack of knowledge about the purpose and appropriate dosage by aquaculture producers. The local government played a minor role in the production of aquaculture in extension services and thus their dependence on feed and drug manufacturers for knowledge.

Most farmers are usually not well informed about the efficient and safe application practices with antibiotics. Dissemination of such information on antibiotics could contribute to a decreased use of antibiotics and decrease impacts on regional human medicine and adjacent coastal ecosystems (Holmström *et al.*, 2003).

2.5 Antibiotic resistance in aquaculture

In addition to the therapeutic role that antibiotics play, administration of antibiotics in fish diet significantly has been proven to be an effective tool for ensuring the development of intensive and large-scale aquaculture production as it enhance feed efficiency, promote growth and to improve the quality of the animal products (Hao *et al.*, 2014). A recent study on the growth promoting capabilities of antibiotics correlated with the decreased activity of bile salt hydrolase, an intestinal bacteria-produced enzyme that exerts negative impact on host fat digestion and utilization (Lin, 2014).

The overuse and misuse of antibiotics in aquaculture and most importantly food production either directly to the water or in fish food, as prophylactics to control infectious disease is undoubtedly associated with the emergence and spread of antibiotic resistance bacteria and antibiotic resistance genes in fish and the aquatic environment (Rolain, 2013) which may lead to the transfer from animals to humans (Stanton, 2013).

Some evidence has indicated that the development of multidrug resistance could be linked to non-therapeutic application of antibiotics (Marshall and Levy, 2011) which results in poor response to treatment which raises a lot of public health concern in a global world and especially in developing country with little or no legislature for enforcement of such regulations to ensure the rational use of antibiotics in aquaculture establishments.

Different types of antibiotics are used in aquaculture of which some are critically used in human medicines such as tetracylines, flouroquinones, aminopenicillins etc. A better understanding of the ecological role of antibiotics and antibiotic resistance in natural environments may eventually help to predict and counteract the emergence and evolution of resistance

2.6 Public health significance of antibiotics in food

Preserving the efficacy of these life-saving medications, as well as their availability for both human and veterinary use, is therefore essential to preserve our future. The development of antimicrobial resistance (AMR) compromises this dual objective and impacts our ability to successfully treat infectious diseases.

The availability and use of antimicrobial medicines has transformed the practice of human and animal medicine. Infections that were once lethal are now treatable, and the use of antimicrobial drugs has advanced global health as well as animal health, which has contributed to a key component of policies to improve animal welfare, food security and food safety (OIE).

The emerging view that antibiotics should be used with more care has prompted strict regulations on the use of antibiotics in aquaculture and on the presence of antibiotic residues in aquaculture products. In most developed countries, governments have set obligatory Maximum Residue Levels (MRLs) for aquaculture products and also the public health risk associated with antimicrobial residues which depends on the quantity of the antimicrobial consumed or exposure.

More concern has been raised for the use of antibiotics in animals that may represent a potential threat to human health through the development of resistance and potential allergies. Recent studies have suggested that some resistant isolates from humans were more easily transmitted from companion animals or birds. There is enough evidence that shows that anti-microbial resistance among bacteria isolated from humans could be the result of using anti-microbial agents in food animals and has led to human consequences (Guardabassi and Kruse, 2008) which include;

- The infections that would not have otherwise occurred
- Increased frequency of treatment failures and increased severity of infection. Increased severity of infection includes longer duration of illness, increased frequency of blood stream infections, increased hospitalization and increased mortality.

- Occupational hazards associated with the usage of antibiotics. The absence of gloves or face mask during the administration of antibiotics has resulted in dermatitis (sulphonamides) and aplastic anaemia (chloramphenicol in animal husbandry).

In the aquatic animal, there are consequences with unjudicious use of antibiotics (Lee *et al.*, 2009; Salem *et al.*, 2011; Zounková *et al.*, 2011) which include;

- Direct antibiotic toxicity
- Adverse interractions with other drugs
- Interference with the protective effect of normal host microflora or the disturbance of the metabolic function of microbial flora in the digestive tract of herbivores
- The selection or promotion of antibacterial resistance
- Tissue necrosis at injection sites
- Drug residues in animal products that are intended for human consumption
- Impairment of the host's immune or defense mechanisms
- Damage to fetal or neonatal tissues

There is therefore the need to ensure the prudent use of antibiotics and the establishment of scientific monitoring of systems in order to limit the adverse effects of the abuse of antibiotics and enhance the safety of animal - derived foods and the environment (Hao *et al.*, 2014).

2.7 Way forward

The association between antibiotic use and resistance is evident both on individual and population levels. In the European Union, countries with large antibiotic consumption have higher resistance rates. Antibiotic resistance leads to failed treatments, prolonged hospitalizations, increased costs and deaths.

With few new antibiotics in the Research & Development pipeline, prudent antibiotic use is the only option to delay the development of resistance. Antibiotic policy consists of prescribing strategies to optimize the indication, selection, dosing, route of administration, duration and timing of antibiotic therapy to maximize clinical cure or prevention of infection whilst limiting the unintended consequences of antibiotic use, including toxicity and selection of resistant microorganisms. There is the urgent global call to value and support of antibiotic committees, guidelines, ID consultants and/or antimicrobial stewardship teams to prolong the efficacy of available antibiotics to reduce healthcare costs without adversely affecting the quality of care.

2.7.1 Regulations regarding antibiotic use in aquaculture

In recent times, the fight against antimicrobial resistance has taken a multi-dimensional approach as various international bodies such as the World Organisation for Animal Health (OIE), World Health Organisation (WHO) and Food and Agriculture Organisation (FAO) have critical roles to play to address non – human antimicrobial usage and its associated antimicrobial resistance and possible public health challenges (Guardabassi and Kruse, 2008).

The Codex Alimentarius is a body under the auspices of FAO and WHO that develops food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme. Its main purpose is to protect the health of consumers and to ensure fair trade practices in the international trade. The World Trade Organisation (WTO) Sanitary and Pytosanitary Agreement (SPS) signed in 1994, established the OIE as the reference organisation standards related to animal health, including zoonosis (OIE, 2016).

In order to minimize the possible impact of animal antimicrobial usage on public and animal health, various international organisations such as the WHO, OIE, FAO and the EU

Commission have consistently emphasized on the importance of prudent and rational antimicrobial use in animal production (Guardabassi and Kruse, 2008).

In 2015, the OIE made a strong contribution to the WHO's Global Action Plan on antimicrobial resistance which seek to ensure, for as long as possible, the treatment of infectious diseases with effective, quality antimicrobial agent (Guardabassi and Kruse, 2008). The OIE has been mandated by its member countries with support from FAO and WHO to create a global database for monitoring the use of antimicrobial agents, which is linked to the OIE's World Animal Health Information System (WAHIS).

The four main objectives of the OIE Strategy include:

- Improve awareness and understanding
- Strengthen knowledge through surveillance and research
- Support good governance and capacity building
- Encourage implementation of international standards

At the recent United Nations General Assembly meeting held on 21st September, 2016, there was a political declaration "One Health" which is in line with the Global Action Plan aimed at combating the global threat posed by the Antimicrobial Resistance (AMR).

At the regional level, the FAO collaborates with competent institutions, both governmental and non- governmental who are mandated by OIE to implement the code of conduct / guidelines to ensure effective monitoring of Antimicrobials in food production. In Ghana, Veterinary Services Commission is the competent authority that is mandated to prescribe and supervised the use of veterinary medicine in animal production. Section 76 of the Fisheries Regulation 2010 states it clear "A person shall not use a chemical or drug in any aquaculture establishment without a valid prescription by a Veterinary officer". Persons found culpable can be sentence to a term of imprisonment not exceeding three months in addition to a fine.

2.7.2 Alternatives to antibiotics

Probiotics have been proven to have positive impact on aquatic animal growth, survival and health. Probiotics are live beneficial micro-organisms unlike any other drug ingredients have the potential for infectivity or toxin production to obtain a desired outcome. The common probiotics used in aquaculture include *Lactobacillus, Lactococcus, Enterobacter, Pseudomonas, Clostridium and Saccharomyces* species among others.

In aquaculture, intestines, gills, skin mucus of aquatic animal and habitats can be used as sources for acquiring appropriate probiotics which can be bacteria and non-bacteria (Hai, 2015). Its safety depends on the nature of the specific microbe being used. Among the numerous health benefits attributed to probiotics, modulation of immune system is one of the most commonly purported benefits of the probiotics and their potency to stimulate the systemic and local immunity under in vitro and in vivo conditions is noteworthy (Nayak, 2010).

Probiotics mixtures have been shown to serve as treatment for Non – alcohol liver disease pathogenesis and improve better lipid profiles, better leptin and resistin levels in rats (Al-muzafar and Amin, 2017).

The use of probiotics in feed remains a growing public health issue as they are usually not prescribed by a competent authority and probiotic safety is characterized by the scarcity of studies to assess the safety of its usage over a long period of time in foods and animals. They are used for long periods in non-specific doses that may result in residues finding their way into food chain.

The holistic approach to avoid the use of antibiotic would involve ensuring biosecurity in all aquaculture establishment and good nutrition and development of fish vaccines for disease prevention.

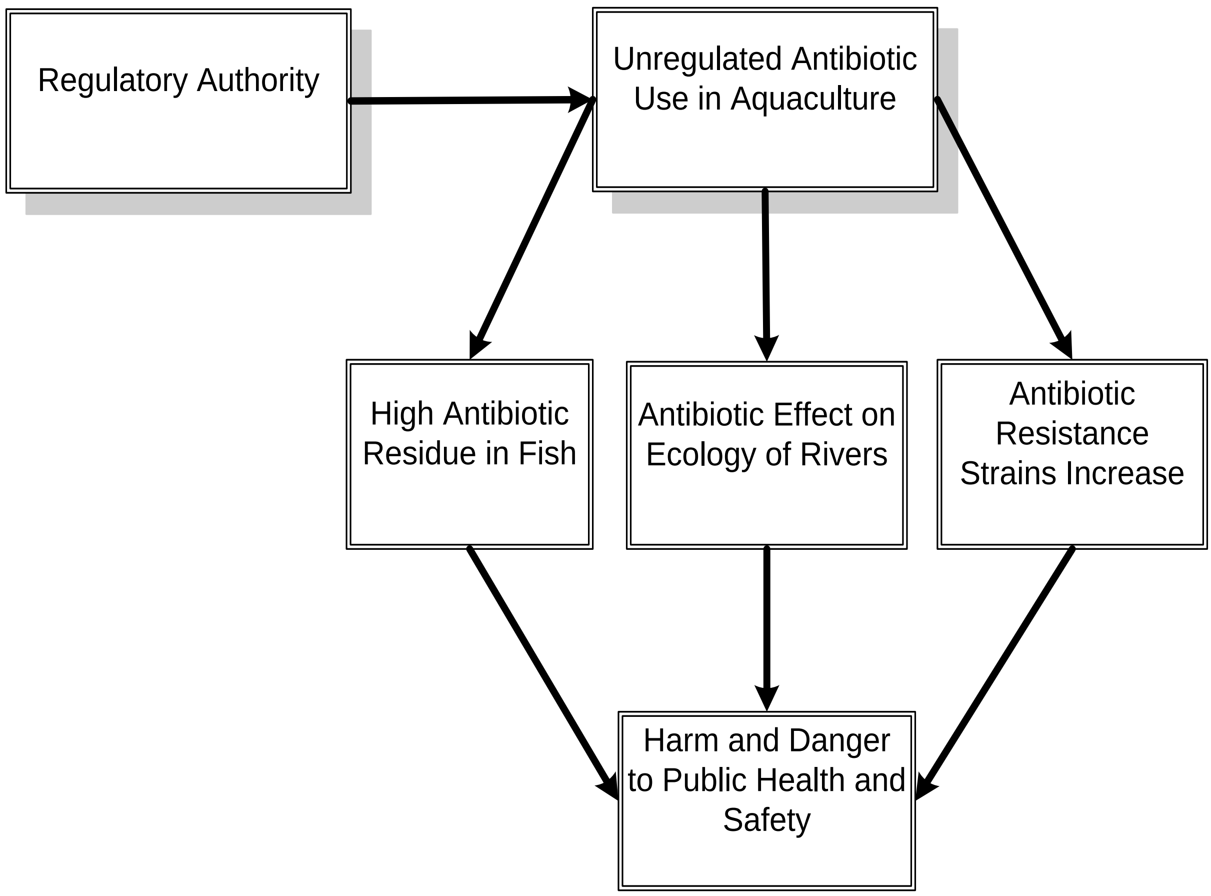

Figure 2.2: Conceptual framework for antibiotic use in aquaculture (MOFAD, 2016)

Chapter 3

Methodology

3.0 Research methods and design

The study involved three major activities: (1) questionnaire survey (quantitative component); (2) fish sample analysis and (3) in-depth Interviews (qualitative component) This chapter details how data was obtained through these approaches and how the data was analysed. A structured questionnaire was administered to managers or owners of selected fish farmers along the South Volta Basin in southern-eastern Ghana. The survey was on the use of antibiotics in fish-farming activities. It assessed farmers' knowledge of procedures for the procurement and use antibiotics in fish farming. In depth interviews were held with veterinarians from the Fisheries Commission to get their views on antibiotic use in aquaculture and their roles and responsibilities in the regulation of this practice. Laboratory analysis of procured fish samples from the selected fish farms was undertaken at the Veterinary Laboratory of the Veterinary Services Division, Accra. The chapter describes the methods used in the survey as well as the analysis of fish samples.

3.1 Study sites

The Asuogyaman District of the Eastern region was the study site. The District is one of the twenty-one (21) districts of the Eastern Region of Ghana. It covers a land area of 1507 sq. km. Its capital is Atimpoku. The district has a population of 74,124 (Ghana Statistical Service GPHS, 2000) which is made up of 35% rural and 65% urban, and has an annual growth rate of 1.7%. The vegetation is mainly forest, savannah woodland and regrowth.

The main water body in the district is the Volta Lake (largest man-made lake in the world). Fishing in and along the Volta Lake contributes 80% of the national output of the fish farming sub sector in Ghana. Fish farming is the primary subsistence and commercial occupation of inhabitants in almost all the communities along the 141 km shore line of the Lake. This includes communities such as Kpong, Dzidzokope, Atimpoku, Akosombo, Survey Line, Small London, South- Senchi and Dodi Asantekrom, Kudi Kope and Sedom.

The choice of this district for the study is based on the high concentration of fish farms and the growing number of fish farms and hatcheries on both large and small scales. These fish farms are owned by both Ghanaians and foreigners. Species of fish cultured include tilapia and catfish. The Fishery Commission maintains a registry of the names and number of fish farms in the area. This list is available at the zonal office of the Commission. As at the December 2016, the number of registered fish farms for the area was 146.

3.2 Study variables

3.2.1 Survey (quantitative component)

Using a list of fish farms obtained from the zonal office of the Fisheries Commission, systematic random sampling was used to select 70 out of the 146 registered farms to be used in the survey. The selected farms were categorized into large, medium and small scale farms, depending on their annual fish outputs.

Between February and March 2017, structured questionnaires were administered to farm managers/owners by trained research assistants within the district. The questionnaire comprised of sections on socio-demographic characteristics of the farm owners, routine operations of the farm, knowledge, attitudes and practices regarding antibiotic use. Farm owners who were not met during the administration of questionnaires were visited again to ensure that selected farms were covered. The interviews were carried out by enumerators who could translate the questions into the local language of the farmers. Each questionnaire was marked with a code.

The questionnaires were reviewed and approved by the Director of the Fisheries Commission. Pretesting of questionnaires was carried in the adjoining Shai-Osuduku District. Pretesting of questionnaires were carried out over 2 days. The draft questionnaire was subsequently revised.

Subsequent to the administration of the questionnaire and on days and times unknown to the famers, “mystery buyers” purchased fish samples from the farms and sales outlets of selected farms where the questionnaires were administered. The code used on the questionnaire was similarly applied to the fish samples to ensure matching of questionnaires to samples.

3.2.2 Survey (qualitative component)

A week following the purchase of fish samples from selected farms and sales outlets, an in-depth interview was conducted with two veterinarians who have specialized in aquatic animal health and have been seconded to the Fisheries Commission by the Veterinary Services Division of the Ministry of Food and Agriculture. They are responsible for the monitoring, regulating and management of Aquatic Animal Health activities in both the marine and aquaculture zones in the country for almost two decades. Issues on antibiotic use in aquaculture with regards to the regulations, challenges and proposed interventions by the relevant institutions were among the questions that were discussed. An interview guide was used in this interview and each interview session lasted for about half an hour. All the interviews were conducted at convenient places at the Head Office of the Fisheries Commission. Interviews were recorded. Notes were also taken on pertinent issues raised to enable follow-up questioning. Interviews were in English, and they were transcribed and typed into a computer. Content analysis was performed based on the thematic areas that formed the basis for the design of the interview guide.

3.3 Sampling

Selected fish samples (50) weighing about 1000g were purchased from pond sites or local sales outlets of the selected farms. They were kept in sealable transparent plastic bags with labels (identification code, date, time, species) stored on ice in ice boxes and transported to the veterinary laboratory within 48 hours of sample collection to prevent any deterioration of samples.

In the laboratory, the fish samples were stored at – 20^{o}C until samples were to be analyzed. The muscle and the liver of the fish samples were taken and analyzed for the presence or absence of antibiotics. All the analyses were conducted in the laboratories of the Veterinary Services Division of the Ministry of Food and Agriculture in Accra.

3.4 Laboratory analysis

The laboratory analysis comprised of preparation of the media based on the manufacturer's instructions. For this analysis, nutrient agar was used. Prior to media preparation, all the petri dishes to be used were washed well and rinsed with distilled water, packed into canisters, wrapped with aluminium foil and sterilized in a hot air oven at 121^{0} C for about 1 hour. After the set time, they are removed and placed in a laminar flow to cool.

3.4.1 Preparation of solid media

28 mg of nutrient agar was measured into 1000ml of distilled water, kept in a microwave for 5 minutes to dissolve and later sterilize in the autoclave for 15 minutes. After sterilization, the colour of the media changed from white to brown and 25ml was poured into the petri dishes to solidify.

3.4.2 Preparation of liquid media

7.5mg of the nutrient agar was measured into 250mls of distilled water, kept in the microwave for 5minutes to dissolve. Content was later sterilized in the autoclave for 15minutes. After sterilization, 5mls of media was poured into tubes and kept in a water bath to prevent it from solidifying.

3.4.3 Preparation of *Bacillus subtilis*

Bacillus subtilis was inoculated on a media plate for 24 hours to get 10^{-7}-fold colony forming unit by striking. A subculture was made and used as the original stock to prepare the 10^{-7} fold colony forming unit. A 10-fold serial dilution was prepared from the original stock by pipetting 100ul of stock and added to 900ul distilled water, well mixed and 500ul was poured onto five new media plate respectively, (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}).

3.4.4 Preparation of test sample

The test samples were thawed for four (4) hours prior to preparation. The samples were then dissected to remove the liver and put on a sterilized petri dish. Muscles from the same sample was also taken and added. This process was repeated for all the test samples.

3.4.5 Antibiotic sensitivity testing

Mueller Hinton (MH) agar plates for the tests were removed from the fridge where they were being stored after passing sterility test and dried in the incubator at 37^{o}C for about 1 hour. Two hundred (200ml) of MH agar was prepared per the manufacturer's instructions and poured 9ml each into 10ml vacutainer tubes and suspended in a rack at 50^{o}C in a water bath. One (1) ml of stock culture of standard reference bacterial (Bacillus subtilis) was pipetted into the first tube of the molten MH agar at 50^{o}C and a tenfold serial dilution was carried out. The last 1ml was discarded into a decontaminating jar of 1% bleach. The dilutions are immediately poured into corresponding solid formed plates of MH. Commercially prepared antibiotic sensitivity disc (Cipro) was placed into the plates at equidistance from the test samples.

The test samples were also prepared and inoculated as a tissue or a saturated disc of the test sample using sterilized forceps. The plates were incubated with the bottom down to prevent moisture from fallen back onto the culture media and for the sample at 37^{o}C for 24 hours. The plates were then removed from the incubator and checked for zones of inhibition around the controls as compared to the test sample sites. A positive test is where the positive standard controls show inhibition and the negative test showed none. All the samples were analyzed using the microbiological inhibition test to screen for samples containing antibiotic residues as described at the laboratory (Dang *et al.*, 2010). Observations were done using the eyes to identify the zone of inhibition.

The samples that tested positive could further undergo a post-screening test in order to identify the antibiotic class of the residues detected. The concentrations of the antibiotics could also be analyzed with High Performance Liquid Chromatography (HPLC) with Ultra Violet (UV) and fluorescence detection using the procedure described (Lee et al., 2007).

3.5 Data handling and analysis

In the quantitative component, data obtained from the questionnaires were entered into Excel spread sheet, checked and cleaned by a data analyst. Fish farms were classified as small, medium or large scale according to their production outputs. Data were analyzed by Stata 14 using descriptive statistics as appropriate.

In the qualitative component, all interviews were recorded and notes were also taken on pertinent issues raised to enable follow-up questioning. Interviews were transcribed and typed into a computer. Content analysis was performed based on the thematic areas that formed the basis for the design of the interview guide. Pertinent and summative quotes were noted and are reported.

3.6 Ethical considerations

In all the fish farms that the study was conducted, participation in the survey was voluntary. Researcher assistants introduced themselves, stated why they were on the farm, read out content of consent form and asked whether the respondents agree to be interviewed at the beginning of each interview. Farm owners/managers were assured that no identifiers will be used in the write-up on the research and farmers' identities will be protected at all times.

The study was approved by the Ethics Review Board of the Ensign College of Public Health and institutional approval was obtained from the Fisheries Commission.

3.7 Limitations of the study

The investigator encountered challenges in getting a laboratory in Accra that could perform the quantification analysis on the fish samples.

Within the scope of this study, quantities of antibiotics were not determined, so further studies could be done to establish the public health risk associated with antimicrobial residues based on the quantity of the antimicrobial encountered or consumed to address the data gap.

3.8 Funding

This study was funded by The World Bank under West African Regional Fisheries Project. The funders were not involved in the design, data collection, analysis and writing up of the study. The added knowledge from the study would assist the Fisheries Commission to strengthen and enforce strict aquatic animal health regulations to ensure safe and quality fish food to safeguard against food security and also to promote trade in aquatic animals in Ghana

and beyond. The study also received partial financial support from the Student Research Award Program (SRAP) at Ensign College of Public Health.

Chapter 4

Results

4.0 Demographic profile

Out of the 70 fish farmers interviewed, majority (95.7%) were males, with years of farm establishment at an average of 4.5 years. Most (95.7%) of the respondents had formal education, ranging from primary school to university. The scale of fish production of the respondents ranged from small scale through medium scale to the large scale. They produced for domestic and commercial purposes. Most (97.1%) of the fish farmers cultured tilapia, and the rest (2.9%) reared catfish. Monoculture was the dominant culture method constituting 97.1%, (table 4.1). Of the 91% of the respondents who were Ghanaians, 58.6% were Ewes and 15.7% Akans. A greater proportion (72.9%) of the respondents have never had any formal training in fish farming (table 4.1).

Table 4.1: Demographic characteristics of surveyed fish farms in Asuogyaman District

Variable	Frequency (%)
Sex	
Male	67 (95.7)
Female	3 (4.3)
Educational level	
No education	3 (4.3)
Primary	6 (8.7)
Secondary	44 (62.7)
Tertiary	17 (24.3)
Years of Farm Establishment	

Less than 1 year	2 (2.9)
1 to 5 years	40 (57.1)
6 to 10 years	23 (32.9)
More than 10 years	5 (7.1)
Nationality	
Ghanaian	64 (91.4)
Non-Ghanaian	6 (8.6)
Ethnicity	
Akan	11 (15.7)
Ewe	41 (58.6)
Ga/Ga Adangme	10 (14.3)
Other	8 (11.4)
Type of Fish Culture	
Catfish	2 (2.9)
Tilapia	68 (97.1)
Type of culture	
Integrated	1 (1.4)
Monoculture	68 (97.1)
Polyculture	1 (1.5)
Scale of Production	
Small scale	4 (5.7)
Medium scale	8 (11.4)
Large scale	58 (82.9)

Note: Data are frequencies (percentages)

4.1 Antibiotic use in aquaculture production

About 67.1% of the respondents admitted to the use of antibiotics in the production cycle to cure infections/diseases on their fishes without engaging the services of a veterinarian for diagnosis, prescription and supervision of the treatments. Majority of the respondents explained the difficulty in assessing the services of aquatic animal health specialists in the sector. Oxytetracycline was the commonest antibiotic that was being used by the respondents for treatment of infections in fish farms. Almost three quarters of the respondents (72.3%) used antibiotics for curative purposes while 23.4% and 4.3% used the antibiotics for preventive and growth stimulation purposes respectively. The antibiotics were mixed with fish feed and fed to the infected fishes in the pond or cage for about five (5) days or more, depending on the severity of the infection/ disease. Out of the 32.9% negative responses to antibiotic use in fish farming, 22.4% used salt, 5.1% used neem leaves, garlic and other herbal extracts and 1.9% used potassium per manganate solution to treat infections and wounds on fishes. About 3.5% of the farmers also added Aminovit to fish feed to enhance the feed intake and feed conversion ratio for their stocks. All the respondents who admitted to the use of antibiotics indicated that the antibiotics are used during the fingerling stage (young fishes) of the fishes' lifecycle as a result of poor handling and stress during their transportation from one farm to the another resulting in low immunity and thus increasing the risk of infections. Prior to harvest, fishes have high immunity so infections/diseases rarely occur unless there is an outbreak and so antibiotics are not used.

4.2 Sources of antibiotics

About 53.2% and 29.8% of the respondents who use antibiotics bought the antibiotics from drug stores or local agro chemical shops, respectively. Hatchery operators also served antibiotics to about 6.4% of the respondents. Other respondents (8.5%) indicated other farmers and relations as their source of antibiotics. Majority (72.3%) of the respondents do not have formal training in the antibiotic or drug administration, so application is based on the fish farmer’s judgement of the severity of the infection.

4.3 Describing the presence of antibiotic residues

Out of the 50 fish samples that were purchased form the farm sales outlet and farm gates and tested for antibiotic residues at the laboratory, a total of 38 (76%) of the liver samples tested positive for antibiotic residues by displaying zones of inhibitions on the cultured media. The samples that tested positive gave the same reaction as displayed by the antibiotic sensitive disc which served as positive controls on the cultured media. However, the muscle samples did not show detectable levels of antibiotic residues and thus there were no zones of inhibition on the culture media. Below is pictorial display of the samples showing zones of inhibition which indicates the presence of antibiotics in the fish samples.

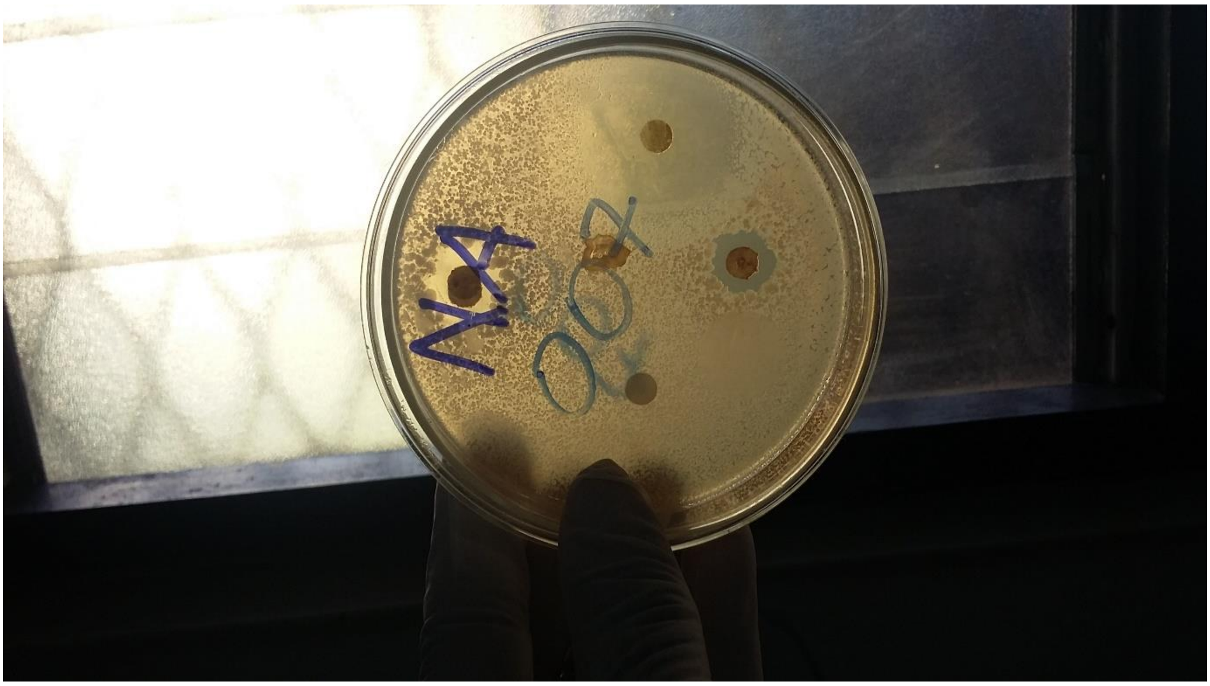

Figure 4.1: Sample showing zone of inhibition

Figure 4.2: Sample showing zone of inhibition

Figure 4.3: Sample showing no zone of inhibition

4.4 Fish farmers' knowledge and perception about antibiotics

Out of the 70 fish farms surveyed, majority (68.6%) confirmed that they knew about government regulations on aquaculture and antibiotic use, and 74.3% of the farmers agreed that antibiotic use without supervision or prescription from an expert was an offence under the regulations. Assessing the farmers' opinion about the purpose of antibiotics in the cure of infections, most (71.4%) responded positively to its effectiveness. Only 17.1% indicated that they do not know about the effectiveness of antibiotics in aquaculture. Majority, (82.9%) were of the strong view that imprudent use of antibiotics would lead to antibiotic resistance in fish and humans. Almost all the farmers (95.7%) also agreed that having enough knowledge on antibiotic use is essential to reduce or prevent abuse.

More than half of the farmers (57.1%) who used the antibiotics confirmed that they depended on their own experience to administer antibiotics in their practice, while 17.1% sought advice from fellow farmers. About one fifth of the farmers consult the fisheries officers and the Aquatic Animal Health specialist for advice. However, (94.3%) of the farmers were of the view that it was important to call an expert when you notice that your fishes are sick. Three quarters of the farmers surveyed agreed that use of antibiotics in aquaculture increases their total cost of production although they would avoid losses to fish stocks. More than half of the farmers (61.4%) indicated the importance of aquaculture health related seminars for fish farmers as good husbandry practices help to prevent fish diseases.

4.5 In-depth interviews with Aquatic Animal Health Specialists (AAHS)

In-depth interviews with two Aquatic Animal Health Specialists from the Fisheries Commission were conducted. The interviews focused on these issues: views on antibiotic use in fish farming in Ghana; specific sources of concern; food safety; common antibiotics;

challenges with enforcement of regulations; concerns about use of hormones in recent times to enhance growth of fishes.

"antibiotic use in fish farming in Ghana is unregulated and thus making it dangerous for all of us".

[First AAHS]

"*The administration of antibiotics in aquaculture is unregulated".*

[Second AAHS]

Regarding the specific ways in which antibiotic use could be a source of concern to the fishes, the aquatic ecosystem and public health

"The accumulation of antibiotic residues in fish and fish products and also the development of antibiotic resistance to certain common antibiotics."

[First AAHS]

"Imprudent use of antibiotics contaminates the aquatic environment, destroys its biodiversity and also enhances the development of antibiotic resistance strains".

[Second AAHS]

The two most common classes of antibiotic that are used in fish farming are the tetracycline (oxytetracycline) and the penicillin (amoxicillin). Accessing these antibiotics for use in fish farming is very easy as fish farmers buy them from drug stores and local agrochemical shops without any prescription, as is in the case with antibiotics for human use. The quantities and duration of administration will vary from farm to farm or individual to individual.

The specialists expressed worrying concern that fish farmers may be using antibiotics wrongly in their activities for any infection observed on their farms which would impact negatively on food safety and public health.

"Whatever they see, thus from a parasite to a unicellular organism, they will use antibiotics to cure regardless of the type of infection/disease or the specific antibiotic required for treatment and thereby abusing the antibiotic. An example is can be said of the imprudent use of antibiotic, Colistin in the pig formulations in the developed countries which has led to development of resistant strains of the antibiotic."

[First AAHS]

"Wrong administration of antibiotics would pollute the water and also increase antibiotic resistance pathogens in the environment which will find its way into the food chain and then to humans."

[Second AAHS]

Discussing the challenges with the enforcement of regulations and how to resolve the current situation to prevent a looming danger, the AAHS outlined certain measures that would help to address the current crisis with antibiotics as diseases will always be present in fish farming.

"There is the need to have enough human resource (aquatic animal health specialists/officers) in every zone of high aquaculture activity to conduct routine inspections of facilities."

"The importance of establishing maximum residue limits for the various antibiotics in the fish and fish products and certification of each harvest for consumption. Upon detection of residues that are above the recommended limits, closure or ban on sale of such fish can be enforced. This would deter fish farmers from using antibiotics on their own without

prescription or supervision. They would engage the services of trained staff to assist them with diagnosis and treatment and thus prevent the misuse of antibiotics on farms."

"Awareness creation on antibiotic use in fish farming on the consuming public would also help to curb the menace as consumers would be empowered to make informed decisions on their choice of fish and fishery products on the market"

[First and Second AAHS]

Chapter 5

5.0 Discussion

Fish farming has a relatively-recent history in Ghana but the pace of development of the industry is impressive; buoyed by increased local demand for freshwater fish. In acknowledgement of potential in this industry, the Government of Ghana has prioritized the development of aquaculture as a high-earning industry with potential for employment generation and foreign exchange earnings. In this thesis, we have applied both quantitative and qualitative data collection techniques to explore the use of antibiotics in fish farming in farms located along the Volta River Basin in the Asuogyaman District of the Eastern Region of Ghana. A thorough search of online scientific databases produced no result on such work in Ghana. This study is possibly the first to explore the practice in the country. It is anticipated that a discussion of the findings of the study will stimulate interest and attention to the regulation of antibiotic use in aquaculture in the country.

This Chapter will discuss the findings of the study, taking into consideration findings in other related studies. Towards the end of the Chapter, we situate the findings within the context of the regulatory framework for aquaculture in Ghana.

5.0 Unregulated use

This study found that there was near universal widespread, unregulated use of antibiotics in fish farms in the study area. About three-quarters of the sampled fish tested positive for the presence of antibiotic residues. On each farm visited, the use of antibiotics was routine and not under any expert directions. The practice does not appear to be consistent with the provisions of the Fisheries Act, 2002 (Act 625) and the Fisheries Regulation, 2010 (L.I.1968). It also contravenes provisions of the Environmental Protection Agency Act 1990 (Act490) and the Food and Drugs Act, 1992 (PNDCL 305B) which require that permission on the use of feed, drugs and chemicals should be sought and obtained from the Fisheries Commission. The findings in this study confirm the concerns of many researchers about the unregulated use of antibiotics in fish farming in developing countries (Naylor *et al.*, 2000; Cabello, 2006). This concern does not however appear to be receiving the required attention. In Ghana, data on antibiotic use in the aquaculture industry are not available as much studies have not been carried out since the commercial production began over a decade ago. There is also very little published work documenting the practice in sub-Saharan Africa. Globally, the industry is coming under immense criticisms due to widespread use of antimicrobial agents that poses the risk of accumulation of antimicrobial residues within the food chain and also the development of antimicrobial resistance which threatens food safety and public health (Pham et al., 2015; Wahlstr, 2003; Rogers & Basurco, 2009; Heuer et al., 2009; Romero et al., 2010). Countries in Europe and America have instituted mechanisms to ascertaining the level of antibiotic residues in aquaculture products.

5.1 Choices of antibiotics

This study has identified the tetracyclines to be the most widely used antibiotics. The responses from the survey revealed that more than half of the fish farmers use tetracycline. Although it was not clear in the study why these antibiotics were the most popular, it could be inferred that they are chosen because of their broad-spectrum properties. It was evident from the survey that the antibiotics are used for therapeutic purposes to treat any microbial infection observed during the production cycle without any diagnosis, thus increasing antibiotic residue risk and food safety consequences.

The use of broad-spectrum antibiotics in aquaculture has serious implications for their continued effectiveness in human health care (Heuer et al., 2009). It has been established that the consumption of antibiotic-contaminated fish and meat products have the potential to alter the human digestive tract in ways that favour infections such as Salmonella (Benbrook, 2002; Aarestrup, 2005; Gauker, 2010). Studies have also shown that the occurrence of resistance strains of bacteria in human pathogens severely limits the therapeutic options in human infection (Cole *et al.,* 2009)

5.2 Farmers' knowledge on regulations on antibiotic use

Despite the relatively high display of knowledge on antibiotic use and food safety issues by the fish farmers in this study, their adherence to the proper administration of antibiotics was very low. Their abuse of the antibiotics was seen to be economically motivated as indicated in other similar studies (Pham et al., 2015; Olatoye & Basiru, 2013). Their main objective for antibiotic use in fish production was to reduce morbidities and mortalities in order to minimise losses and regain their investments. This is similar to all intensive animal production establishment and thus the regular use of drugs (Gauker, 2010; Holmström et al., 2003; Asare & Gyansa-Lutterodt, 2015; Cabello, 2006; Caruso, 2016).

A greater proportion of the farmers (especially the large scale) knew about the government regulations that prohibits the use of drugs in aquaculture as the educational level of farmers from the survey was varied. Referring to the LI 1968 of 2010, the sub-section (1) regulation (75) clearly states that "a person shall not use a chemical or drug in any aquaculture establishment without a valid prescription by a veterinary officer". In addition, the sub-section (2) states that "a person who contravenes sub-regulation (1) commits an offence and is liable, on summary conviction, to a fine of not more than two hundred penalty units or to a term of imprisonment not exceeding fifteen months or to both". In spite of the above regulations on drug use in the industry, the farmers still use antibiotics without supervision or prescription from a veterinarian as there is very low enforcement and compliance of regulations as the regulatory agency has inadequate resources to effectively carry out this activity.

Farmers have unrestricted access to antibiotics (over the counter) mostly from local agrochemical stores and drug stores, thus making their frequent application very easy as indicated in studies from other countries where the industry is fast growing with low enforcement of regulations (Olatoye & Basiru, 2013; Pham et al., 2015). Some farmers highlighted the negative effect of imprudent use of antibiotics on the aquatic environment through pollution, making the industry unsustainable for future generations as documented in other studies (Li et al., 2016; Hernandez Serrano, 2005; Guardabassi & Kruse, 2008). Few farmers (the large scale farmers) in the study kept records of the antibiotics used and even retained prescription which is a mandatory practice in well-developed aquaculture industries (Rogers et al., 2009; Larson, 2006; Sarmah et al., 2006). A strict enforcement of the above regulations will be effective if sources of information on antibiotic use for farmers are not influenced by drug store operators, local agrochemical shops and other farmers in the industry (Pham *et al.*, 2015) but the regulatory agency. The regulatory agency must ensure good husbandry practices (biosecurity) in aquaculture establishments, encourage adequate feed composition, restrict movement of aquatic animals through regulations and develop vaccines for disease prevention.

5.3 Public awareness

When awareness on the use of antibiotics and its negative consequences are created using relevant mass media platforms, consumers would make informed decisions in their choice and consumption of fish and fish products, and this would force the industry to end this menace with imprudent use of antibiotics. The industry would gradually collapse with people losing their source of livelihoods and food security becomes threatened. Observations made during the study were consistent with other works in which some farmers operated under very unhygienic conditions and thus serve as a predisposing factor to infections / disease outbreaks and the abuse of antibiotics, disregarding its negative human impacts (Holmström et al., 2003; Marshall & Levy, 2011; Wahlstr, 2003; Heuer et al., 2009; Gauker, 2010)

5.4 "Mystery buying"

There is the urgent need for the regulatory agency to establish maximum residue levels or range for antibiotic used in aquaculture in order to make residue monitoring in the industry very effective. One of the important findings of this study is the fact that antibiotic residues were detected in fish products procured from producers who had denied their use of antibiotics. This is in contrast to a study which was conducted in Vietnam where farmers provided honest and reliable responses on the survey to monitor antibiotic use (Pham et al., 2015). It is therefore important to resource the aquatic animal health officers to engage the services of 'mystery buyers' during routine sampling of fish and also employ readily available scientific ways for screening and quantification of antibiotic residues in fish samples in aquaculture. In Ghana, there have not been any survey on the quantification of antibiotic residues in fish samples in aquaculture, as has been done in other developed countries (Defoirdt et al., 2011; Sekkin & Kum, 2011). The practice of regulating the use of antibiotics in aquaculture is likely to meet with some resistance, denial and scepticism from farmers. A culture of certification will need to be developed. In addition, ingredients for fish feeds and the feeds from the open markets would occasionally have to be sampled and tested.

5.5 Regulatory capacity

The market for aquaculture in Ghana is largely the domestic market and sub-regional. Within this scope, products hardly undergo any strict testing for antimicrobials/antibiotics to check for the residues, as in the case of other fish products that are bound for the export markets in the EU and the United States. In most developed nations like Norway, the United States and the EU, all fish products for both domestic and export consumption undergo series of testing and certified as wholesome for consumption, prior to sale, in order to ensure food safety and protect public health (Sekkin & Kum, 2011).

One of the major challenges encountered in carrying out this study was the lack of capacity to ascertain the levels of antibiotics in fish samples. None of the laboratories at the various regulatory agencies had the capacity to quantify levels of antibiotic residues. This is a major drawback to the enforcement of the provisions of the laws regulating the use of drugs and other chemicals in the industry. The detection of antibiotics in the sampled fishes cannot be regarded as completely unexpected. The lack of capacity to determine the levels of the residues undermines the ability to make a definite conclusion on the potential threat to food safety.

In developed countries such as in the EU, they have built institutional capacities to trace and quantify the levels of antimicrobial residues in aquaculture products to affirm quality and value of product prior to product sale. These processes affect the trade of aquatic products (Rogers & Basurco, 2009). The aspiration of the Government of Ghana to make aquaculture a major foreign exchange earner stands to be undermined by the capacity of regulatory agencies to check the levels of antibiotics in fish products from the country. The local consuming public are also equally at risk.

Chapter 6

Conclusions and Recommendations

6.0 Conclusion

The survey has generated information on the unregulated use of antibiotics in aquaculture, farmers' knowledge regarding antibiotic use in the Asuogyaman zone and the challenges that confront the regulatory agency as a whole. Inappropriate use of antibiotics is still highly prevalent in aquaculture in Asuogyaman, though the fish farmers expressed high knowledge in the subject matter during the data collection.

The results from the study gave an indication that those fish farmers operating on the small scale should be the main target of future awareness creation or campaign against antibiotic usage. The regulatory agency should institute capacity to monitor the proper use of approved antibiotics as it will continue to be necessary in animal production, including aquaculture, for consumers to be reassured that the use of approved antibiotics which may be administered without posing any hazard or risk to consumers

It is the hope of the investigator that the results from this will stimulate innovative and highly specific policy strategies and actions from the regulatory agency (Fisheries Commission) to provide appropriate preventive measures and/ or sanctions to the fish farmers aimed at improving husbandry practices through biosecurity and also educate them on the consequences of misuse of antibiotics on the fish and fishery products and associated public health risk. In addition, the use of immunostimulants and probiotics for disease control and vaccination options will also help to reduce the use of antimicrobial agents in the aquaculture industry.

The government of Ghana under the Ministry of Fisheries and Aquaculture should enforce strict legislations and implementation on use of antimicrobial drugs in aquaculture, provide a functional fish diagnostic laboratory and train more aquatic animal health professionals to render efficient and effective extension services in order to safeguard the fish, humans and the environment against the development and spread of antimicrobial resistance as a result of antimicrobial misuse and residues in aquaculture.

6.1 Recommendation

There is the need for support for the Fisheries Commission laboratory to have the capacity to perform quantification analysis for antimicrobial residues without which enforcement of regulations would be impossible in the sector.

It is also recommended that the Fisheries Commission would establish institutional collaborations with the FAO to build its capacity in the development of a better national data on usage of different antimicrobials, the occurrence on residues of various antimicrobials, prevalence of antimicrobial resistance in various bacteria, spread of resistance genes to human pathogens, effects of antimicrobials residues on intestinal microflora, and the pharmacological and pharmacokinetical aspects of antimicrobials used in order to create public awareness on antimicrobials in aquaculture.

References

Aarestrup, F. M. (2005) ‘Veterinary Drug Usage and Antimicrobial Resistance in Bacteria of Animal Origin’, *Basic and clinical pharmacology and toxicology*, 96(4), pp. 271–281.

Al-muzafar, H. M. and Amin, K. A. (2017) ‘Probiotic mixture improves fatty liver disease by virtue of its action on lipid profiles , leptin , and inflammatory biomarkers’, *BMC Complementary and Alternative Medicine*. BMC Complementary and Alternative Medicine, pp. 1–9.

Aminov, R. I. (2013) ‘Biotic acts of antibiotics’, *Frontiers in Microbiology*, 4(AUG), pp. 1–16.

Asare, B. A. and Gyansa-Lutterodt, M. (2015) ‘The Ghana example: The challenges of antimicrobial use in low and Middle income countries’.

Bagal, V. L., Khatta, V. K., Tewatia, B. S. and Sangwan, S. K. (2016) ‘Relative efficacy of organic acids and antibiotics as growth promoters in broiler chicken’, *Veterinary World*, 9(4), pp. 377–382.

Beebe and S (2010) ‘Impacts of Agriculture on Human Health and Nutrition’.

Benbrook, C. M. (2002) *Antibiotic Drug Use in U . S . Aquaculture*. Sandpoint, Idaho.

Bieranye, S., Martin, B., Adu, F., Agyare, C., Gbedema, S. Y., Boamah, V. E. and George, D. F. (2013) ‘Antibiotic resistance patterns of strains of Staphylococcus aureus isolated from patients in three hospitals in Kumasi , Ghana’, *Journal of Bacteriology Research*, 5(3), pp. 35–40.

Bosma, R. H. and Verdegem, M. C. J. (2011) ‘Sustainable aquaculture in ponds: Principles, Practices and Limits’, *Elsevier*, 139, pp. 58–68.

Braykov, N. P., Eisenberg, J. N. S., Grossman, M., Zhang, L., Vasco, K., Cevallos, W., Moser, K. A., Marrs, C. F., Foxman, B., Trostle, J., Trueba, G. and Levy, K. (2016) ‘Antibiotic Resistance in Animal and Environmental Samples Associated with Small-Scale Poultry Farming in Northeastern Ecuador’, *mSphere*, 1(1), pp. 1–15.

Brown, K., Zaytsoff, S. J. M., Uwiera, R. R. E. and Inglis, G. D. (2016) ‘Antimicrobial growth promoters modulate host responses in mice with a defined intestinal microbiota’, *Scientific Reports*, 6(1), p. 38377.

Burridge, L., Weis, J. S., Cabello, F., Pizarro, J. and Bostick, K. (2010) *Chemical Use in Marine Finfish Aquaculture in Canada: A Review of Current Practices and Possible Environmental Effects, Fisheries and Oceans Canada.*

Cabello, F. C. (2006) ‘Heavy use of prophylactic antibiotics in aquaculture : A growing problem for human and animal health and for the environment’, 8(7), pp. 1137–1144.

Cabello, F. C., Godfrey, H. P., Tomova, A., Ivanova, L., Dölz, H., Millanao, A. and Buschmann, A. H. (2013) ‘Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health’, *Environmental Microbiology*, 15(7), pp. 1917–1942.

Caruso, G. (2016) 'Antibiotic Resistance in Fish Farming Environments: A Global Concern Antibiotic Resistance in Fish Farming', *Journal of FisheriesSciences.Com Journal*, 10(104), pp. 9–13.

Cháfer-Pericás, C., Maquieira, Á. and Puchades, R. (2010) 'Fast screening methods to detect antibiotic residues in food samples', *TrAC Trends in Analytical Chemistry*, 29(9), pp. 1038–1049.

Cole, D. W., Cole, R., Gaydos, S. J., Gray, J., Hyland, G., Jacques, M. L., Powell-Dunford, N., Sawhney, C. and Au, W. W. (2009) 'Aquaculture: Environmental, toxicological, and health issues', *International Journal of Hygiene and Environmental Health*, 212(4), pp. 369–377.

Dang, P. K., Degand, G., Danyi, S., Pierret, G., Delahaut, P., Ton, V. D., Maghuin-Rogister, G. and Scippo, M.-L. (2010) 'Validation of a two-plate microbiological method for screening antibiotic residues in shrimp tissue', *Analytica Chimica Acta*, 672(1–2), pp. 30–39.

Defoirdt, T., Sorgeloos, P. and Bossier, P. (2011) 'Alternatives to antibiotics for the control of bacterial disease in aquaculture', *Current Opinion in Microbiology*, 14(3), pp. 251–258.

Done, H. Y., Venkatesan, A. K. and Halden, R. U. (2015) 'Does the recent growth of aquaculture create antibiotic resistance threats different from those associated with land animal production in agriculture?', *The AAPS Journal*, 17(3), pp. 513–524.

Edward J. Noga (2010) *Fish Disease: Diagnosis and Treatment*. 2nd edn. Wiley-Blackwell.

FAO (2010) 'Fisheries and Aquaculture Department Food And Agriculture Organization Of The United Nations', *The State of World Fisheries and Aquaculture*.

Faruk, M. A. R., Ali, M. M. and Patwary, Z. P. (2010) 'Evaluation of the status of use of chemicals and antibiotics in freshwater aquaculture activities with special emphasis to fish health management', *Journal of the Bangladesh Agricultural University*, 6(2), pp. 381–390.

Guardabassi, L. and Kruse, H. (2008) 'Principles of prudent and rational use of antimicrobials in animals', in Lars Bogø Jensen Luca Guardabassi, H. K. (ed.) *Guide to Antimicrobial Use in Animals*. Oxford, UK: Blackwell Publishing Ltd, pp. 1--12.

Gupta, M.V. and Acosta, B. . (2004) 'A review of global tilapia farming practices.', *Aquaculture Asia*, 9, pp. 7–12.

Gyansa-Lutterodt, M. (2013) 'Antibiotic resistance in Ghana', *The Lancet Infectious Diseases*, 13(12), pp. 1006–1007.

Gyssens, I. C. (2017) 'Antibiotic policy', *International Journal of Antimicrobial Agents*. Elsevier, 38, pp. 11–20.

Hai, N. V (2015) 'The use of probiotics in aquaculture', *Journal of Applied Microbiology*, 119(4), pp. 917–935.

Hao, H., Cheng, G., Iqbal, Z., Ai, X., Hussain, H. I., Huang, L., Dai, M., Wang, Y., Liu, Z. and Yuan, Z. (2014) 'Benefits and risks of antimicrobial use in food-producing animals', *Frontiers in Microbiology*. Frontiers Research Foundation, pp. 1–11.

Hernandez Serrano, P. (2005) *Responsible use of antimicrobials in aquaculture, FAO*

Fisheries Technical Paper.

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(8), pp. 1248–1253.

Hollis, A. and Ahmed, Z. (2014) ‘The path of least resistance: Paying for antibiotics in non-human uses’, *Health Policy*. Elsevier Ireland Ltd, 118(2), pp. 264–270.

Holmström, K., Gräslund, S., Wahlström, A., Poungshompoo, S., Bengtsson, B.-E. and Kautsky, N. (2003) ‘Antibiotic use in shrimp farming and implications for environmental impacts and human health’, *International Journal of Food Science & Technology*. Blackwell Science Ltd, 38(3), pp. 255–266.

Holmstrom K, Graslund S, Wahlstrom A, Poungshompoo S, Bengtsson B, K. N. (2003) ‘Antibiotic use in shrimp farming and implications for environmental impacts and human health’, *International Journal of Food Science and Technology*, 38(3), pp. 255–266.

Huang, L., Xu, Y.-B., Xu, J.-X., Ling, J.-Y., Chen, J.-L., Zhou, J.-L., Zheng, L. and Du, Q.-P. (2017) ‘Antibiotic resistance genes (ARGs) in duck and fish production ponds with integrated or non-integrated mode’, *Chemosphere*, 168, pp. 1107–1114.

Huchzermeyer, D. (2016) *Aquatic Animal Health Policy Inception Report*.

Jiang, H., Zhang, D., Xiao, S., Geng, C. and Zhang, X. (2013) ‘Occurrence and sources of antibiotics and their metabolites in river water, WWTPs, and swine wastewater in Jiulongjiang River basin, south China’, *Environmental Science and Pollution Research*, 20(12), pp. 9075–9083.

Kathleen, M. M., Samuel, L., Felecia, C., Reagan, E. L., Kasing, A., Lesley, M. and Toh, S. C. (2016) ‘Antibiotic Resistance of Diverse Bacteria from Aquaculture in Borneo’, *International Journal of Microbiology*. Hindawi Publishing Corporation, 2016.

Kwadwo Frimpong, S., Adwani, A. (2015) ‘The Challenges and Prospects of Fish Farming in Ghana : A Project Management Perspective’, *International Journal of ICT and Management*, (2), pp. 29–34.

Larson, E. (2006) ‘regarding antibiotic use among Latinos in the United States : Review and recommendations’, *American Journal of Infection Control*, 34(8), pp. 495–502.

Lee, J.-B., Chung, H.-H., Chung, Y.-H. and Lee, K.-G. (2007) ‘Development of an analytical protocol for detecting antibiotic residues in various foods’, *Food Chemistry*, 105(4), pp. 1726–1731.

Lee, S., Najiah, M., Wendy, W. and Nadirah, M. (2009) ‘Chemical composition and antimicrobial activity of the essential oil of Syzygium aromaticum flower bud (Clove) against fish systemic bacteria isolated from aquaculture sites’, *Frontiers of Agriculture in China*, 3(3), pp. 332–336.

Li, J., Zhang, H., Chen, Y., Luo, Y. and Zhang, H. (2016) ‘Sources identification of antibiotic pollution combining land use information and multivariate statistics’, *Environmental Monitoring and Assessment*, 188(7), p. 430.

Lin, J. (2014) ‘Antibiotic growth promoters enhance animal production by targeting intestinal bile salt hydrolase and its producers', *Frontiers in Microbiology*. Frontiers Research Foundation, pp. 1–4.

Lundebye, A.-K., Lock, E.-J., Rasinger, J. D., Nøstbakken, O. J., Hannisdal, R., Karlsbakk, E., Wennevik, V., Madhun, A. S., Madsen, L., Graff, I. E. and Ørnsrud, R. (2017) 'Lower levels of Persistent Organic Pollutants, metals and the marine omega 3-fatty acid DHA in farmed compared to wild Atlantic salmon (Salmo salar)', *Environmental Research*, 155, pp. 49–59.

Lupin, H. (2009) 'Human health aspects of drug and chemical use in aquaculture', *CIHEAM Options Méditerranéennes*, 103(86), pp. 95–103.

Marshall, B. M. and Levy, S. B. (2011) 'Food animals and antimicrobials: Impacts on human health', *Clinical Microbiology Reviews*, 24(4), pp. 718–733.

Meek, R. W., Vyas, H., Jane, L. and Piddock, V. (2015) 'Nonmedical Uses of Antibiotics : Time to Restrict Their Use ?', *PLoS Biology*, 1(10), pp. 1–11.

MOFAD (2016) *Annual Report*. Accra.

Moriarty, D. J. W. (1999) 'Disease Control in Shrimp Aquaculture with Probiotic Bacteria', *Proceedings of the 8th International Symposium on Microbial Ecology - Microbial Interactions in Aquaculture Disease*, 2013, p. 424123.

Nayak, S. K. (2010) 'Probiotics and immunity: A fish perspective', *Fish and Shellfish Immunology*, pp. 2–14.

Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N., Beveridge, M. C. M., Clay, J., Folke, C., I, J. L., Mooney, H. and Troell, M. (2000) 'Effect of aquaculture on world ® sh supplies', *Nature*, 405(6790), pp. 1017–1024.

OIE (2016) 'The OIE Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials', (November).

Olatoye, I. O. and Basiru, A. (2013) 'Antibiotic Usage and Oxytetracycline Residue in African Catfish (Clarias gariepinus in Ibadan, Nigeria)', *World Journal of Fish and Marine Sciences*, 5(3), pp. 302–309.

Paterson, I. K., Hoyle, A., Ochoa, G., Baker-austin, C. and Taylor, N. G. H. (2016) 'Optimising Antibiotic Usage to Treat Bacterial Infections', *Scientific Reports*. Nature Publishing Group, 6(1), p. 37853.

Pham, D. K., Chu, J., Do, N. T., Degand, G., Delahaut, P., Pauw, E. De, Douny, C., Nguyen, K. Van, Vu, T. D., Scippo, M. and Wertheim, H. F. L. (2015) 'Monitoring Antibiotic Use and Residue in Freshwater Aquaculture for Domestic Use in Vietnam', *EcoHealth*, 12(3), pp. 480–489.

Picklo, M. J. (2016) 'Chapter 13 - Farmed Fish: A Valuable Source of Lipid Based Nutrients A2 - Raatz, Susan K.', in Bibus, D. M. B. T.-F. and F. O. in H. and D. P. (ed.). San Diego: Academic Press, pp. 161–167.

Pulcini, C., Mohrs, S., Beovic, B., Gyssens, I., Theuretzbacher, U., Cars, O., Beovic, B., Bosevska, G., Bruch, M., Bush, K., Cars, O., Cizmovic, L., Daneman, N., Demoré, B., Deptuła, A., Dumpis, U., Fleming, A., Frimodt-Møller, N., Giamarellou, H., Gojkovic-

Bukarica, L., Gudnason, T., Gyssens, I., Hanberger, H., Harbarth, S., Harxhi, A., Kantardjiev, T., Kostalova, D., Krcmery, V., Kurvits, K., Ludwig, E., Lyytikäinen, O., MacGowan, A., Mohrs, S., O'Connor, S., Pagani, L., Petrikkos, G. L., Popescu, G. A., Pulcini, C., Raka, L., Paño Pardo, J. R., Dias, L. C. R., Salvesen-Blix, H., Simonsen, G. S., Tacconelli, E., Theuretzbacher, U., Turnidge, J., Valintėlienė, R., Vlahović-Palčevski, V., Zarb, P. and Zemlickova, H. (2017) 'Forgotten antibiotics: a follow-up inventory study in Europe, the USA, Canada and Australia', *International Journal of Antimicrobial Agents*. Elsevier B.V., 49(1), pp. 98–101.

Rigby, B., Davis, R., Bavington, D. and Baird, C. (2017) 'Industrial aquaculture and the politics of resignation', *Marine Policy*, 80, pp. 19–27.

Rogers, C. and Basurco, B. (2009) 'in Antimicrobial agents in aquaculture : practice , needs and issues', *Rogers C. (ed.), Basurco B. (ed.). The use of veterinary drugs and vaccines in Mediterranean aquaculture. Zaragoza : CIHEAM*, 59, pp. 41–59.

Rolain, J. M. (2013) 'Food and human gut as reservoirs of transferable antibiotic resistance encoding genes', *Frontiers in Microbiology*. Frontiers Research Foundation, pp. 1–10.

Romero, J., Feijoó, C. G. and Navarrete, P. (2012) 'Antibiotics in Aquaculture – Use , Abuse and Alternatives', *Health and Environment in Aquaculture*, pp. 160–198.

Salem, R., Lewandowski, R. J., Kulik, L., Wang, E., Riaz, A., Ryu, R. K., Sato, K. T., Gupta, R., Nikolaidis, P., Miller, F. H., Yaghmai, V., Ibrahim, S. M., Senthilnathan, S., Baker, T., Gates, V. L., Atassi, B., Newman, S., Memon, K., Chen, R., Vogelzang, R. L., Nemcek, A. A., Resnick, S. A., Chrisman, H. B., Carr, J., Omary, R. A., Abecassis, M., Benson III, A. B. and Mulcahy, M. F. (2011) 'Radioembolization Results in Longer Time-to-Progression and Reduced Toxicity Compared With Chemoembolization in Patients With Hepatocellular Carcinoma', *Gastroenterology*. Elsevier, 140(2), pp. 497–507.

Sapkota, A., Sapkota, A. R., Kucharski, M., Burke, J., McKenzie, S., Walker, P. and Lawrence, R. (2008) 'Aquaculture practices and potential human health risks: Current knowledge and future priorities', *Environment International*, 34(8), pp. 1215–1226.

Sarmah, A. K., Meyer, M. T. and Boxall, A. B. A. (2006) 'A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment', *Chemosphere*, 65, pp. 725–759.

Sekkin, S. and Kum, C. (2011) 'Antibacterial Drugs in Fish Farms: Application and Its Effects', *Chapter*.

Singer, A. C., Shaw, H., Rhodes, V., Hart, A. and Balcazar, J. L. (2016) 'Review of antimicrobial resistance in the environment and its relevance to environmental regulators', *Frontiers in Microbiology*. Frontiers Research Foundation, pp. 1–22.

Stanton, T. B. (2013) 'A call for antibiotic alternatives research', *Trends in Microbiology*. Elsevier Ltd, pp. 111–113.

Subramani, P. A. and Michael, R. D. (2017) 'Chapter 4 - Prophylactic and Prevention Methods Against Diseases in Aquaculture A2 - Jeney, Galina BT - Fish Diseases', in *Fish Diseases*. Academic Press, pp. 81–117.

Troell, M., Kautsky, N., Beveridge, M., Henriksson, P., Primavera, J., Rönnbäck, P., Folke,

C. and Jonell, M. (2017) ‘Aquaculture BT - Reference Module in Life Sciences’, in. Elsevier.

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(2), pp. 425–432.

Zhang, Q., Jia, A., Wan, Y., Liu, H., Wang, K., Peng, H., Dong, Z. and Hu, J. (2014) ‘Occurrences of Three Classes of Antibiotics in a Natural River Basin: Association with Antibiotic-Resistant Escherichia coli’, *Environmental Science & Technology*. American Chemical Society, 48(24), pp. 14317–14325.

Zounková, R., Klimešová, Z., Nepejchalová, L., Hilscherová, K. and Bláha, L. (2011) ‘Complex evaluation of ecotoxicity and genotoxicity of antimicrobials oxytetracycline and flumequine used in aquaculture’, *Environmental Toxicology and Chemistry*. John Wiley & Sons, Inc., 30(5), pp. 1184–1189.

Appendices

Appendix A: Participant Consent Form

Part 1. Participant Information

Introduction

I am from Ensign College of Public Health in Kpong and conducting a study to explore Antibiotic use in Aquaculture in the Asuogyaman zone. I will be explaining all about the study to you and you will also receive a copy of the leaflet that explains all about this research that you are being asked to join in. Please take all the time you need to read it carefully. You may ask me any questions about anything you do not understand at any time. You are a volunteer. You can choose not to take part and if you join, you may quit at any time. There will be no penalty if you decide to quit the study.

Rationale

It has been realized that the antibiotics used by humans are also being administered in fish farms for various reasons without consultations with the relevant authority and thus could contribute to antibiotic resistance in future in the industry if not addressed immediately, hence the reason for such a study in Asuogyaman zone which supplies about 80% of the total national fish output.

Why you are being asked to participate

You have been asked to take part in this study because you engage in aquaculture activities in the Asuogyaman zone.

Procedures

A trained research assistant will ask you a series of survey questions alone for approximately 30 - 45 minutes. Your responses will be recorded on paper and later entered into a computer database by study staff.

Risk and Benefits

I anticipate minimal or no risk to you. There is no direct benefit to you for being in the study; however, study outcomes may lead to better understanding of factors that may contribute to the antimicrobial resistance and the emergence of fish diseases in Aquaculture and provide effectively interventions to protect public health.

Confidentiality

All data will be de-identified and will be kept private. Data will be entered without any information that will make it possible for your identity to be known. The information you provide will be kept strictly confidential and will be available only to persons related to the study. (myself and my supervisors) The Fisheries Commission and Office of Ethical Review Board of Ensign College may also have access to study records upon their request.

Your responses will not be shown to other participants or community members. The original survey forms will be destroyed once data entry is complete.

Voluntariness and Withdrawal

Your participation in the study is completely voluntary and you reserve the right not to participate, even after you have taken part, to withdraw. This is your right and the decision you take will not be disclosed to anyone. It will not affect the extension services that will be offered to you in future. Please note however, that some of the information that may have been obtained from you without identifiers may inform certain policy decisions and may be used in publications.

Cost/Compensation

Your participation in this study will not lead to you incurring any monetary cost during or after the study.

Who to contact

This study has been approved by the Fisheries Commission and the Institutional Review Board of Ensign College. If you have any concern about the conduct of this study, your welfare or your rights as a study participant or if you wish to ask questions, or need further explanations later, you may contact me. Mary Nkansa (0246 634120) of Ensign College of Public Health, or My Supervisor Dr. Frank Baiden (0204591181) and Dr. Ziddah (0244254048). You may also contact the Administrator of the Institutional Ethics Committee of the Ensign College of Public Health at (+233245762229).

Thank you.

Do you have any questions?

Part 2. CONSENT DECLARATION

"I have read the information given above, or the information above has been read to me. I have been given a chance to ask questions concerning this study; questions have been answered to my satisfaction. I now voluntarily agree to participate in this study knowing that I have the right to withdraw at any time without affecting future extension services"

Left thumbprint of participant

Name of **Participant** ____________________________________

Signature of **Participant** ____

Date: / / 2017

Name of **Witness** ____

Signature of **Witness** ____

Date: / / 2017

Name of **Investigator** ____

Signature of **Investigator** ____

Date: / / 2017

Appendix B: Fish farm Questionnaire

Section 1: GENERAL INFORMATION

No.	Question	
1.	Date of Survey	………. / ………. / ……….. (DD/MM/YYYY)
2.	Name / Tel. No. of Enumerator	………………………………………………..

Section 2: LOCATION

3.	District	
4.	Community/Town	

Section 3: FARMER/FARM IDENTIFICATION

5.	How many years has the farm been operational	1. 1ess than 1 year [ ] 2. 1 – 5 years [ ] 3. 6 - 10 years [ ] 4. More than 10 years [ ]
6.	Telephone Number of Farm/Farmer	
7.	Sex of Farm Manager/Owner	1. Male [ ] 2. Female [ ]
8.	Nationality of Owner	1. Ghanaian [ ] 2. Non – Ghanaian [ ]
9.	If Ghanaian, tick ethnicity	1. Akan [ ] 2. Ga/ Ga Dangme [ ] 3. Ewe [ ] 4. Hausa [ ] 5. Other, specify ………………………………………..
10	Educational Level of Farm Manager/Owner	1. No Education [ ] 2. Primary [ ] 3. JHS/MSLC [ ] 4. Secondary/Vocational/Technical [ ] 5. Degree/Diploma/HND [ ] 6. Postgraduate [ ] 7. Others (state)________________________________

Section 4: PRODUCTION

11.	Types of Fish cultured	1. Tilapia [ ] 2. Catfish [ ] 3. Others (specify) [ ]
12.	Type of Culture	1. Monculture [ ] 2. Polyculture [ ] 3. Intergrated [ ]
13.	Type of Production System	1. Extensive [ ] 2. Semi-intensive [ ] 3. Intensive [ ]
14.	Scale of Production	1. Small Scale [ ] 2. Medium Scale [ ] 4. Large Scale [ ]
15.	Have you ever been trained in Fish farming?	1. Yes [ ] 1. No [ ]
16..	If Yes, what kind of training have you received? (choose all that apply)	1. Hatchery Management [ ] 2. Water Quality Management [ ] 3. Feed and Feeding [ ]

		4. Fertilization and Manuring [ ] 5. Sanitation and Diseases [ ] 2. Others (Specify) ………………………………….
18..	If Yes, which organization has provided training to you before?	1. Fish farmers' association [ ] 2. Government Agencies [ ] 3. Drug manufacturing companies [ ] 3. Drug sellers [ ]
19.	When did you receive your last training?	1. Last six months 2. One to two years now 3. Two and above years 4. Other specify ……………………………
20.	What kind of feed do you most/usually feed to your fishes	1. Formulate own feed [ ] 2. Use already formulated feed [ ] 3. Both [ ]
21.	State all the ingredients you use in formulating your feed (Tick as applicable)	1. Wheatbran [ ] 2. Soyabean [ ] 3. Fishmeal [ ] 4. Ricebran [ ] 5. Others, specify………………………….
22	Which of the following sanitary methods do you do	1 Liming of ponds [ ] 2 Fallowing of ponds [ ] 3 Defouling of nets [ ] 4. Frequent draining of water [ ] 5 Use of aerators [ ] 6 Others, specify…….
23	Who do you call when you notice that your fishes are sick	1. Fish Vet 2. Fisheries Officer 3. Buy antibiotics from Agro-Vet shop to treat 4. Other farmers for help 5. Others (state)……………

Section 5: SOURCE AND USE OF ANTIBIOTICS

24	Have you ever used antibiotics in your fish farming?	1. Yes [ ] 2. No [ ] 3. Don't know or decline to answer [ ]
25	What kind of antibiotics did you use on your farm?	1. Tetracycline [ ] 2. Penicillin [ ] 3. Suphonamides [ ] 4. Others (specify)……..
26	If you use antibiotics, what (did) do you use it for?	1. Preventive drug [ ] 2. Curative drug [ ] 3. Feed formulation [ ] 4. Others (specify) ……………………………………
27	Where (did) do you mostly get your antibiotics from?	1. Local Agrochemical Shops [ ] 2. Importation [ ] 3. Veterinary Services [ ] 4. Fingerling seller [ ] 5. Others (specify) …………………………………
28	Who administers (d) antibiotics to the fish?	1 Farm Owner [ ] 2 Farm Manager [ ] 3 Other Farm hands [ ]
29	Is this person who administers drugs trained to do so?	1. Yes [ ] 2. No [
30	Have you ever attended a seminar/field day/field demonstration sponsored by a drug company?	1 Yes [ ] 2 No [ ]
31	If yes to above, what are some of the information you were given on drug and	1. Name of drugs [ ] 2 Dosage [ ]

	antibiotics.	3 Route of administration [ ] 4 Withdrawal period [ ] 5 Others, specify …………
32	When do you decide to use antibiotics?	1 Immediately [ ] 2 After a week [ ] 3 After a month [ ] 4 Never [ ]
33	Do you apply antibiotics to fish prior to harvesting?	1. Yes [ ] 2 No [ ]
34	**If yes**, why?	
35	**If no**, why?	
36	Whom do you sell your fish to?	1. Restaurants [ ] 2. Individuals consumers [ ] 3. Retailers [ ] 4. Others, specify………………………..

Section 6: KNOWLEDGE AND PERCEPTION ABOUT ANTIBIOTICS

37	Good sanitation and hygiene help to prevent animal disease.	1. Agree [ ] 2. Disagree [ ] 3. Don't Know [ ]
38	It is always important to call an expert when you notice that your fishes are sick	1 Agree [ ] 2 Disagree [ ] 3 Don't Know [ ]
39	Having enough knowledge or information on a particular antibiotic is essential to reduce or prevent abuse.	1. Agree [ ] 2. Disagree [ ] 3. Don't Know [ ]
40	Antibiotics are effective against infections	1 Yes [ ] 2 No [ ] 3. Don't Know [ ]
41	Imprudent use of antibiotics lead to antibiotic resistance in fishes and also in humans	1 Yes [ ] 2 No [ ] 3. Don't know [ ]
42	There is government regulation(s) on the use of antibiotics	1 Yes [ ] 2 No [ ] 4 Don't Know [ ]
43	It is an offence to use drugs (antibiotics) without the supervision or prescription from a fish vet	1 Agree [ ] 2 Disagree [ ] 3 Don't Know [ ]
44	The use of antibiotics increases your cost of production	1 Agree [ ] 2 Disagree [ ] 3. Don't Know [ ]
45	It is not important to attended any aquaculture health related seminar in fish farming.	1 Agree 2 Disagree 3 Don't Know
46	It is important to have a good relationship with the Agro-vet shop?	1 Agree [ ] 2 Disagree [ ] 3 Don't Know [ ]

Appendix C: Questionnaire for an in depth interview on antibiotics use in aquaculture

1) Demographic information

a. Tel. No.
b. Age
c. Sex
d. Ethnicity
e. Years of experience

2) Antibiotic use in aquaculture farms

a. How do you see the use of antibiotics in fish farming in Ghana?
b. Any sources of concern? In what specific ways could there be concern?
c. Can you describe how farmers get access to antibiotics in this area?
d. What are the common antibiotics that they commonly use? And in what quantities and duration?
e. Do you have concern that farmers may be using antibiotics wrongly?
f. How may such wrong use be a problem for food safety?
g. Can you describe how you wish to see the challenges with antibiotic use resolved? Training? Sample analysis? Sanctions?
h. What is the nature of challenges you face in getting farmers to use antibiotics correctly?

Appendix D: Ethics Approval Letter

ENSIGN COLLEGE OF PUBLIC HEALTH - KPONG

OUR REF: ENSIGN/IRB/M2
YOUR REF:
Tel: +233 245762229
Email: irb@ensign.edu.gh
Website: www.ensign.edu.gh

P. O. Box AK 136
Akosombo
Ghana

21st November, 2016.

INSTITUTIONAL REVIEW BOARD SECRETARIAT

Mary Nkansa,
Ensign College of Public Health.

Dear Ms Nkansa,

OUTCOME OF IRB REVIEW OF YOUR THESIS PROPOSAL

At a meeting of the INSTITUTIONAL REVIEW BOARD (IRB) of Ensign College of Public Health held on 16th and 17th November 2016, your proposal entitled **"Antibiotic Use in Fish Farming in the Asuogyaman District of The Eastern Region Of Ghana** "was considered.

Your proposal has been approved for data collection in the following settings:

1. Recruit and train Research Assistants for optimal performance.
2. Consider other practices that may influence food quality.

We wish you all the best.

Sincerely,

Dr (Mrs) Acquaah-Arhin

(Chairperson)

Cc. Dean of Ensign College.

Cc: Ag. Academic Registrar, Ensign College.