KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

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THE USE OF ANTIBIOTICS AND RESISTANCE PATTERNS OF BACTERIAL ISOLATES FROM SELECTED FISH FARMS IN THE ASHANTI REGION OF GHANA

A THESIS SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICS IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF PHILOSOPHY (MPHIL) IN PHARMACEUTICAL MICROBIOLOGY

BY

ESTHER EYRAM AGOBA

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DECLARATION

I, Esther Eyram Agoba, hereby declare that this thesis "The use of antibiotics and resistance patterns of bacterial isolates from selected fish farms in the Ashanti Region of Ghana" consists entirely of my own work produced from research undertaken under supervision and that, no part of it has been published or presented for another degree elsewhere, except for the permissible excerpts/references from other sources, which have been duly acknowledged.

ESTHER EYRAM AGOBA

Student (20285735) ……..... …….....…….....

Signature Date

Certified by

MR. FRANCIS ADU

(S**upervisor**) ……..... …….....…….....

Signature Date

Certified by

DR. CHRISTIAN AGYARE

(Co-Supervisor) ……..... …….....…….....

Signature Date

Certified by

PROF. K. OFORI- KWAKYE

(**Head of Department**) ……..... …….....…….....

Signature Date

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LIST OF ABBREVIATIONS

AMP	Ampicillin
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
CHL	Chloramphenicol
CLSI	Clinical and Laboratory Standards Institute
COT	Trimethoprim/Sulphamethoxazole
CPR	Ciprofloxacin
CRX	Cefuroxime
DHFR	Dihydrofolate reductase
DHPS	Dihyropteroate synthase
DNA	Deoxyribonucleic acid
EMB	Eosin Methylene Blue
ESBLs	Extended Spectrum β - lactamases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization
GEN	Gentamicin
MDR	Multi - drug resistant
MRSA	Methicillin Resistant *Staphylococcus aureus*
MRVP	Methyl-Red Voges - Proskaeur
MSA	Mannitol salt agar
PBP	Penicillin-Binding Protein
PCR	Polymerase Chain Reaction

PDR	Pan Drug Resistant
RBC	Red Blood Cells
RNA	Ribo-nucleic acid
RND	Resistance Nodulation-Type
SPSS	Statistical Package for Social Science
SSA	Salmonella-Shigella agar
TET	Tetracycline
TSI	Triple Sugar Iron
WHO	World Health Organization
XDR	Extensively Drug Resistant
XLD	Xylose lysine dextrose

ABSTRACT

Antibiotics may be used in fish farms to prevent or treat bacterial infections especially in hatcheries. This affects a wide range of bacteria and has potential impact on receiving water bodies and fish pathogens and has been reported to contribute to antibiotic resistance in other parts of the world but not reported in Ghana due to the fact that there are no studies conducted. This study was carried out to assess some fish farming practices among catfish and tilapia farmers which may contribute to antibiotic resistance as well as to determine the susceptibilities of *Staphylococcus aureus*, *Escherichia coli*, *Shigella species*, *Salmonella typhi* and *Pseudomonas aeruginosa* isolated from fish pond water, catfish gut and tilapia gut from 11 farms and 2 hatcheries to penicillin, ampicillin, flucloxacillin, erythromycin, tetracycline, sulphamethoxazole/trimethoprim, cefuroxime, gentamicin, ciprofloxacin and chloramphenicol using the disc diffusion method. Validated questionnaires were administered to 63 fish farmers in six zones of the Ministry of Fisheries-Ashanti Region. 73% of farmers claimed not to use antibiotics on their farms. Three farmers (4.8%) used tetracycline on the fish farms whilst two hatchery farmers add antibiotics (tetracycline or chloramphenicol) to fish feed. 93.6% of respondents who use manure on fish farms use poultry manure from commercial poultry farms and use it mainly to fertilize fish ponds. With the exception of gentamicin and ciprofloxacin, there was varying resistance of more than 60% to the other antibiotics. Generally, isolates showed high resistance to penicillin, ampicillin, flucloxacillin and tetracycline whilst low resistance was observed in all isolates to gentamicin (1.7% to 5.6%) except in *Pseudomonas aeruginosa*. 44.9% to 92.9% of isolates of organisms showed resistance to more than 3 antibiotics. In conclusion, even though there was no recent history of antibiotic use in most of the farms studied, there was multidrug resistance in isolates.

CHAPTER ONE

1.0 INTRODUCTION

Bacterial diseases in fish which usually occur under stress conditions result in high morbidities and mortalities leading to significant economic loss (Sudheesh *et al*., 2012). To avoid such huge losses, fish farmers use antibiotics and various antimicrobial agents not only for prevention and treatment of diseases but also to control external parasites and fungi, aquatic weeds and molluscs. They may also be used to ensure good water quality and to disinfect eggs and equipment (Rodgers and Furones, 2009). The broad use of antibiotics on fish farms has been reported and this has resulted in multidrug resistance in bacteria isolates in many studied farms (Samuel *et al*., 2011; Cabello *et al*., 2013; Chenia and Vietze, 2012). Antibiotic resistant bacteria from fish farms effluents have also been studied and shown to be transferrable to adjoining water bodies (Gordon *et al*., 2007). The World Health Organization (WHO) defines antibiotic resistance as the resistance of a microorganism to an antimicrobial medicine to which it was previously sensitive (Walsh, 2013). Antibiotic resistance is one of the major health challenges, which is largely attributed to varying factors such as indiscriminate use of antibiotics both in humans and in food producing animals (Huttner *et al*., 2013; Laxminarayan *et al*., 2013; Darwish *et al*., 2013).

Antibiotic resistance is the ability of bacteria to withstand usually achievable systemic concentration of an antibiotic with normal dosage schedule and or fall in the minimum inhibitory concentration ranges (Bisht, 2009) or as the World Health Organization (WHO) defines it, the resistance of a microorganism to an antimicrobial medicine to which it was previously sensitive (Walsh, 2013). Globally, studies have shown high levels of antibiotic resistance in bacterial isolates from hospitals as reported even in

developed countries including United States of America, France and Korea (Tenover *et al*., 2012; Hawser *et al*., 2012; Cholley *et al*., 2011).

The World Health Organization (WHO) in 2014, reported in its maiden global report on antibiotic resistance an alarming increase in infectious disease treatment failures as a result of increasing antibiotic resistance. The WHO report focusing on resistance to third generation cephalosporins, fluoroquinolones, carbapenems, penicillins in *Klebsiella pneumoniae, Escherichia coli*, *Streptococcus pneumoniae*, non-typhoidal Salmonella, *Shigella species* and *Neisseria gonorrhea* reported high resistance in these isolates to the antibiotics mentioned. Methicillin resistant *Staphylococcus aureus* (MRSA) proportions were reported to be between 20 and 80% in most regions in the world, and even exceed 80% in some areas (WHO, 2014). In Ghana, resistance to antibiotics has been reported and has been attributed to poor antibiotic use, monitoring and surveillance systems as well as unreported treatment failures in Ghana (Gyansa-Lutterodt, 2012). A study by Newman *et al.* (2006) in nine regions in Ghana indicated high level of resistant bacterial isolates in both teaching and regional hospitals. In another study in various hospitals in Ghana, including two teaching hospitals, seven regional hospitals, and two district hospitals, bacterial isolates including *Staphylococcus aureus*, *Salmonella typhi* and *other Salmonellae species* showed high resistance (73-82%) to tetracycline, cotrimoxazole, ampicillin and chloramphenicol (Newman *et al*., 2011).

Antibiotic resistant genes in food-producing animals have been shown to be transferrable to humans through the food chain (Sarter *et al*., 2007). Bacteria may be transferred from the aquatic environment to humans through direct contact with water and in the handling processes of the fish as well as direct consumption (Chenia and Vietze, 2012, Lowry and Smith, 2007). Antibiotics, some of which are used in humans,

have been used in aquatic environments mainly to prevent diseases or to treat diseases in fish production and may be administered through feed or direct application in pond water (Romero *et al*., 2012). Antibiotics as well as pesticides used in fish farms may accumulate in the water and sediments of fish farms and receiving water bodies. These residues in fish tissues may consequently affect consumers (AbuBakar *et al*., 2010, Pouliquen *et al*., 2009).

The use of antibiotics in aquaculture affects a wide range of bacteria and has potential impact on other components of the aquatic system such as receiving water bodies as well as in fish pathogens (Romero *et al*., 2012; Stachowiak *et al*., 2010; Gordon *et al*., 2007).

Fish farming practices such as the use of animal manure, waste water, human excreta in fish farms and disposal of untreated effluents from fish farms may contribute to antibiotic resistance in fish farms and adjoining water bodies (Dang *et al*., 2011; Stachowiak *et al*., 2010; WHO, 2006a). The use of antibiotics in fish farms is of importance to human health as resistant bacteria in these farms could be transferred to other bacteria or directly to human pathogens especially taking into consideration the similarity between fish pathogens and human pathogens such that humans can be colonized with pathogens from fish. (Heuer *et al.*, 2009).

Human pathogens such as *Staphylococcus aureus, Salmonella spp, Shigella spp, Escherichia coli* and *Pseudomonas aeruginosa* have been isolated from fish farms and some of the isolates showed high resistance to commonly used antibiotics in humans. These antibiotics include the penicillins, tetracyclines, cephalosporins, sulphonamides, quinolones, and macrolides (Karki *et al*, 2013; Chenia and Vietze, 2012; Newaj-Fyzul *et al*., 2008; Su *et al*., 2011).

Ghana has a thriving freshwater aquaculture industry with tilapia and catfish being the most farmed species in freshwater farms. Tilapia farming alone contributes 88% of total fish farming in Ghana (Onumah *et al*., 2010). Few studies have been done on the bacteria flora of the fish farms in Ghana, including the bacteria flora of fish feed and on farms using agricultural waste as well as flora of sewage treatment plant used as fish pond (Ampofo and Clerk, 2003b; Ampofo and Clerk, 2003a; Ampofo and Clerk, 2010).

1.1 JUSTIFICATION

Fish farming is a growing industry in Ghana. Antibiotic use extends from use in humans to use also in animals and there have been several studies and reports of antibiotic resistance in clinical isolates and also in isolates from terrestrial animals (Hutner *et al*.,2013; Tenover *et al*., 2012). Antibiotic use in food-producing animals has contributed to the increasing antibiotic resistance globally (Laxminarayan *et al*., 2013; Donkor *et al*., 2012). Many studies have reported the use of antibiotics in fish farms in several parts of the world and have examined the contribution of such practices to antibiotic resistance. In the face of increasing antibiotic resistance in Ghana (Newman *et al*, 2011), it is necessary to also examine the non-human use of antibiotics and consider the contribution of such practices to antibiotic resistance in the country. Bacteria have been isolated from fish farms but data on resistance of these isolates is rare or non-existent. This study seeks to determine the presence or otherwise of antibiotic resistant bacteria of human health importance and also examine certain practices on selected fish farms which may contribute to the dissemination of antibiotic resistance.

CHAPTER TWO

LITERATURE REVIEW

2.1 ANTIBIOTICS

The discovery of antibiotics was undoubtedly one of the major breakthroughs in modern medicine with great impact on human health (Högberg *et al*., 2010). With the increase in infectious diseases worldwide particularly in developing countries such as those in Africa, antibiotics have been a great source of relief as they have helped control several of these infectious diseases (Kimang'a, 2012; Huttner *et al*., 2013). However, the successful treatment of infections with antibiotics has given rise to antibiotic resistance (Karisetty *et al*., 2013b).

Organisms may be classified as multi-drug resistant (MDR), extensively drug resistant (XDR) or pan drug resistant (PDR). Multidrug resistant organisms are defined as organisms which are non-susceptible to at least one agent in three or more classes of antibiotics. Extensively drug resistant organisms are non-susceptible to at least one antimicrobial agent in all but remain susceptible to two or fewer antimicrobial classes. Pan drug resistant organisms are non-susceptible to all agents in all antimicrobial classes (Magiorakos *et al*., 2012).

2.2 MECHANISMS OF ACTION OF ANTIBIOTICS

There are four main mechanisms of action of antibiotics: including interference with cell wall synthesis, protein synthesis inhibition, disruption of processes in synthesis of nucleic acid and inhibition of a metabolic pathway (Kohanski *et al.*, 2010; Tenover, 2006).

2.2.1 Inhibition of bacterial cell wall synthesis

Beta-lactam antibiotics (penicillins, cephalosporins, carbapenems, monobactams) and glycopeptides inhibit cell wall synthesis of bacteria by binding to the enzymes (collectively called penicillin-binding proteins) of the peptidoglycan cell wall thereby inhibiting transglycosylation and transpeptidation (McDermott and White, 2014). Peptidoglycans are important in the resistance of the bacteria to intracellular pressure. Penicillin-binding proteins (PBPs) play a significant role in the catalysis of the polymerization of the glycan strand (transglycosylation) and the cross-linking between glycan chains (transpeptidation) (Sauvage *et al*., 2008).

2.2.2 Protein synthesis inhibition

Antibiotics make use of the difference in structure of bacterial ribosomes from proeukaryotic cells to selectively inhibit protein synthesis in bacterial cells. Poor accumulation of these antibiotics by the eukaryotic cells and the weak inhibition of ribosomal activity in these cells contribute to the selective action of such antibiotics (Chopra and Roberts, 2001). This is exhibited by the binding of chloramphenicol to the 50S subunit of the ribosomes whereas macrolides, tetracyclines and aminoglycosides bind to the 30S subunit of the ribosomes (Tenover, 2006; Hermann, 2007).

2.2.3 Interference of nucleic acid synthesis

The quinolones and fluoroquinolones interfere with DNA synthesis by targeting topoisomerase II and topoisomerase IV which are involved in chromosomal functions in DNA replication. Fluoroquinolones specifically target DNA gyrase and topoisomerase IV. This is exhibited in aerobic Gram positive bacteria such as *Staphylococcus aureus* and in some aerobic Gram-negative organisms such as *Escherichia coli* (Kohanski *et al*., 2010; Blondeau, 2004).

2.2.4 Inhibition of metabolic pathway

Trimethoprim and sulphonamides inhibit dihydrofolate reductase (DHFR) and dihyropteroate synthase (DHPS) respectively which are critical enzymes required for the folic acid synthesis pathway, which is very important in DNA synthesis in both Gram positive and Gram negative organisms. These inhibitors may also interfere in the binding of products of the DHFR or DHPS enzymes or substrates of similar substructure eventually resulting in a disruption in the DNA synthesis (Tenover, 2006; Bourne, 2014).

2.3 MECHANISMS OF ANTIBIOTIC RESISTANCE

Resistance of bacteria to antibiotics may be intrinsic (inherent) or acquired. Bacteria species may have the inherent ability to resist the action of an antimicrobial agent through its natural structural or functional characteristics and may be due to a lack of affinity of the antibiotic for the target site of the bacteria (Blair *et al*., 2015). Lack of access of the antibiotic into the cell of the bacteria, expulsion of the antibiotic by active exporters in the chromosome and the presence of inherent antibiotic inactivating enzyme production such as β-lactamase in *Klebsiella spp, Pseudomonas Aeruginosa(P. aeruginosa)* and *Acinetobacter baumannii* also contribute to inherent resistance (Cox and Wright, 2013; Rice, 2009).

Acquired resistance is resistance of bacteria to antibiotics it was previously susceptible to. This could occur due to mutation of existing genetic material or by obtaining new genetic material from another source (Karisetty *et al*., 2013a).

Antibiotics may exert selective pressure on the population of bacteria resulting in the vertical transfer of resistance which may also be acquired by other strains. This leads to accumulation and subsequently multiple drug resistance (Kumar and Singh, 2013;

Alanis, 2005). Resistant genetic elements may also be transferred horizontally to other bacteria of the same species, or to another species or a different genus through plasmids, transposons or integrons (Raghunath, 2008). For instance, *Acinetobacter spp* has been known to transfer resistant genes from environmental microorganisms to clinical microorganisms (Riesenfeld *et al*., 2004).

Generally, mechanisms of resistance of bacteria to antibiotics may be due to inactivation of drugs by enzymes, expulsion of antibiotics from bacterial cell, reduction in antibiotic permeability and uptake and alteration in drug target site (Poole, 2004).

2.3.1 Inactivation of drugs by enzymes:

Enzymes such as β-lactamases may inactivate antibiotics thus making them ineffective. β-lactamases such as the penicillinases, metallo- β-lactamases, cephalosporinases and oxacillinases have been the main mechanism of resistance to βlactams (Tang *et al*., 2014). Carbapenems, which used to be the last resort of the βlactams, has been threatened by serine carbapenemases and metallo-β-lactamases leading to a rise in carbapenem resistance in many organisms (Bonomo, 2011). Of the carbapenemases, metallo- β lactamases are currently known to be the most problematic being able to hydrolyze almost all β –lactams (Cornaglia *et al*., 2011). The extended spectrum β-lactamases (ESBLs) commonly found in the

enterobacteriacae especially *Escherichia coli* and *Klebsiella pneumoniae* are equally important and particularly problematic because of the large range of antibiotics they affect (Poole, 2004).

2.3.2 Expulsion of antibiotics from bacterial cell:

Efflux pumps present in certain bacteria expel drugs from the cell resulting in low intracellular levels of the drug which may not be effective. Efflux pumps are usually associated with tetracyclines (Tet A, Tet B and Tet K) and fluoroquinolones. Efflux

pumps to macrolides have also been described in enterobacteriacae and also to chloramphenicol (Alanis, 2005). *Pseudomonas aeruginosa* has been reported to have at least four efflux pumps which confer resistance to fluoroquinolones and other antibiotics (Jacoby, 2005). Efflux pumps are also responsible for resistance to a broad range of antibiotics in *Acinetobacter spp* notably, Ade ABC and Ade IJK (Poirel *et al*., 2011; Rumbo *et al*., 2013) as well as in Gram-positive bacteria such as *Streptococcus pneumonia* (Jacoby, 2005). The resistance nodulation-type (RND) efflux pumps are known to cause resistance in about fifteen species of Gram-negative bacteria and because of non-specificity are able to expel different classes of antibiotics including aminoglycosides, fluoroquinolones, penicillins and tetracyclines (Kamicker *et al*., 2008; Kim *et al*., 2011).

2.3.3 Reduction in antibiotic permeability:

In some Gram-negative bacteria, membrane porins are modified either by a reduction in the numbers or by replacement with less selective porins to reduce permeability to antibiotics, thus, antibiotics such as aminoglycosides may not reach the target ribosomes while β-lactams will also not reach the penicillin binding proteins (Kumar and Singh, 2013, Blair *et al*., 2015). The under-expression of the porins CarO and Omp33 have been implicated as one of the resistance mechanisms in organisms such as *Acinetobacter baumannii* to β-lactams (Gordon and Wareham, 2010).

2.3.4 Alteration in drug target site:

Resistance to macrolides may be mediated by alterations in the antibiotic target site which consequently limit antibiotic action by reducing affinity for the altered antibiotic target. Point mutations on the ribosomes account for resistance to macrolides and where there are mutations on the DNA gyrase (i.e. gyrA and par C), quinolone activity is affected (Hawkey, 2003, Lambert, 2005). This is exhibited by *Mycobacterium*

tuberculosis in which resistance to rifampicin is as a result of mutations in the RNA polymerase (Brandis *et al*., 2012). In β-lactams, penicillin- binding proteins (PBPs) may become altered thus promoting resistance for instance of *Staphylococcus to* methicillin (Ba *et al*., 2014). In *Mycobacterium spp*, mutations in the 16sRNA confer resistance to the aminoglycosides (Lambert, 2005).

2.4 ANTIBOTIC USE IN FOOD-PRODUCING ANIMALS

Antibiotics have been used in food-producing animals for disease prevention and treatment, as well as to promote growth (Mathew *et al*., 2007; Angulo *et al*., 2009). Over the past three decades, studies have implicated the use of antibiotics in food animals as a contributing factor to the ever increasing problem of antibiotic resistant bacteria in humans (Marshall and Levy, 2011). In response to the rising threat of antibiotic resistance as a result of use in food-producing animals, the World Organization for Animal Health, together with the US Food and Drug Administration and the World Health Organization, called for the regulation of veterinary antibiotic use in over 100 developing countries (Gilbert, 2012).

About 80% of all food-producing animals have been reported to receive medication, mostly antibiotics (Darwish *et al*., 2013). These include tetracyclines, β-lactams, aminoglycosides, macrolides and sulphonamides (Kemper, 2008). The use of antibiotics in food–producing animals may result in the selection of resistant microorganisms which may transfer resistant genes to other organisms thereby increasing the level of resistant microorganisms in the environment (Landers *et al*., 2012). Not only could there be accumulation of antibiotic residues in food samples but also antibiotic resistant isolates may be spread as a result of antibiotic use in foodproducing animals (Kim *et al*., 2013; Kemper, 2008). Multidrug resistant genes

from food-producing animals have been found in humans (Winokur *et al*., 2001; Lester *et al*., 2006; Graveland *et al*., 2010).

Data on the extent of antibiotic use in animals in developing countries remain sparse. Mitema *et al.* (2001) reported that antibiotics such as tetracyclines, sulphonamides and aminoglycosides were the most commonly used antimicrobials for veterinary purposes in Kenya (Mitema *et al*,. 2001). A study in Ghana by Donkor *et al.* (2011) indicated residues of β-lactams, tetracyclines, chloramphenicol, macrolides, aminoglycosides, sulphonamides, and quinolones in food from animal sources. An epidemiological study of *Escherichia coli* isolates from some livestock farmers in Ghana showed high level resistance to tetracyclines and the penicillins. Ninety-eight percent of farmers interviewed used antibiotics regularly in animal production mainly for the prevention of infections in the animals. A correlation was observed between resistant isolates from animals and resistant *E .coli* isolates in humans, possibly suggesting the transfer of resistant isolates from animals to humans (Donkor *et al*., 2012).

2.5 AQUACULTURE

The Food and Agriculture Organization (FAO) defines aquaculture as the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants. Farming in aquaculture, refers to a deliberate interference in the rearing process of aquatic organisms to increase production, such as regular stocking, feeding and protection from predators (Kümmerer, 2009, FAO, 2015a). Considering the recent boom in the aquaculture industry with over 67% of production in China, the wide use of antimicrobials in aquaculture has become a source of serious concern of development of antibiotic resistance in bacteria (Marshall and Levy, 2011).

Formulated feeds, antibiotics, antifungal, and agrochemicals are the core requirements of aquaculture production in recent times. These are economically burdensome on farmers, especially in the developing countries and as such to supplement these, human and animal excreta may be used in the fish farms (Sapkota *et al*., 2008).

Hatcheries from which fingerlings are sourced require good management practices to prevent disease outbreak and contamination. Hatcheries are to be disinfected before the hatchery season as well as disinfecting eggs before transferring them into the hatchery and the use of pathogen-free water for hatchery ponds is encouraged (Small, 2006). Though private commercial farmers and the Ministry of Fisheries are usually the suppliers of fingerlings, some farmers, however, obtain the fingerlings from their own hatcheries (FAO, 2006).

It is reported that fish supplies over 20% of the total protein to Ghanaians (Jacquet and Alder, 2006). Two major species of fish, tilapia (*Oreochromis niloticus*) and catfish (*Clarias garienpinus* and *Heterobranchus longifilis*) are the most produced fishes in Ghana. Fish in ponds in Ghana are fed mostly on artificial and formulated feed. The ban on imports of tilapia into Ghana necessitated the increase in production of tilapia species to meet demands (Onumah *et al*., 2010).

Fish farms are distributed throughout the southern and middle belts of the country with most farmers relying on the seeping of water in earthen-dug ponds as source of water and thus most fish farms are located in marshy lands. The ponds are therefore not dried completely as water would be needed to seep into the ponds and the dug-out earthen pond is the most used by farmers with few using concrete ponds (FAO, 2006). Most of Ghana's fish farmers have little knowledge in fish farming and so depend largely on agriculture extension officers from the Ministry of Fisheries or other people with formal training in fish farming for advice (FAO, 2006).

2.6 USE OF ORGANIC FERTILIZERS AND WASTEWATER

The use of organic fertilizers on fish farms is thought to be for ecological and economic benefits. Most farmers use this to boost phytoplankton growth in the ponds (Kang'ombe *et al*., 2006, Mischke and Zimba, 2004). In the Asian countries, both human and animal excreta as well as waste water are known to be used in fish farming. Waste water use in fish ponds also serves as the sole means of waste water disposal in some countries such as Vietnam (WHO, 2006a).

Animal manure could serve as reservoirs of resistant bacteria and antibiotic residues which could account for selective pressure in environments such as fish ponds when applied (Heuer *et al*., 2011; Mlejnková and Sovová, 2013; Dang *et al*., 2011). This presents a public health risk as the fish may harbour disease–causing pathogens particularly enterobacteria which could be transferred to humans directly through contact or indirectly through the fish or contaminated fish pond water (Elsaidy *et al*., 2015; Hoa *et al*., 2011). A study by Ampofo and Clark (2003) reported the use of organic manure including cow manure, poultry manure, pig manure and cow blood from abattoirs in fertilizing fish ponds in Ghana with poultry manure being the most used. The use of these agricultural waste products in aquaculture poses a public health risk (Ampofo and Clerk, 2003).

2.7 ANTIBIOTIC USE IN FISH FARMS

In fish farming, antimicrobials are not only used to prevent and treat diseases but also to control external parasites and fungi, aquatic weeds and molluscs. They may also be used to ensure good water quality and to disinfect eggs and equipment (Rodgers and Furones, 2009).

Antibiotics may be used in fish farms for prophylactic or therapeutic use. Antibiotics are used as prophylaxis in healthy fish in order to prevent diseases hence promoting growth (Sapkota *et al*., 2008). In fish farm hatcheries, prophylactic treatment is often employed usually with tetracycline or oxytetracycline (Dietze *et al*., 2005).

Therapeutic treatment may involve both infected and uninfected fish in the population in which case, treatment is metaphylactic. This is often the case in fish farms when there is increased mortality during an outbreak of infection as opposed to therapeutic treatment (Serrano, 2005; McEwen and Fedorka-Cray, 2002). The commonly used antibiotics in fish farms in a study by Sapkota *et al*. (2008) were oxytetracycline and chloramphenicol. Oxytetracycline is used by 92% of the world's top thirteen countries in aquaculture production while 69% use chloramphenicol and oxolinic acid. Twenty six antibiotics belonging to nine classes of antibiotics are reportedly used in aquaculture by the FAO (2010).

Table 2.1: Classes of antibiotics used in aquaculture

Class of antibiotics	**Examples**
Sulphonamides	sulfamerazine, sulfadimidine and sulfadimethoxine
Potentiated sulphonamides	trimetoprim/Sulfadiazine combination
Tetracyclines	chlortetracycline, oxytetracycline
Penicillins	ampicillin, amoxycillin and benzyl penicillin
Quinolones	ciprofloxacin, enorfloxacin, norfloxacin, oxolinic acid, perfloxacin, flumequine and sarafloxacin
Nitrofurans	furazolidone
Macrolides	erythromycin and spiramycin
Aminogycosides	gentamicin

Others	chloramphenicl, florfenicol, thiamphenicol, tiamulin, nalidixic acid, miloxacin

The possibility of spread of infections on fish farms due to unhygienic practices such as increased fish densities in a pond and lack of barriers between farms may result in the use of antibiotics on fish farms (Naylor and Burke, 2005). The antibiotics may be administered as injections or in feeds or bath (Cabello, 2006; Yanong, 2010).

There are very few antibiotics developed specifically for use in aquaculture production thus the use of specific antibiotics on fish farms may be determined by regulations governing its use in countries (Costa *et al*., 2012). Most of the antibiotics currently known to be used in fish farming such as oxytetracycline and chloramphenicol are also used in humans (Sapkota *et al.*, 2008). Regulations on the use of antibiotics in fish farming are strict in the European and some Asian countries as well as in North America. However, in most developing countries where significant fish production occurs, there seems to be little or no regulations on the use of antimicrobial agents on fish farms (Smith, 2008; Serrano, 2005).

2.8 ANTIBIOTIC RESIDUES IN WATER AND SEDIMENTS

Antibiotics administered to fish may accumulate in the water and sediments of fish ponds. About 80% of ingested antimicrobials eventually pass out in faeces or in urine and other fluids and may accumulate in the sediments of the fish pond and receiving water bodies as well as plants (Pouliquen *et al*., 2009). Uningested antibiotics and food could leach into the water sediments as well as in fish tissues and may result in selective pressure in the pond environment leading to antimicrobial resistance of fish pathogens.

They may also be carried to other aquaculture bodies and thus exert pressure in such environments (Marshall and Levy, 2011; Kümmerer, 2009; Hoa *et al*., 2011). Residues of antibiotics such as oxytetracycline and flumequine have been found present in pond sediments and in fish tissues even after long withdrawal periods (Lalumera *et al*., 2004; Bebak-Williams *et al*., 2002).

2.9 ANTIBIOTIC RESISTANT BACTERIA FROM FISH AND FISH FARMS

Bacteria which are resistant to antibiotics have been isolated from different sites of fish farms and their environs in several studies (Samuel *et al*., 2011; Cabello *et al*., 2013; Chenia and Vietze, 2012). Bacteria showing high multidrug resistance were isolated on Chilean salmon farms both from salmon fingerlings and pelletized feed (Miranda and Zemelman, 2002).

Antimicrobials and antibiotic resistant bacteria from fish farms effluents have also been studied and shown to be transferrable to rivers as depicted in a study by Gordon *et al*. (2007).

Studies have also shown antibiotic resistance in motile aeromonads from fresh water fish to amoxicillin, oxytetracycline, ampicillin, novobiocin and polymixin-B (Hatha *et al*., 2005).

Isolates from harvested fish in markets have also been found to show multidrug resistance to antibiotics. A study in Malaysia of *Salmonella* isolates from catfish and tilapia markets and farms showed multidrug resistance to chloramphenicol, clindamycin, rifampicin, streptomycin and tetracycline. Plasmids of various sizes from the isolates of *Salmonella* serovars from tilapia and catfish samples were also detected in the study (Budiati *et al*., 2015).

Antibiotic resistant bacteria have also been isolated from integrated fish farms where animal manure and waste have been used even though there was no direct use of antibiotics on such farms (Su *et al*., 2011; Dang *et al*., 2011). In a study by Karki *et al.* (2013), 56.25% of hatchery-raised tilapia yielding ampicillin resistant bacteria from fish gut were sensitive to gentamicin but showed varying resistance between 3.3%-20% to chloramphenicol, vancomycin, tetracycline and streptomycin.

Hatcheries studied had indicated that no antibiotics were used in their farms.

2.10 FISH BACTERIAL FLORA AND DISEASES

Both pathogenic and non-pathogenic bacteria may be found in many fish species and rarely cause diseases unless in the presence of stress conditions such as high stocking densities, poor water quality and intercurrent disease which may make the fish immuno-compromised (Osungbemiro *et al*., 2014). However, of all the infectious diseases that affect fish, bacterial infections result in high morbidities and mortalities (Sudheesh *et al*., 2012).

Escherichia coli, Bacillus spp, Shigella spp, Staphylococcus spp, Micrococcus spp, Pseudomonas spp, Enterococcus spp, Salmonella spp, Vibrio sp., Serratia sp., Klebsiella sp. and *Proteus sp* have been isolated from fish ponds, gills, skin and intestine, with some showing high resistance to antibiotics (Samuel *et al*., 2011; Koonse *et al*., 2005; Shah *et al*., 2012). *Aeromonas spp, Pseudomonas spp, Streptococcus spp, Vibrio spp, Enterococcus spp, Staphylococcus spp,* and enterobacteria are known to be pathogenic to fish and may result in massive outbreak of diseases when fish are immuno-compromised (Gisain *et al*., 2013).

Some of these diseases such as *Streptococcus* infections which result in the 'pop-eye effect', ascites, haemorrhage and enteritis and other conditions result in high mortalities

and may be treated with antibiotics such as erythromycin and amoxicillin (Yanong and Francis-Floyd, 2010). Furunculosis which is caused by aeromonads and may present with ulcerations and internal haemorrhages in fish results in low mortalities but scars remain in fish that survive (Austin and Austin, 2007).

Bacteria such as *E.coli, Shigella spp, Salmonella spp* and *S. aureus* are not ingenious to fish and thus their presence in fish samples and their environs may be as a result of contamination from human or animal sources. *S. aureus* may however be found in 50% of fish populations (Huss, 2007; Elsaidy *et al*., 2015). Fungi such as *Achlya spp*. and *Saprolegnia spp, Branchiomyces spp*. *Aphanomyces spp* are also reported as fish pathogens under stress conditions as they are rarely found on healthy fish and they cause various fungal infections characterized by skin lessions (Osman *et al*., 2010; Karunasagar *et al*., 2003).

There are few facilities available to investigate viral agents in tropical fishes. Viruses such as rhabdovirus, rheo-like virus and the infectious pancreatic necrosis virus found particularly in the tropical snakehead fish have been reported. Spring viraemia of carp virus, Infectious salmon anaemia virus and others have been identified as pathogens in finfish (Karunasagar *et al*., 2003).

2.11 TRANSFER OF RESISTANT BACTERIA AND GENES

Aquaculture system may interact with other ecological systems resulting in the likely transfer of antimicrobial resistant bacteria and their genes to animals and humans. This may occur most likely through the food chain and processes such as physical examination of fish, handling and treatment of diseases (Teuber, 2001; Lowry and Smith, 2007; Ucko and Colorni, 2005). These pathogens include Gram-positive bacteria such as *Staphylococcus aureus (S. aureus), Clostridium botulinum* and *Streptococcus iniae.* The Gram- negative pathogens include *Aeromonas spp, Vibrio cholerae,*

Escherichia coli (E.coli) and *Salmonella spp* (Lehane and Rawlin, 2000; Novotny *et al*., 2004). *Vibrio spp* including *Vibrio cholerae* is an important pathogen acquired from freshwater bodies through poor handling of fish products. The pathogenic non cholera *Vibrio species* can also be transmitted through various routes such as open wounds or the consumption of fish (Tantillo *et al*., 2004). Pathogens from fish may be transmitted to humans during handling procedures like cleaning ponds with bare hands, exposure to the pond water, injuries by fins of fish, fish bites and through fish processing procedures such as scaling and evisceration as was reported in *Streptococcus iniae* infections in fish farmers in Northern America, China and Taiwan (Novotny *et al*., 2004; Weinstein *et al*., 1997; Lau *et al*., 2003; Sun *et al*., 2007). They may also be acquired orally after ingestion of contaminated or infected fish (Dvorak, 2009).

Resistant bacteria from aquaculture may transfer genes horizontally to other bacteria of human health importance (Heuer *et al*., 2009). There are reports of antibiotic resistant bacteria isolated from fish farms showing similar resistance determinants as that isolated from humans (Furushita *et al*., 2003). A study by Rhodes *et al.* (2000) showed that *Aeromonas* isolates from hospital effluents and fish tanks had transferred oxytetracycline- encoding plasmids to *E .coli* and were found to be similar to plasmids from fish farms in Norway and Scotland.

2.12 BACTERIA UNDER STUDY

2.12.1 *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive, non-flagellated, non-motile, non-spore forming cocci of the family Micrococcacae (Gillespie and Bamford, 2012). *S. aureus* is the most virulent of the *Staphylococci* (Forbes *et al*., 2007). *S. aureus* is usually coagulase-positive and pathogenic, being differentiated from the non-pathogenic

Staphylococci which are mostly coagulase negative. *S. aureus* is also catalase positive and produces β-haemolysis of blood (Cowan *et al*., 2004). Initially susceptible to penicillin, it is increasingly becoming resistant due to β-lactamase activity.

S. aureus is known to cause life-threatening infections including respiratory and skin infections in hospitals and community settings (Klevens *et al*., 2007). The upsurge of methicillin-resistant *Staphylococcus aureus* (MRSA) has further threatened successful treatments of *Staphylococci* infections (Gould *et al.*, 2012). Resistant *S. aureus* isolates, including MRSA have been isolated from tilapia (Atyah *et al*., 2010).

2.12.2 *Salmonella spp*

Salmonella spp, a member of the enterobacteriaceae are Gram-negative, rod shaped bacteria that possess flagella and are motilelike all enterobacteria. They are glucose fermenters and grow on a variety of selective media including MacConkey agar, Xylose lysine dextrose (XLD) agar and Salmonella-Shigella agar (SSA) and are usually identified routinely using the indole, methyl-red Voges-Proskaeur (MRVP) and citrate test (IMViC) as well as on triple sugar iron (TSI) (Forbes *et al*., 2007).

In humans, *Salmonella spp* cause enteric fever and gastroenteritis (Acheson and Hohmann, 2001). They are generally non-pathogenic to fish but could cause salmonellosis and other infections in humans (Lowry and Smith, 2007). Transfer of resistant genes from fish to human pathogens can occur through plasmids such as found in isolates of *Salmonella spp* from tilapia and catfish (Budiati *et al*, 2013; Budiati *et al*., 2015).

2.12.3 *Shigella spp*

Shigella spp is also belongs to the enterobacteriacae, Gram-negative and rod-shaped.

It ferments glucose and also grows on media such as MacConkey agar, XLD and SSA.

It causes shigellosis (formerly bacterial dysentery) in humans and is transmitted via the

oral-faecal route (Forbes *et al*., 2007). Just like other enterobacteria, its presence in aquacultural environments is a sign of faecal contamination (Novotny *et al*., 2004). Resistant *Shigella spp* have been isolated from aquaculture environments, more often in integrated fish farms (Surendraraj *et al*., 2009). The transmission of multidrug resistant *Shigella spp* is widespread and has been found in many studies (Shiferaw et al., 2012; Qiu *et al*., 2012).

2.12.4 *Escherichia coli*

Escherichia coli is a Gram-negative, rod-shaped facultative anaerobe and is usually found in the lower intestines of warm-blooded animals (Singleton, 1999). Its presence in fish and fish pond environments is usually used to test faecal contamination of food samples because they can survive long hours outside the body (Samuel *et al*., 2011). It is known to cause gastroenteritis and toxic strains of *E. coli* have been isolated from fish (Novotny *et al*., 2004). Multidrug resistant isolates of *E. coli* are widespread globally and have been isolated both in clinical and animal samples (Sanchez *et al*., 2012; Ho *et al*., 2011; Nordmann *et al*., 2012).

2.12.5 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative bacteria distributed widely in habitats including water and soil (Khan *et al*., 2007). The production of fluorescent compounds (pyoverdin, pyocyanin, pyorubin, pyomelanin) enables it to chelate and dissolve iron. It is non-fermentative and oxidase positive (Parija, 2014). In fish, *P. aeruginosa* causes septicaemia under stress conditions and have been isolated from catfish and tilapia (Najiah *et al*., 2009). *P. aeruginosa* causes a wide range of infections in humans including cystic fibrosis which are very difficult to treat due to the resistance shown to almost all clinically important antibiotics (Rossolini and Mantengoli, 2005). Its lower outer membrane permeability makes it less susceptible to most antibiotics and also

readily acquires resistant genes from other bacteria (Breidenstein *et al*., 2011; Pena *et al*., 2013).

2.13 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing is done to determine the effectiveness of antimicrobial agents against test microorganisms. Antimicrobial susceptibility testing is performed with phenotypic or genotypic methods (Reller *et al*., 2009; EUCAST, 2015). The phenotypic methods of determining antimicrobial susceptibility include measuring bacterial growth by micro-broth dilution, antimicrobial gradient methods (E-TEST) or disc diffusion methods (Kirby-Bauer method). The determination of minimum inhibition concentrations (MICs) is the principle for the phenotypic tests. Bacteria isolates are then identified as susceptible, intermediate or resistant to the antibiotic based on standardized reference methods by Clinical and Laboratory Standards Institute (CLSI)or European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Pulido *et al*., 2013). There are also automated methods such as the Phoenix, Sensititre ARIS 2X, Vitek 1 and 2, and WalkAway systems (Reller *et al*., 2009).

Genotypic methods of antimicrobial susceptibility testing employ the use of molecular techniques, including PCR, DNA microarrays, and molecular probes which offer alternatives to conventional phenotypic tests (Liu *et al*., 2014). These genotypic tests target known resistance genes such as gyr A, ESBLs etc. and are thus used to identify specific pathogens such as methicillin-resistant *Staphylococcus aureus*. They are usually reserved for confirmation of phenotypic resistance but a combination of phenotypic and genotypic testing may be useful for surveillance purposes (Liu *et al*., 2014; Zankari *et al*., 2012; Turnidge and Paterson, 2007).

2.14 FISHES UNDER STUDY

2.14.1 TILAPIA (*Oreochoromis niloticus*, Linnaeus, 1758)

Tilapia is native to East Africa and inhabits brackish water mainly at temperatures between 8 to 42º C. Considered a hardy species and very tolerant of high salinities, it is omnivorous and feeds on algae, insects and crustaceans. In some areas, it is used to control aquatic plants (Gómez-Márquez *et al*,2003; FAO, 2015c). Tilapia is described as the second most important farmed fish worldwide and also the most important aquaculture species of the 21st century with about 98% farmed outside their original habitats (Gupta and Acosta, 2004).

2.14.2 CATFISH (*Clarias gariepinus*, Burchell, 1822)

The African catfish, *Clarias gariepinus* (Burchell, 1822) one of the most commercially important fishes of Africa is native to Africa. The habitat of catfish is freshwaters of pH 6.5-8.0 but can tolerate extreme environmental conditions and can breathe air when active or under dry conditions due to the presence of an accessory breathing organ. It is a general scavenger and feeds on insects, crabs, plankton as well as other fishes, small birds and plants. *C. gariepinus* has mostly been used to control over-breeding in mixed-sex tilapia culture in earthen ponds. Catfish are typically fed in fertilized ponds for 6 to 11 months before they are harvested (FAO, 2015b; Musa *et al*.,2013)

2.15 SURVEY AREAS

Survey was conducted in six zones of the Fisheries commission of Ghana in the Ashanti Region. The region lies in the southern half of the country and occupies 24,389 sq. km. or 10.2 percent of the total land area of Ghana. The region has several water bodies including Lake Bosumtwi and many rivers such as Offin, Pra, Afram 2 and Owabi which serve as sources of drinking water for residents of many localities in the

region.There are 30 metropolitan, municipals and districts which have been designated into six zones (Kumasi, Atwima, Ejura-sekyere, Amansie, Adansi and Kwabre) by the Ashanti region Fisheries commission. The region has a population of 4,780,380 representing the highest proportion (19.4 percent) of the total population (GSS, 2012.) Available water surface area in Ashanti for fisheries development about 125.3 hectares and produces about 564.1 metric tons of fish annually (http://www.mofad.gov.gh/?q=content/ashanti-region-leads-pond-fish-farming-ghana-0).

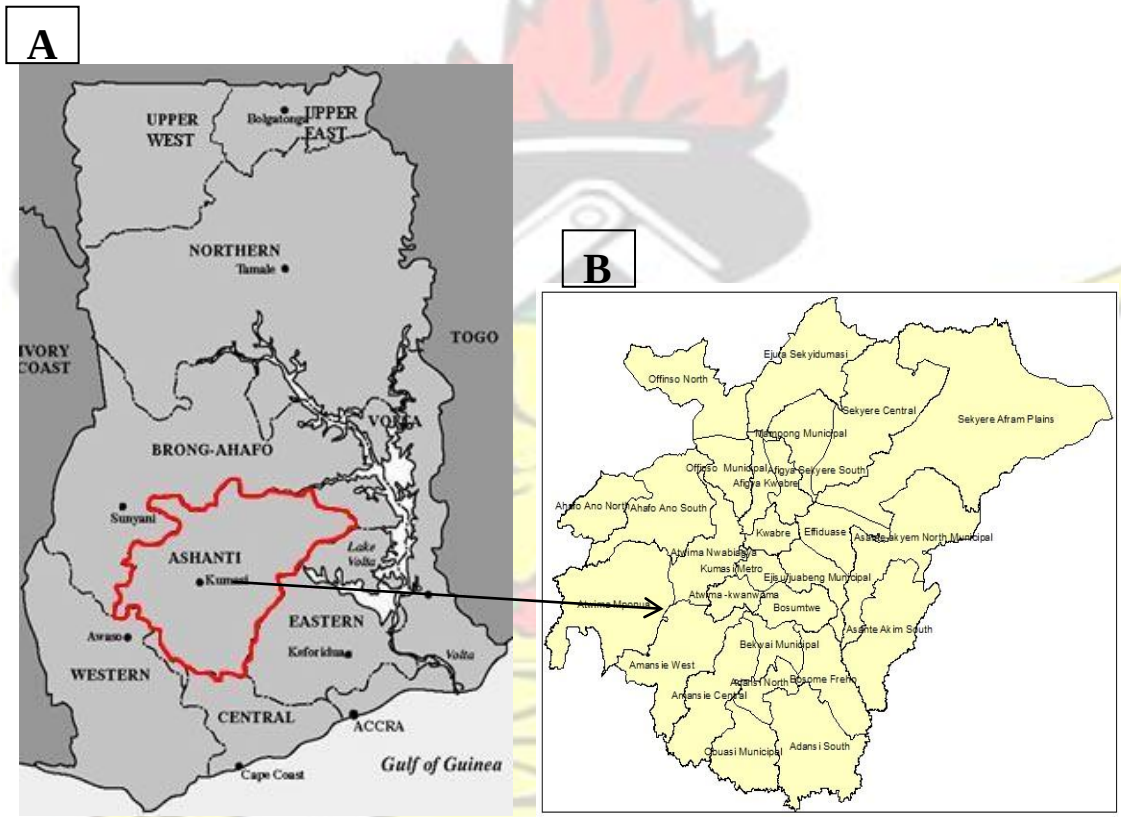

Figure 2.1: Map showing the study area of survey

A. Map of Ghana Source: http://www.pcaf.com.gh/gspd_contact.htm

(27/04/2016)

B. Map of the Ashanti region showing districts (Bonyah *et al.*, 2013)

AIM

To find out the extent of use of antibiotics in selected fish farms in the Ashanti Region of Ghana and determine the antibiotic susceptibility profile of some bacterial isolates from selected farms to selected antibiotics.

OBJECTIVES

1. To find out the extent of use of antibiotics on fish farms in selected farms in the Ashanti Region through administration of validated questionnaires.
2. To determine the effect of some fish farming practices on antimicrobial resistance.
3. To identify and isolate *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigella spp* and *Pseudomonas aeruginosa* from fish ponds, tilapia and catfish samples.
4. To determine antimicrobial susceptibility of *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigella spp* and *Pseudomonas aeruginosa* isolates to selected antibiotics.

CHAPTER THREE

3.0 MATERIALS AND METHODS 3.1

MATERIALS

Table 3.1.1 List of instruments and equipment

Item	Manufacturer/Company/Place
Disk dispenser	Oxoid Ltd, Basingstoke, UK
Dry bath incubator	Light Labs, Dallas, USA
Incubator(Gallenkamp Plus II)	Sanyo Corporation, UK
Thermostatically controlled water bath	New Brunswick, Edison, USA
Autoclave	Systec, Wettenberg, Switzerland
Laminar air flow cabinet	Skan AG, Allschill, Switzerland
Haraeus oven	Amscope, New York, USA
Microscope	Biorad, California, USA
Petri dishes	Fisher Scientific GmbH, Schwerte, Germany
Test tubes	Fisher Scientific GmbH, Schwerte, Germany

Table

3.1.2: List of culture media and chemicals for microbiological studies

Item	Manufacturer/Company/Place
Mannitol Salt (MS) Agar	Oxoid Ltd, Basingstoke, USA
Eosin methylene blue (EMB)	Oxoid Ltd, Basingstoke, USA
Salmonella- Shigella (SS) Agar	Oxoid Ltd, Basingstoke, USA
Pseudomonas Cetrimide Agar	Oxoid Ltd, Basingstoke, USA
Blood agar base	Oxoid Ltd, Basingstoke, USA
Triple sugar Iron agar	Oxoid Ltd, Basingstoke, USA
Baird-Parker agar base	Oxoid Ltd, Basingstoke, USA
Mueller-Hinton agar	Oxoid Ltd, Basingstoke, USA
Tryptone Soya Broth	Oxoid Ltd, Basingstoke, USA
Koser Citrate medium	Oxoid Ltd, Basingstoke, USA
Peptone water	Oxoid Ltd, Basingstoke, USA
MRVP broth	Oxoid Ltd, Basingstoke, USA
Oxidase disc	Abtek biologicals Limited, Liverpool, UK
Kovac's reagent	Oxoid Ltd, Detroit, MI, USA
Koser's citrate medium	Oxoid Ltd, Detroit, MI, USA

3.1.3: List of antibiotics for sensitivity testing

Item	Manufacturer/Company/Place

Table

Penicillin (10 units)	Abtek Biologicals Limited, Liverpool, UK
Ampicillin(10µg	Abtek Biologicals Limited, Liverpool, UK
Flucloxacillin(5µg)	Abtek Biologicals Limited, Liverpool, UK
Erythromycin(15µg)	Abtek Biologicals Limited, Liverpool, UK
Tetracycline(30µg)	Abtek Biologicals Limited, Liverpool, UK
Trimethoprim/Sulphamethoxazole(1.25/23.75µg)	Abtek Biologicals Limited, Liverpool, UK
Cefuroxime(30µg)	Abtek Biologicals Limited, Liverpool, UK
Ciprofloxacin(5µg)	Abtek Biologicals Limited, Liverpool, UK
Chloramphenicol(30µg)	Abtek Biologicals Limited, Liverpool, UK
Gentamicin(10µg)	Abtek Biologicals Limited, Liverpool, UK

Table

3.1.4: Standard for antibiotic sensitivity testing

Item	Manufacturer/Company/Place
0.5 McFarland Standard	Thermoscientific, Copenhagen, Denmark

Table 3.1.5: Reference organisms for antibiotic sensitivity testing

Organism	Source
E.coli ATCC 25922	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
S.aureus ATCC 25923	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
P.aeruginosa ATCC 29213	Department of Veterinary Disease Biology, University of Copenhagen, Denmark

3.1.1 Antibiotics

The selection of antibiotics for the antimicrobial susceptibility tests was based on the different classes of antibiotics reportedly used in fish farms as well as in livestock production in other countries (Sapkota *et al.*, 2008). They are also antibiotics which are critically and highly important in human health and are commonly used in Ghana to treat infections (Organization, 2011). These include penicillin, ampicillin, flucloxacillin, tetracycline, cefuroxime, cotrimoxazole (trimethoprim-sulfamethoxazole) gentamicin, ciprofloxacin and chloramphenicol.

3.2 Survey

A survey of fish farming practices among fish farmers in six zones of the Fisheries Commission of the Ashanti Region was conducted in March, 2014. These zones are Kumasi, Atwima, Ejura-Sekyere, Amansie, Adansi and Kwabre. Preliminary questionnaires were developed after conducting a literature search on fish farming practices and informal interviews with few target respondents and non-respondents (fisheries veterinary officer and some lecturers). A pilot study was done to validate the questionnaire. Validated questionnaires were administered to 63 fish farmers from the six different zones as well as to Government fisheries officers superintending the six zones. Farmers were selected from the six zones based on their availability at the time of the survey. Inclusion criteria for selection of farmers include: Officially registered by the Fisheries commission, existing ponds, produces either catfish or tilapia, available at the time of survey. Farmers whose farms were not officially registered by the fisheries commission or unavailable at the time of survey were excluded. Fish farmers who had no existing ponds for production were excluded. Structured questionnaires addressed the type of antibiotics used on fish farms, source of antibiotics and method of administration if used, record of any disease outbreak on farms, use and source of manure for fish farming, how pond waste is disposed of, type of feed and additives used, and other uses of antibiotics on farm aside fish farming. Biases were reduced by interviewer-administration of questionnaires and avoidance of extreme response and ambiguous questions. Samples of questionnaires administered are in appendix I. For fisheries officers, questionnaires were self-administered (i.e. questionnaires were answered by the interviewee).

3.3 Farms studied for sampling

From the survey conducted, 10 farms from the Kumasi zone and one from the Atwima zone were chosen for sampling of water and fish. Farms which had not been in active production for at least 6 months were not considered for the study. The bias in this selection was the selection of 3 out of the 6 zones in the Ashanti Region for survey. Two fish hatcheries, located in the Kumasi and Ejura-Sekyere zones which serve as the official source of fingerlings (mainly tilapia) for majority of farmers in the Ashanti Region were also studied (Table 3.2).

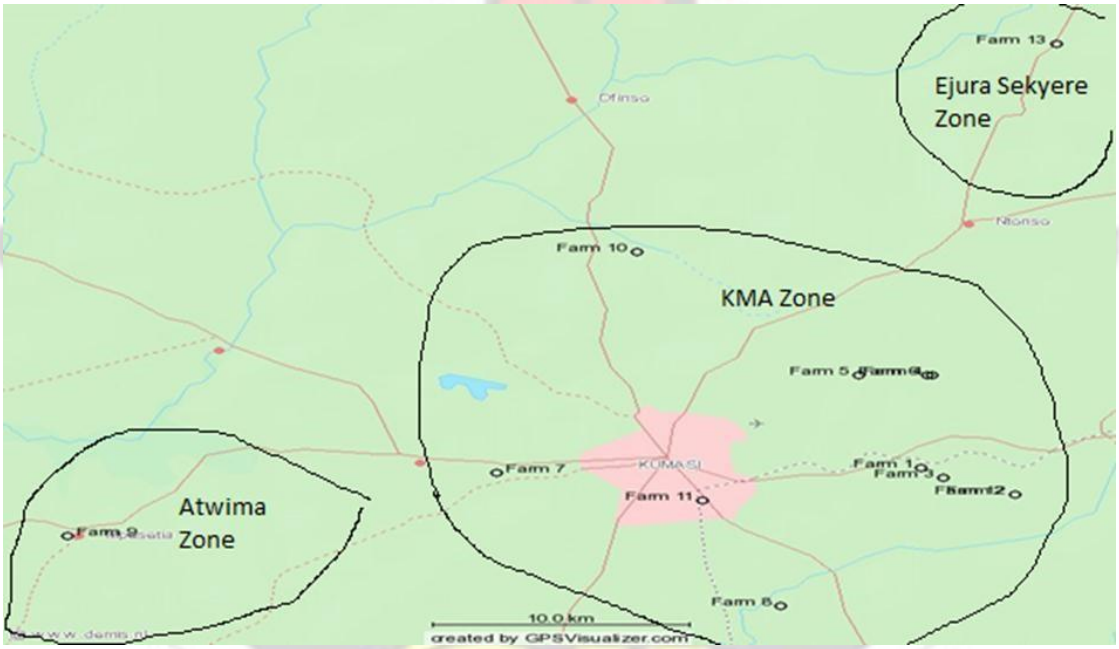

Figure 3.1 Geographical representation of studied farms

Table 3.2: Location of Studied Farms and Samples Collected

FARM	ZONE	GPS LOCATION	SAMPLE		
	Kumasi	6^0	WATER	TILAPIA	CATFISH
1		41’33.2”N, 1o 32’4.9”W	✓	✓	X

2	Kumasi	6o 40’39.1”N, 1o 30’5.2”W	✓	✓	✓
3	Kumasi	6o 41’12.7”N,1o 31’37.2”W	✓	✓	X
4	Kumasi	6o 44’44”N,1o 31’50”W	✓	✓	✓
5	Kumasi	6o 44’44.7”N,1o 33’23.4”W	✓	✓	X
6	Kumasi	6o 44’44.2”N, 1o31’56.2”W	✓	✓	✓
7	Kumasi	6o 41’22.6”N,1o 41’5.6”W	✓	✓	✓
8	Kumasi	6o 36’50.4”N,1o 35’2.9”W	✓	✓	✓
9	Atwima	6o 39’13.8”N,1o 50’13.3”W	✓	✓	✓
10	Kumasi	6o 48’57.5”N, 1o 38’7.3”W	✓	✓	✓
11	Kumasi	6o 40’27.4”N,1o 36’43.3”W	✓	✓	X
12	Kumasi	6o 40’39.1”N, 1o 30’5.2”W	✓	✓	✓
13	Ejura Sekyere	6o 56’4.93”, 1o 29’13.15”W	✓	✓	X

✓ - Sample available on farm X– sample not available on farm

3.4 Collection of samples from water

One hundred millilitres (100mL) of water samples were collected approximately 15 to 20 cm below water surface into sterile glass bottles with stoppers from a minimum of four different sites (Figure 3.2) of all fish farms (ponds) between 8:00 and 11:00 GMT. The water samples were transported to the laboratory in boxes with ice and work was carried out on them within 24 h of picking the water samples (Gordon *et al*., 2007).

3.5 Preparation of culture media and isolation of bacteria

One milliliter (1mL) each of composite samples from each farm was aseptically transferred into 10 mL sterile tryptone soya broth, an enrichment medium and incubated for 18 h at 37°C (Elhadi, 2014). After incubation, the tubes were checked for turbidity which indicated microbial growth. With the aid of a sterile inoculating loop, samples from various farms were aseptically streaked unto Mannitol salt agar (MSA), eosin methylene blue (EMB) agar, Salmonella-Shigella agar (SSA) and Pseudomonas cetrimide agar to isolate *S. aureus, E. coli, Shigella spp* and *S. typhi and P. aeruginosa,* respectively. Remaining composite samples were sterilized at 115°C for 30 min and re-checked for the presence or absence of viable aerobic bacteria, anaerobic bacteria and fungal by aseptically cultivating 1 mL of sterilized composite sample in 10 mL freshly prepared sterile nutrient broth, Brewer's medium and sabouraud agar, respectively, before discarding the samples to ensure aseptic disposal of microorganisms (Karki *et al*., 2013).

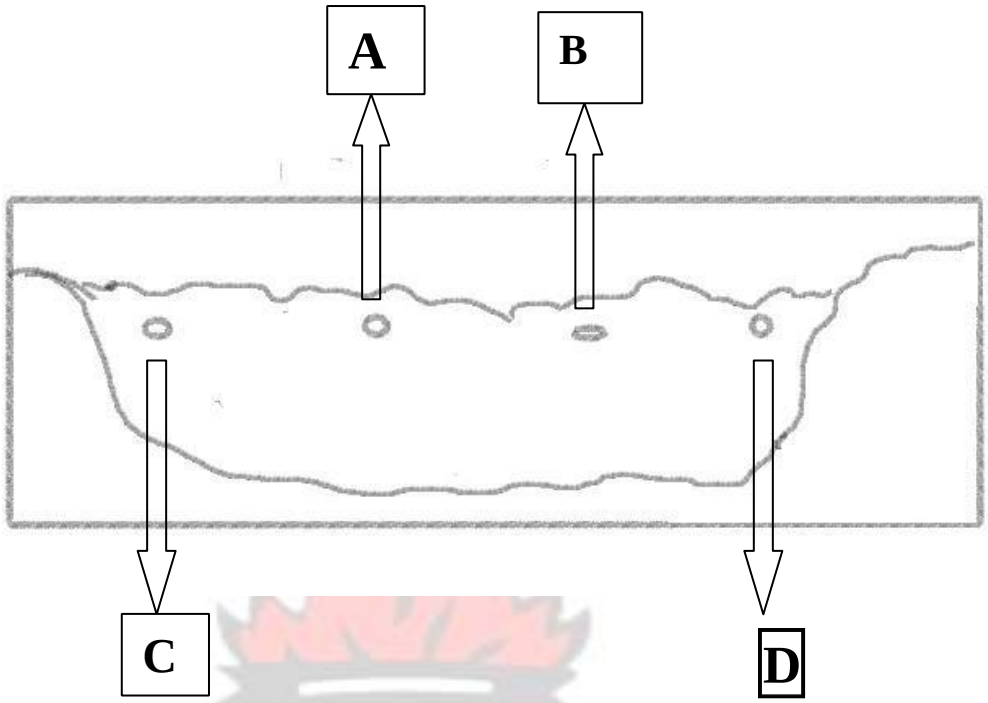

Figure 3.2: Schematic representation of a fish pond showing relative positions of sampling sites (A-site 1, B-site 2, C-site 3, and D-site 4) from which water samples were collected.

3.6 Collection of Bacterial Samples from fish

Non-symptomatic healthy fish samples (tilapia and catfish) were obtained from the eleven selected farms and two hatcheries with the aid of cast net for fish from fish farms and scoop nets for hatcheries. The fishes were dissected aseptically using a sterile scalpel and approximately 2.5 cm of the gut excised. Contents of the excised guts were transferred into 10 mL sterile tryptone soya broth and incubated for 24 h at 37°C. The samples were aseptically streaked unto respective media for the isolation of bacteria including *S. aureus, E.coli, Shigella spp, S. typhi and P. aeruginosa* using a sterile inoculating loop. Remaining samples were sterilized at 115°C for 30 min and re-checked for the presence or absence of viable aerobic bacteria, anaerobic bacteria and

fungal by aseptically cultivating 1 mL of sterilized composite sample in 10 mL freshly prepared sterile nutrient broth, Brewer's medium and sabouraud agar respectively before discarding the samples to ensure aseptic disposal of bacteria and fungi (Karki *et al*, 2013).

3.7 Isolation of Bacteria, Gram Staining and Biochemical tests

3.7.1 Isolation of bacteria from samples

Different selective media were streaked with the samples to isolate the organisms of interest. Mannitol Salt (MS) Agar for *Staphylococcus aureus*, Eosin Methylene Blue (EMB) was used to isolate *Escherichia coli*, Salmonella-Shigella (SS) Agar for *Salmonella spp* and *Shigella spp* and Pseudomonas Cetrimide Agar for *Pseudomonas aeruginosa*. Blood agar was used to demonstrate haemolysis characteristics of *Staphylococcus aureus* isolates (Benson, 2002).

Using a calibrated sterile loop, a loopful (0.1 µL) of sample was aseptically streaked on a 20 mL plate of mannitol salt agar (MSA) and incubated for 24 h at 37°C. *S. aureus* isolates were identified as bright yellow colonies. *S. aureus* ATCC 25923 was used as positive control organism.

A loopful (0.1 µL) of sample was aseptically streaked unto a 20 mL plate of eosin methylene blue (EMB) agar and incubated for 24 h at 37C. ^{0} *E.coli* isolates were identified as pink colonies. *E. coli* ATCC 25922 was used as a positive control organism.

On a 20 mL plate of Salmonella-Shigella agar (SSA), a loopful (0.1 µL) of sample was aseptically streaked. *Salmonella spp* were identified as black colonies and *Shigella spp* as pink colonies after incubation for 24 h at 37C ^{0} (Mikoleit, 2010; Perilla

et al., 2003). *S. typhi* ATCC 14028 and *Shigella flexneri* ATCC 12022 were used as positive control organisms.

A loopful (0.1 µL) of sample was streaked onto a 20 mL plate of Pseudomonas cetrimide agar and incubated for 24 h at 37C. ^{o} *P. aeruginosa* was identified as yellow-green colonies (Benson, 2002). *P. aeruginosa* ATCC 29213 was as a positive control organism.

3.7.2 Gram staining

Colonies on selective media were examined microscopically to determine the morphology of cells. A loopful of isolate was fixed on a slide and flooded gently with ammonium oxalate crystal violet solution for 20 sec. The slide was rinsed with water and Gram's iodine was added for 1 min and washed again. Ethyl alcohol (95%) was used to decolorize the smear and then washed with water and stained with the contrast stain, safranin for 20 sec. The slide was washed with water, blotted dry and observed at a magnification of 40X under the microscope (Brown, 2012).

3.8 Biochemical tests

Biochemical tests conducted included coagulase, catalase, oxidase, citrate, indole, Methyl red (MR) Voges Proskaeur (VP), Baird-Parker, Triple Sugar Iron (TSI) agar tests.

3.8.1 Confirmatory test for S. aureus

3.8.1.1 Coagulase test

To 0.5 mL 24 h broth culture of *S. aureus*, 0.5 mL of 1 in 10 citrated rabbit plasma was added, incubated at 37°C and observed every 30 mins for the first four hours and after

24h for coagulum. The presence of a clot in the test tube indicated a coagulase positive test. *S. aureus* ATCC 25923 was used as positive control organism.

3.8.1.2 Catalase test

Catalase mediates the breakdown of hydrogen peroxide H_2O_2 into oxygen and water. The presence of catalase enzyme in some bacteria is illustrated by the rapid formation of bubbles as a result of the release of oxygen from the breakdown of H_2O_2.

$$2\ H_2O_2 \longrightarrow 2H_2O + O_2$$

To 0.5mL of 3% hydrogen peroxide solution, 0.5mL of a 24 h broth culture of *S. aureus* was added. Bubble formation after 10 sec indicated catalase positive test. *S. aureus* ATCC 25923 was used as positive control organism.

3.8.1.3 Baird Parker

The ability of staphylococci to reduce tellurite to tellurium and to detect lecithinase from egg lecithin is the principle for the formulation of Baird Parker agar medium. The tellurite additive is toxic to egg yolk-clearing strains other than *S. aureus* and imparts a black colour to the colonies. *S. aureus* isolates were streaked on a stabilized plate of Baird Parker agar and incubated for 24 h at 37°C. Growth of grey-black shiny colonies indicated lipolytic and coagulase activities of *S. aureus* (de los Santos *et al*., 2014). *S. aureus* ATCC 25923 was used as a positive control organism.

3.8.1.4 *Haemolysis on blood agar*

Certain bacterial species produce extracellular enzymes that lyse red blood cells in the Blood agar (hemolysis). These hemolysin (exotoxin) radially diffuses outwards from the colony (or colonies) causing complete or partial destruction of the red cells (RBC) in the medium and complete denaturation of hemoglobin within the cells to colorless products. *S. aureus* isolates were streaked onto 5% blood agar plates and incubated for

24 h at 37°C. Cream colonies showed β-haemolytic activity indicated by a clearing of zones around growth as a result of complete haemolysis. (de los Santos *et al*., 2014). *S. aureus* 25923 was used as a positive control organism.

3.8.2 Confirmatory test for enterobacteria

3.8.2.1 Indole test

Tryptophan is hydrolysed by tryptophanase to produce indole. Indole test is used to determine the ability of an organism to spilt amino acid tryptophan to form the compound indole.

Ten microlitres of a 24-h broth culture of *E.coli* was inoculated into 100 µL of peptone water in a 96-well plate. After 24 h of incubation at 37°C, 10 µL of Kovac's reagent was added and observed after 20 min. A pink to red ring (formed as a result of the reaction of 4 p-dimethylamino benzaldehyde in the Kovac's reagent with indole to produce a red coloured compound) formation indicated a positive indole test. This was repeated for isolates of *S. typhi* and *Shigella spp* (Perilla *et al*., 2003). *E. coli* ATCC 25922 was used as a positive control organism.

3.8.2.2 Citrate Test

The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source.

A straightened platinum wire was used to inoculate *E. coli* at the bottom of a 5 mL Koser's citrate medium in a test tube. The caps of the tubes were tightened and tubes incubated at 37°C for 24 h. Blue colour formation (due to the reaction of the carbon dioxide that is released by pyruvate with water and the sodium ion in the medium to produce sodium carbonate, an alkaline compound that will raise the pH) indicated citrate positive and green colour, citrate negative. This was repeated for *S. typhi*,

Shigella spp and *P. aeruginosa* isolates (Harley, 2004). *Klebsiella aerogenes* ATCC 9621 was used as positive control organism and *E. coli* 25922 as negative control organism.

3.8.2.3 Oxidase Test

The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain.

A colony of *E. coli* from a 24-h culture was rubbed onto an oxidase disc. Blue colouration of oxidase disc as a result of the oxidization of the reagent (tetramethyl-pphenylenediamine) by the cytochrome C oxidase oxidizes to (indophenols) purple color end product within 10 sec indicated a positive oxidase test. This was repeated for *S. typhi, Shigella spp* isolates (Shields and Cathcart, 2013). *P. aeruginosa* ATCC 29213 was used as positive control organism and *E. coli* 25922 as negative control organism.

3.8.2.4 Methyl Red Voges Proskauer (MRVP) test

Methyl Red (MR) test determines whether the microbe performs mixed acids fermentation when supplied glucose. With modification to the standard MRVP test, 100 µL of MRVP broth was inoculated with 10 µL of a 24-h *E.coli* broth culture and incubated for 24 h at 37℃. The media was divided into two and further tests carried out. For the methyl red (MR) test, 10 µL of 0.05% methyl red was added. A red coloration (because of a pH at or below 4.4 from the fermentation of glucose) indicated MR positive. For the Voges-Proskauer (VP) test, to the other half of the broth culture, 10 µL of 0.3% creatine solution and 10 µL of 40% KOH solution were added. The absence of a bright pink colour (indicating the absence of diacetyl, the oxidation product of acetoin) indicated negative VP test. This was repeated for *S. typhi* and *Shigella spp* isolates (Feng *et al*., 2002). *E.coli* ATCC 25922 was used as positive control organism.

3.8.2.5 Triple Sugar Iron (TSI) Test

Using an inoculating loop, a 24-h culture of *S. typhi* was inoculated by stabbing the butt of a 20 mL TSI agar slant and then streaking upwards along the surface of the slant. The neck of the TSI tube was capped and the tube incubated at 37°C for 24 h. The tubes were observed for colour change; a yellow colouration indicated acid production (due to fermentation of glucose, lactose or sucrose) whereas a red colouration indicated alkaline production (due to non-fermentation of the sugars; glucose, lactose or sucrose) on butt and slant. The tube was also observed for gas production indicated by cracks in both slant and butt. H_2S production was observed as black precipitate on the medium (Forbes *et al.*, 2007). The test was repeated for *Shigella spp* and *E. coli*. *E.coli* ATCC 25922, *S. typhi* ATCC 14028 and *Shigella flexneri* ATCC 12022 were used as positive controls.

3.9 Antimicrobial susceptibility testing

The susceptibility of at least one biochemically confirmed isolate from each sample to selected antibiotics was performed by the disk diffusion method according to CLSI (2014) guidelines using antibiotic discs. This method of antimicrobial susceptibility testing is highly validated and reproducible. Twenty millilitres (20mL) of sterile Mueller-Hinton agar was poured aseptically into petri dishes and allowed to dry under a class II laminar air flow cabinet. Twenty-four hour colonies of bacteria were suspended into 3 mL sterile distilled water and turbidity adjusted to correspond to 0.5 McFarland standard (≈1.5 x 10^8 CFU/ml) and was streaked uniformly over the surface of the Mueller-Hinton agar using a sterile cotton swab. (Hudzicki, 2012). Antibiotic discs were administered using a disc dispenser on the inoculated plates and incubated for 24 h at 37°C. Antibiotic discs used were: penicillin (10 units), ampicillin (10 µg),

flucloxacillin (5 µg), tetracycline (30 µg), cefuroxime (30 µg), trimetoprimsulphamethoxazole (1.25/23.75 µg), gentamicin (10 µg), ciprofloxacin (5 µg) and chloramphenicol (30 µg).

In accordance with the CLSI guidelines for efficiency of the disk, *E.coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 29213 were used as controls. Zones of growth inhibition were measured from the edge of the disc to the start of growth after 24-h incubation at 37℃ and in accordance with the CLSI (2014) breakpoints, results were interpreted as isolates being susceptible, intermediate or resistant.

3.10 Statistical analysis

Microsoft Excel and Statistical Package for Social Science (SPSS, Chicago, Illinois, USA) version 22 was used to analyze the data on the survey of antimicrobial use on fish farms as well as the frequency of detection of resistant bacteria isolates from different farms and sources. The level of resistance to antibiotics from the various sources was compared using the chi-squared and students t-test at a 0.05 level of significance with 95% confidence interval.

CHAPTER FOUR

RESULTS

4.1 Survey of fish farms- analysis of questionnaires

A total of 63 farmers in the Ashanti Region were interviewed of which 73% of farmers reported no use of antibiotics on the fish farms. Three farmers, representing 4.8% use tetracycline on the fish farms whilst one (1.6%) used chloramphenicol.

These antibiotics were sourced from pharmacies, chemical sellers and veterinary shops and were mainly used for disease treatment in fish. They were administered either by mixing with the pond water or with feed.

Fifty-five percent of farmers used underground water (mainly springs) as source of water for the ponds while the remaining used rivers or streams. 82.9% of farmers had never experienced any disease outbreak on their farms. 27% prevent diseases on their farm by hygienic practices and best management practices which include water reuse, adequate aeration and circulation of ponds, moderate stocking levels and use of good quality fish feed.

47.6% of farmers fed their fish with both commercially formulated feed and food residues such as groundnut husks and rice bran. Two hatcheries added antibiotics (tetracycline or chloramphenicol) to fish feed. 93.6% of respondents who used manure on fish farms used poultry manure from commercial poultry farms and used it mainly to fertilize fish ponds.

Seven respondents used antibiotics for livestock farming around the pond. 25.4% of farmers discarded water from ponds into rivers or streams whilst 58.7% had outlets leading to drains for discarding water (Table 4.1).

Table 4.1: Antibiotic use in fish farms

Antibiotic use practice	Response	Number of farmers	%
Antibiotic used in farm	Tetracycline	3	4.8
	Chloramphenicol	1	1.6
	None	56	73
	Non-response	3	4.8
Source of antibiotic	Pharmacy	1	1.6
	Chemical seller	1	1.6
	Veterinary shops	2	3.2
Method of antibiotic administration	Feed	1	1.6
	Water bath	2	3.2
	Feed and water bath	1	1.6

Table 4.2: Source of water and disposal practices among fish farmers

Water source and disposal practice	Response	Number of farmers	%
Source of water	River	17	27
	Stream	11	17.5
	Wells, springs, boreholes	35	55.6
Frequency of water change	1-3 months	4	6.3
	4-6 months	6	9.5
	7-12 months	14	22.2
	>12 months	23	36.5
	Never	8	12.7
	Non-response		
Method of discarding water	Outlets into river/stream	16	25.4
	Outlets into bush	3	4.8
	Outlets into drains and pond	38	60.3
	Non-respondents	6	9.5

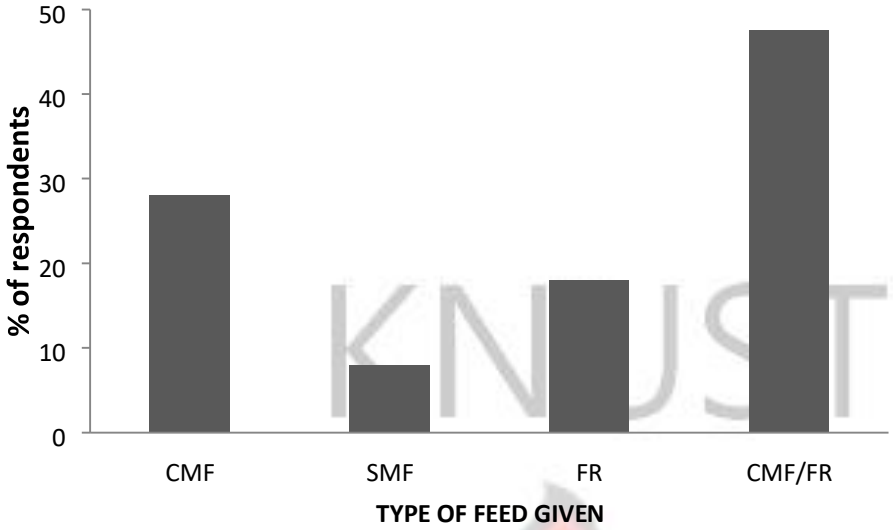

Figure 4.1: Type of feed used in fish farms

CMF- Commercially manufactured feed; SMF- Self manufactured feed; FR(Food residues-rice bran, maize meal, groundnut husk); CMF/FR(Commercially manufactured feed plus food residues)

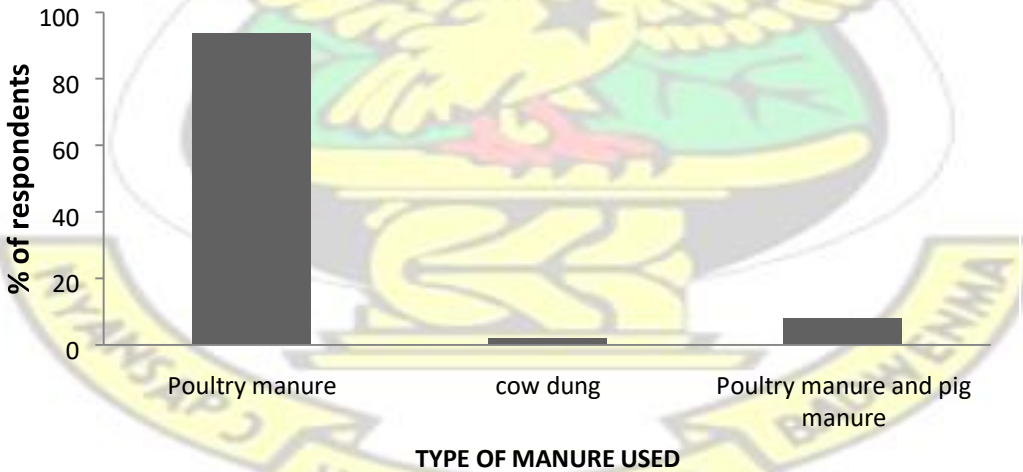

Figure 4.2: Type of manure used in fish farms

ANTIBIOTIC USE IN FISH HATCHERIES

Six out of nine fisheries officers confirmed the use of antibiotics in fish farming to either prevent or treat diseases in fish especially in hatcheries. According to 5 out of the 9 officers, the antibiotics are administered either by mixing with feed or water. The fisheries officers recommended either poultry or pig manure to farmers for use on the main farms. (Table 4.3; 1)

Table 4.3: Responses on use of antibiotics in fish farms by fisheries officers

Question	Response	Number of officers
Reason for antibiotic use in fish farms	Prophylaxis	3
	Therapeutic	1
	Both	3
Recommended stage	Hatcheries	6
Method of administration	Feed	1
	Water	1
	Feed and water bath	3

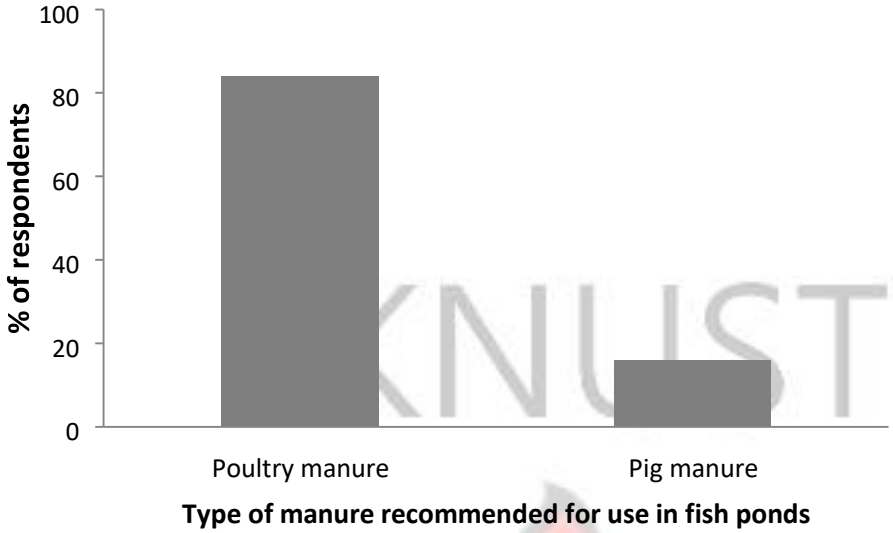

Figure 4.3: Type of manure recommended for use in fish ponds by fisheries officers

4.2 Sample collection

Samples were collected from a total of 13 fish farms including 2 hatcheries. These farms were selected randomly from the 63 fish farmers interviewed and included farms which had been in active production for at least six months. At least, one composite sample each of water and fish gut contents was collected from each farm depending on the types of fish farmed on respective farms. A total of 44 composite samples (Discrete samples from the various ponds on respective farms were combined, thoroughly homogenized, and treated as a single sample) were collected from the 13 farms. These include 20 water samples, 8 composite catfish gut samples and 16 composite tilapia gut samples (Table 4.4). Out of 645 isolates from these samples, 288 were confirmed through various biochemical tests as *S. aureus, E.coli, Shigella spp, S. typhi* and *P. aeruginosa* (Table 4.4).

Table 4.4: Isolation of organisms from selected farms

Farm	NCWS	NCTS	NCCS	NI	% number of isolates
Farm 1	1	1	0	14	4.9
Farm 2	4	3	2	56	19.4
Farm 3	1	1	0	13	4.5
Farm 4	1	1	1	11	3.8
Farm 5	1	1	0	6	2.1
Farm 6	1	1	1	21	7.3
Farm 7	1	1	0	10	3.5
Farm 8	1	1	1	13	4.5
Farm 9	1	1	1	8	2.8
Farm 10	1	1	0	24	8.3
Farm 11	1	1	1	17	5.9
Farm 12	4	1	1	55	19.1
Farm 13	2	2	0	40	13.9
Total	**20**	**16**	**8**	**288**	**100.0**

NCWS- Number of composite water samples; NCTS- Number of composite tilapia gut samples; NCCS- Number of composite catfish samples; NI- Number of isolates from farm

4.3 Identification of isolated organisms

The microorganisms of interest were identified using biochemical tests and Gram staining technique (Brown, 2012). Biochemical test aid in the identification of organisms based on their unique metabolic or fermentation process which are catalyzed by specific enzymes found in these organisms (Perilla *et al*., 2003). The reaction leads to changes in colour. In gram staining, organisms are identified based on the thickness of their cell wall as Gram-positive or Gram-negative (Figure 4.4 and 4.5).

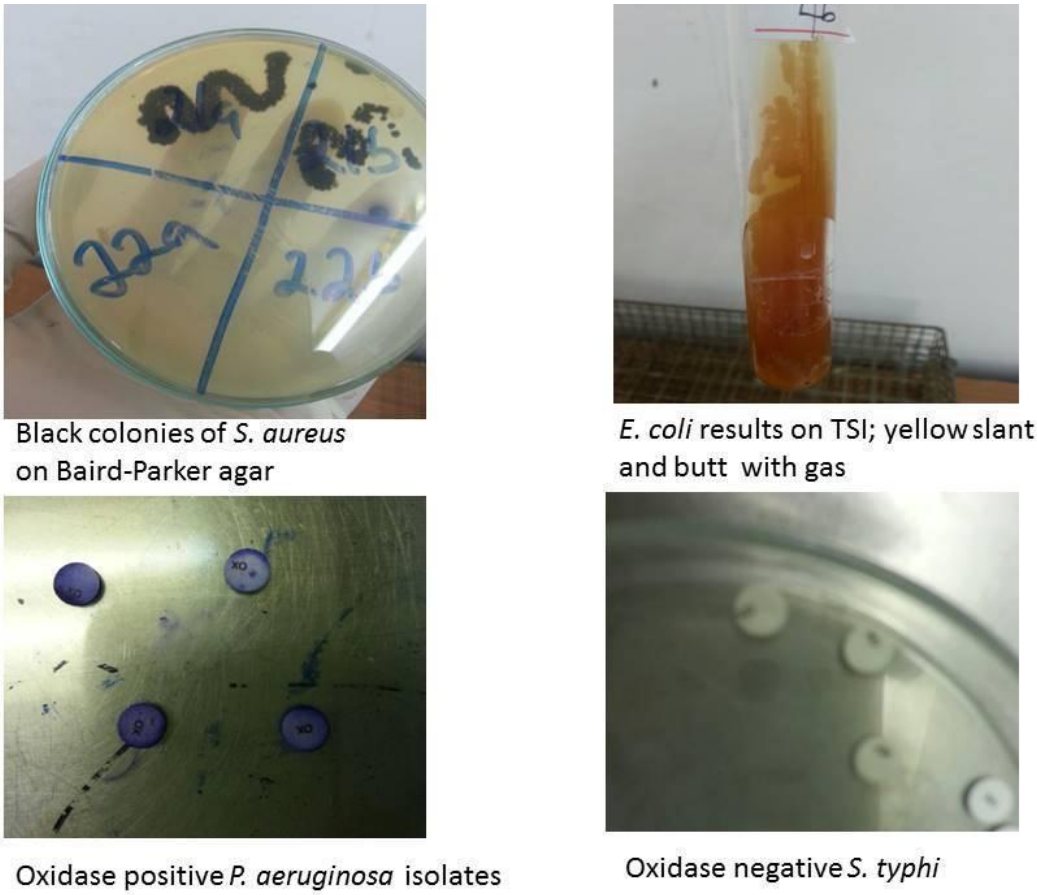

Figure 4.4: Sample results from biochemical tests performed on some isolates

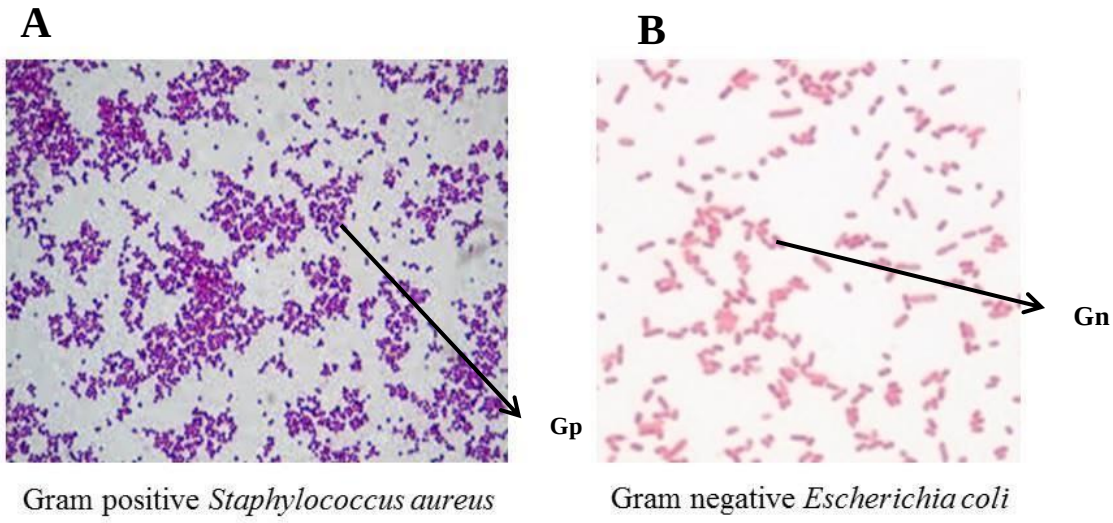

Figure 4.5: A. Gram staining of *Staphylococcus aureus* B. Gram-staining of *Escherichia coli*

Gp: purple –stained Gram positive cocci Gn: Pink- stained Gram-negative rods

Seventy two (25%) of the isolates were identified as *S. aureus* whereas 58 (20.1%), 47

(16.3%), 49 (17%), 62 (21.5%) of the isolates were *E.coli*, *Shigella spp*, *S. typhi* and *P. aeruginosa*, respectively (Table 4.5).

Table 4.5: Frequency of isolation of organisms

	Organism					
	S. aureus	*E. coli*	*Shigella spp*	*S .typhi*	*P. aeruginosa*	Total
Frequency	72	58	47	49	62	288
Percent	25.0	20.1	16.3	17.0	21.5	100.0

Again, *S. aureus* (23.2%), *E. coli* (19%), *P. aeruginosa* (22.5%), *S. typhi* (18.3%) *and Shigella spp* (16.9%) were isolated from water samples from fish ponds. Tilapia samples for the study were also observed to habour *S. aureus* (26.3%), *E. coli* (22.1%), *P. aeruginosa* (23.2%), *S. typhi* (12.6%) *and Shigella spp* (15.8%). In the intestine of catfishes, *S. aureus* (27.5%), *E. coli* (19.6%), *P. aeruginosa* (15.7%), *S. typhi* (17.6%) and *Shigella spp* (19.6%) were isolated. Overall, *S. aureus* was the most isolated and *Shigella spp* was the least isolated in water sample from fishponds as well as tilapia and catfish samples (Figure 4.6).

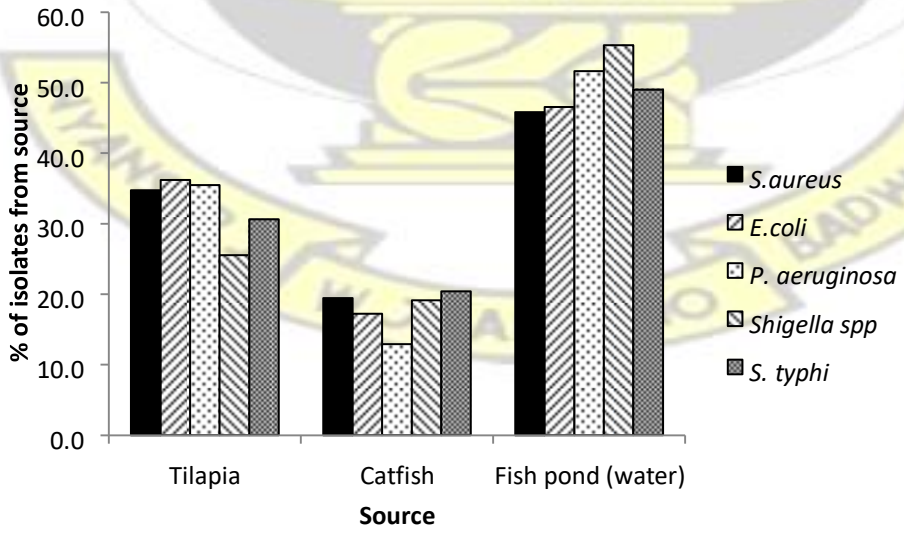

Figure 4.6: Microbial isolates from water (fish pond), tilapia and catfish.

4.4 Antibiotic susceptibility tests

The confirmed isolates were studied for their susceptibility to penicillin, ampicillin, flucloxacillin, erythromycin, tetracycline, sulphamethoxazole/trimethoprim, cefuroxime, gentamicin, ciprofloxacin and chloramphenicol using the disc diffusion method (Hudzicki, 2012). This method was chosen because it is simple and reproducible. The results were interpreted according to CLSI (2014) guidelines.

All isolates of *S. aureus*, *E.coli, S. typhi* and *Shigella spp* were resistant to ampicillin. All *S. aureus* isolates showed 100% resistance to penicillin, ampicillin and flucloxacillin. With the exception of gentamicin and ciprofloxacin, there was varying resistance of more than 60% to the other antibiotics (Table 4.3). Generally, isolates showed high resistance to penicillin, ampicillin, flucloxacillin and tetracycline. Low resistance was observed in all isolates to gentamicin (1.7% to 5.6%) except in *P. aeruginosa* with 29.0% resistance to gentamicin.

All *E.coli* isolates were resistant to ampicillin. Resistance to tetracycline, cotrimoxazole, cefuroxime and chloramphenicol was between 62.1 to 96.6%. Resistance to gentamicin and ciprofloxacin was however 3.4% and 19.0%, respectively (Figure 4.6).

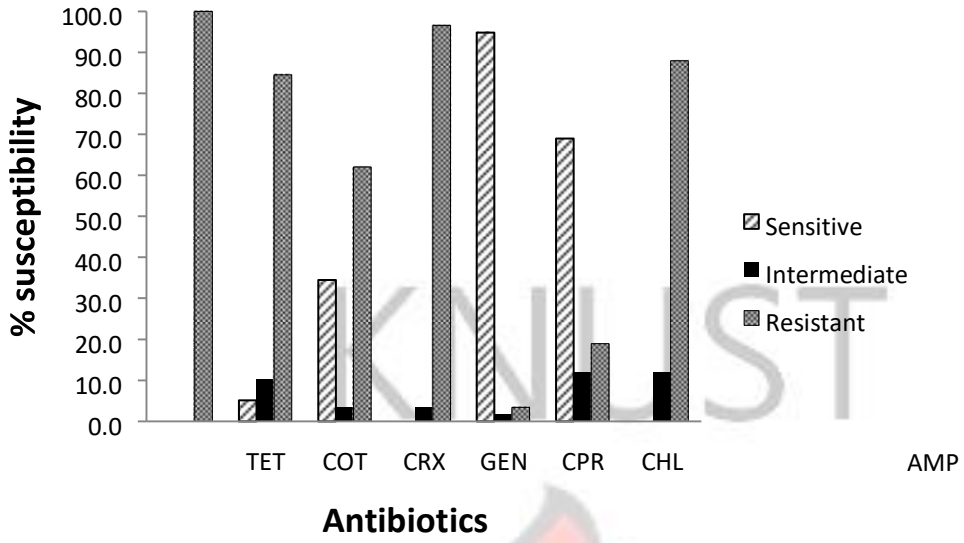

Figure 4.7: Susceptibility of *E. coli* isolates from fish farms to selected antibiotics.

AMP:Ampicillin(10µg); TET: Tetracycline (30µg); COT :Trimethoprim/Sulphamethoxazole (1.25/23.75µg) CRX:Cefuroxime(30µg); GEN:Gentamicin(10µg); CPR: Ciprofloxacin(5µg); CHL: Chloramphenicol(30µg).

Resistance of *Shigella spp* isolates to ampicillin was 100%. Resistance to ciprofloxacin, tetracycline, cotrimoxazole, cefuroxime and chloramphenicol was between 36.3% - 95.7%. All *Shigella spp* isolates were susceptible to gentamicin

(Figure 4.7).

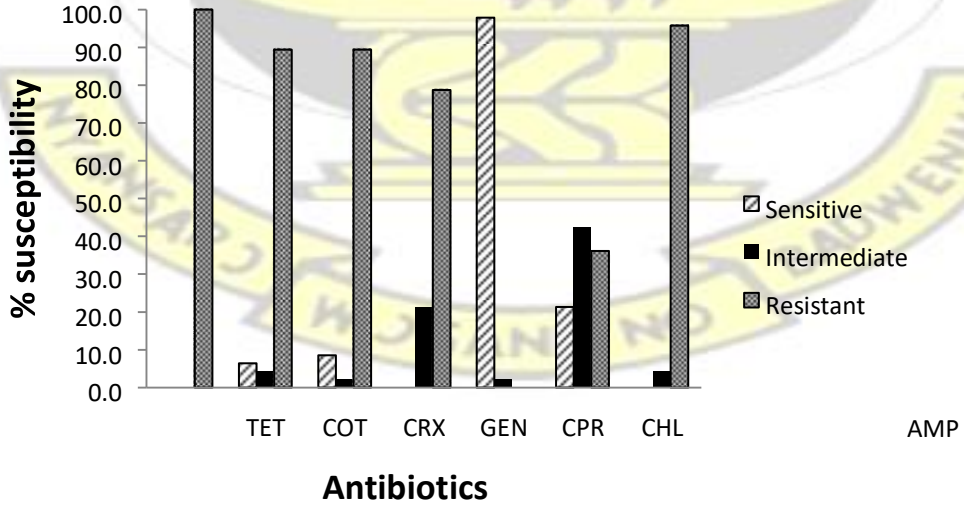

Figure 4.8: Antibiotic susceptibility of *Shigella spp* isolates from fish farms.

AMP:Ampicillin(10µg); TET: Tetracycline(30µg); COT :Trimethoprim/ Sulphamethoxazole (1.25/23.75µg) CRX: Cefuroxime(30µg); GEN:Gentamicin (10µg) ; CPR: Ciprofloxacin(5µg); CHL: Chloramphenicol(30µg).

S.typhi isolates were 100%, 89.8% and 87.8% resistant to ampicillin, tetracycline and chloramphenicol respectively. Resistance to cotrimoxazole, cefuroxime, ciprofloxacin and gentamicin was 61.2% and 59.2%, 18.4% and 4.1%, respectively (Figure 4.8).

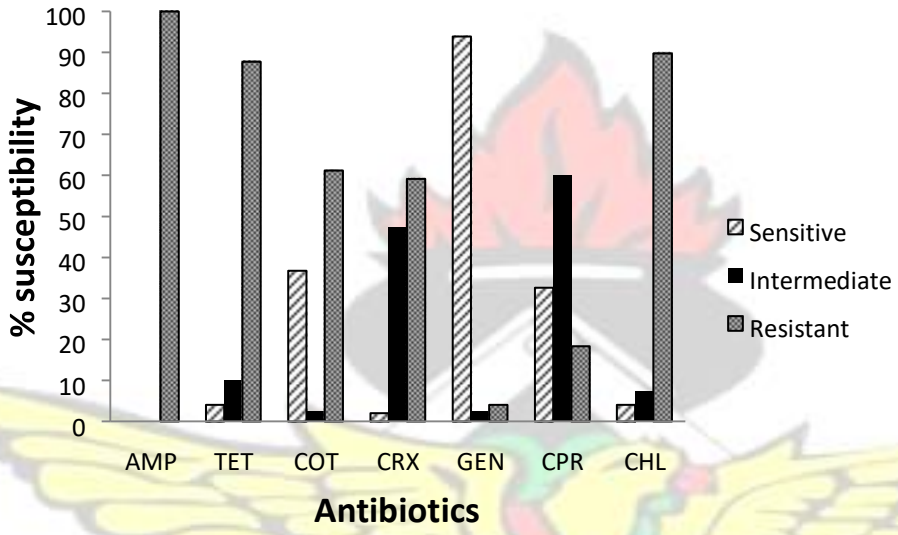

Figure 4.9: Antibiotic susceptibility of *S. typhi* isolates from fish farms.

AMP:Ampicillin(10µg); TET: Tetracycline(30µg); COT :Trimethoprim/ Sulphamethoxazole (1.25/23.75µg) CRX:Cefuroxime(30µg); GEN:Gentamicin (10µg); CPR: Ciprofloxacin(5µg); CHL: Chloramphenicol(30µg).

P. aeruginosa isolates showed resistance of 29.03% and 51.61% to gentamicin and ciprofloxacin, respectively (Figure 4.9)

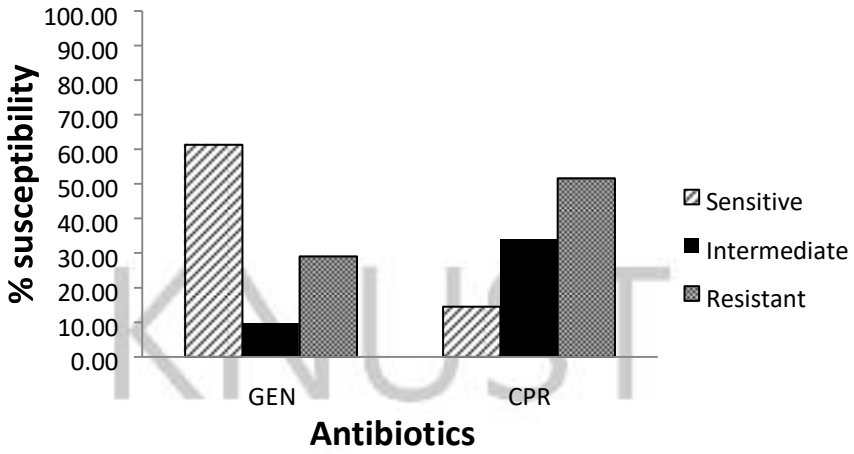

Figure 4.10: Antibiotic susceptibility of *P. aeruginosa* isolates from fish farms.

GEN: Gentamicin (10µg); CPR: Ciprofloxacin (5µg)

S. aureus isolates showed 100% resistance to penicillin, ampicillin and flucloxacillin respectively. Resistance to erythromycin, tetracycline, cotrimoxazole and cefuroxime was 62.5% - 86.1%. Resistance to gentamicin and ciprofloxacin was 5.6% - 29.2% respectively. (Figure 4.10)

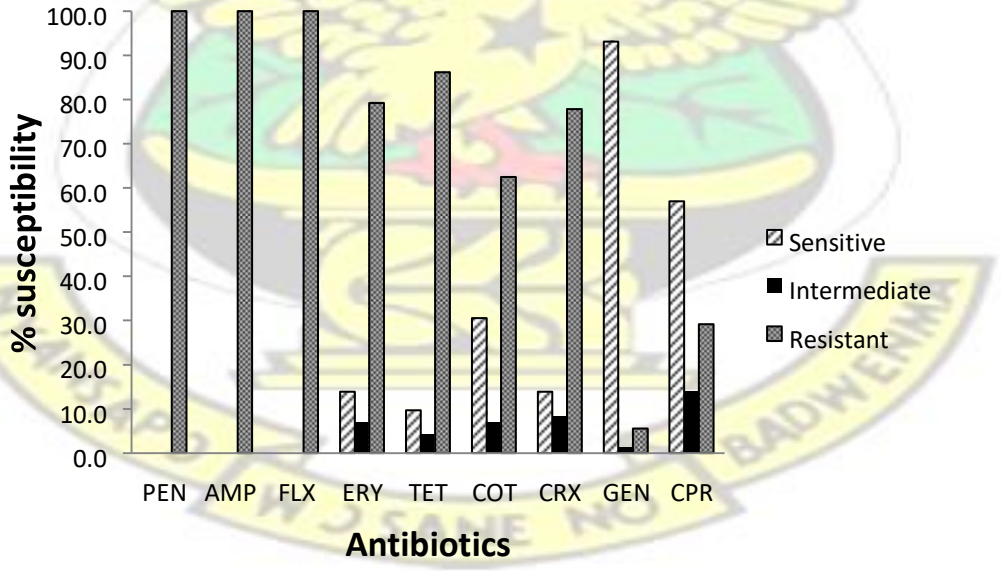

Figure 4.11: Antibiotic susceptibility of *S. aureus* isolates from fish farms.

PEN: Penicillin(10 units); AMP:Ampicillin(10µg); FLX: Flucloxacillin(5µg); ERY: Erythromycin (15µg) TET: Tetracycline(30µg); COT :Trimethoprim/ Sulphamethoxazole (1.25/23.75µg) CRX:Cefuroxime(30µg); GEN:Gentamicin(10µg); CPR: Ciprofloxacin(5µg)

4.4.2 Multidrug resistant isolates

Multidrug resistant (MDR) isolates show resistance by various mechanisms to different classes of antibiotics. (Magiorakos *et al*., 2012). MDR bacteria were defined as isolates with acquired resistance to two or more antibiotics indicated in their respective CLSI (2014) panels. 87.3% of isolates of organisms showed resistance to more than 3 antibiotics, with the exception of *P. aeruginosa*. *S. aureus* isolates showed multidrug resistance to up to 8 antibiotics (Figure 4.11).

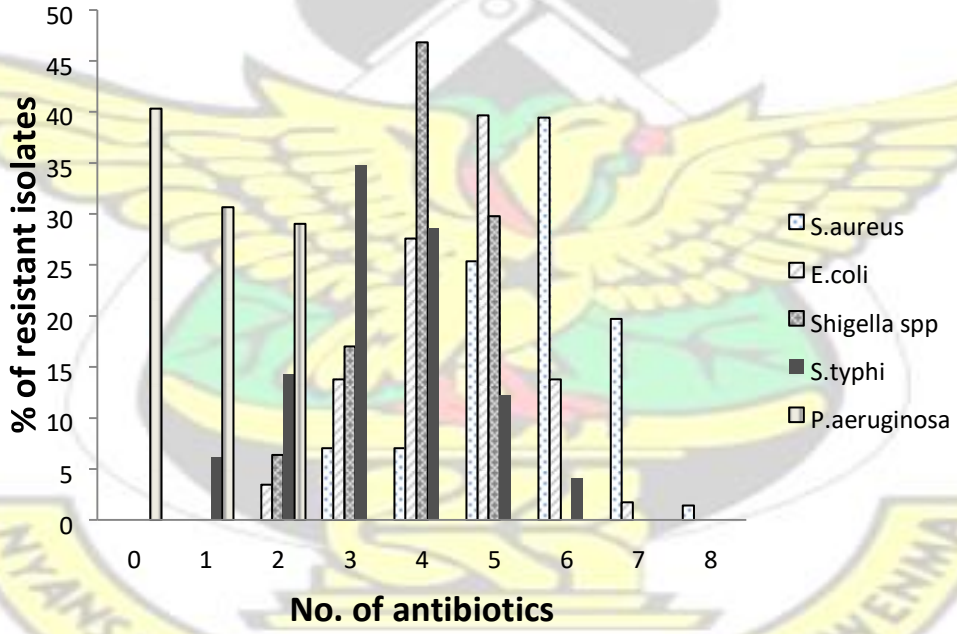

Figure 4.12: Number of antibiotics to which isolates are resistant

4.4.3 Resistant isolates from farms and hatcheries

Isolates from fish farms were compared to isolates from hatcheries to determine if there was any difference in resistance patterns between isolates from either source. Isolates

from both main farms and hatcheries exhibited >50% resistance to antibiotics with the exception of gentamicin and ciprofloxacin. Isolates from both hatcheries and fish farms were 100% resistant to penicillin, ampicillin and flucloxacillin. Tetracycline resistance in main farms was 84.70% as compared to 90.80% resistance in isolates from the hatcheries. Resistance to gentamicin was the lowest at 10.40% and 5.20% in fish farms and hatcheries, respectively.

Table 4.6: Resistance of isolates to antibiotics from fish farms and hatcheries

	FARM				
ANTIBIOTIC	Fish farm		Hatchery		
PEN	54	100.00	18	100.00	-[b]
AMP	150	100.00	76	100.00	-[b]
FLX	54	100.00	18	100.00	-[b]
ERY	40	74.10	17	94.40	0.167
TET	127	84.70	69	90.80	0.440
COT	102	68.00	51	67.10	0.780
CRX	119	79.30	56	73.70	0.161
GEN	20	10.40	5	5.20	0.332
CPR	65	33.90	25	26.00	0.367
CHL	90	93.80	50	86.20	0.057
	Number	Fish farm %	Number	Hatchery %	(CHI-SQ P)[a]

PEN: Penicillin(10 units); AMP:Ampicillin(10µg); FLX: Flucloxacillin(5µg); ERY: Erythromycin(15µg) TET: Tetracycline(30µg); COT :Trimethoprim/Sulphamethoxazole(1.25/23.75µg) CRX:Cefuroxime(30µg); GEN:Gentamicin(10µg); CPR: Ciprofloxacin(5µg) [a]=p-value comparing antibiotic resistance of isolates from main farm to isolates from hatchery [b]= p-value not computed.

Using the students' t-test, the observed differences in resistance to antibiotics in main farms compared to hatcheries were insignificant for all antibiotics (Table 4.6).

4.4.4 Resistant isolates from water, catfish and tilapia

A one-way analysis of variance (ANOVA) (to determine differences between the means of more than two independent groups) was employed to determine whether there was significant differences in source of isolates and their resistance to the antibiotics tested revealed a statistically significant difference (p=0.033) in resistant isolates from water, catfish and tilapia, only for tetracycline resistance (F=3.455, p=0.033). Post-hoc Tukey's tests (to determine if any two groups within the study are related after initial ANOVA tests) on tetracycline resistant isolates from the three sources revealed statistically significant difference (p=0.027) between resistant isolates from water and isolates from tilapia). However, there was no significant difference between tetracycline resistant isolates from water and catfish (p=0.874) as well as between isolates from tilapia compared to catfish (p=0.256). For the other antibiotics, there was no significant difference (p>0.05) in resistance of isolates from water compared to isolates from tilapia and catfish (Table 4.7).

Table 4.7: Resistance of isolates from water, catfish and tilapia to different antibiotics

	SOURCE		

		Water	Tilapia		Catfish		
ANTIBIOTIC	N	%	N	%	N	%	(p=value)[b]
PEN	33	100	25	100	14	100.0	-
AMP	111	100	72	100.0	43	100.0	-
FLX	33	100	25	100.0	14	100.0	-
ERY	27	81.8	20	80.0	10	71.4	0.612
TET	101	91.0	56	77.8	39	90.7	0.033
COT	79	71.2	48	66.7	26	60.5	0.402
CRX	83	74.8	60	83.3	32	74.4	0.390
GEN	16	11.2	8	8.5	1	2.0	0.255
CPR	38	26.6	35	37.2	17	33.3	0.423
CHL	71	91.0	42	89.4	27	93.1	0.840

PEN: Penicillin(10 units); AMP:Ampicillin(10µg); FLX: Flucloxacillin(5µg); ERY: Erythromycin(15µg) TET: Tetracycline(30µg); COT :Trimethoprim/Sulphamethoxazole(1.25/23.75µg) CRX:Cefuroxime(30µg); GEN:Gentamicin(10µg); CPR: Ciprofloxacin(5µg)[b]= p-value comparing resistant isolates from water to catfish and tilapia N=number of isolates

4.4.5 Resistant isolates per farm

The number of resistant isolates on each farm was computed to determine the resistance pattern of isolates to antibiotics on the individual farms studied. This is important in relating the resistant isolates on the farms to the practices observed. The number of resistant isolates from respective farms to antibiotics was calculated as a percentage of the total number of antibiotic resistant isolates. Farms 2 and 12 had the highest number of resistant isolates. 26.1% of trimethoprim/sulphamethoxazole resistant isolates were from Farm 12. 24.1% of gentamicin resistant isolates were from Farm 2. Almost 20% and 1% of tetracycline resistant isolates were from Farm 12 and Farm 5 respectively. Isolates from Farms 4, 5 and 8 were all susceptible to gentamicin (Table 4.8).

Table 4.8: Resistant isolates per farm

		ANTIBIOTICS									
FARM	Number of isolates	PEN %[a]	AMP %	FLX %	ERY %	TET %	COT %	CRX %	GEN %	CPR %	CHL %
Farm1	14	5.6	4.4	5.6	7.0	5.1	4.6	4.0	4.0	4.4	4.3
Farm 2	56	18.1	17.7	18.1	19.3	18.9	17.6	19.4	24.0	18.9	17.1
Farm 3	14	4.2	5.3	4.2	5.3	5.1	6.5	6.9	8.0	7.8	6.4
Farm 4	11	4.2	4.0	4.2	5.3	4.1	5.2	5.1	0.0	6.7	4.3
Farm 5	6	5.6	2.7	5.6	1.8	1.0	3.3	2.9	0.0	1.1	1.4
Farm 6	20	9.6	7.1	9.6	10.4	7.7	5.9	6.9	8.0	8.9	6.4
Farm 7	10	2.8	3.5	2.8	3.5	3.6	5.2	3.4	12.0	2.2	3.6
Farm 8	13	8.3	4.9	8.3	7.0	4.6	3.9	3.4	0.0	3.3	2.9
Farm 9	8	2.8	2.7	2.8	1.8	2.0	1.3	2.3	8.0	2.2	2.9
Farm 10	23	8.3	7.5	8.3	3.5	7.1	5.9	7.4	12.0	10.0	7.1
Farm 11	18	5.6	7.1	5.6	5.3	6.1	7.2	6.9	4.0	6.7	8.6
Farm 12	55	13.8	19.0	13.8	15.8	19.9	26.2	20.0	16.0	24.4	22.9
Farm 13	40	11.1	14.1	11.1	14.0	14.8	7.2	11.4	4.0	3.4	12.1
Total	**248**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Penicillin(10 units); AMP:Ampicillin(10µg); FLX: Flucloxacillin(5µg); ERY: Erythromycin(15µg) TET: Tetracycline(30µg); COT :Trimethoprim/ Sulphamethoxazole (1.25/23.75µg) CRX:Cefuroxime(30µg); GEN:Gentamicin(10µg); CPR: Ciprofloxacin(5µg**).** [a]%=% number of isolates from farm resistant to antibiotic/Total number of antibiotic resistant isolates.

CHAPTER FIVE

5.1 DISCUSSION

Antibiotics are used on fish farms for prophylaxis and treatment of diseases and some which are also used in humans, have been used in fish farming and may be administered through feed or direct application in pond water (Romero *et al*., 2012). Antibiotics administered to fish may accumulate in the water and sediments of fish ponds and may result in selective pressure in the pond environment leading to antimicrobial resistance of fish pathogens (Marshall and Levy, 2011). From the survey on antibiotic use in fish farms in the Ashanti region, only 7.8% of respondents used antibiotics on fish farms. This may be because they had not experienced any disease outbreaks on their farms and depend on fisheries officers to make

interventions in the event of outbreaks of diseases. It could also be due to the fact that most of the farms visited engaged in practices such as maintaining water hygiene, using appropriate stock densities and the use of quality feed. Faruk *et al.* (2004) reported these practices to be associated with prevention of diseases on fish farms and thus eliminate the need for use of antibiotics on the farms.

Diseases in fish have been associated with high stocking densities and poor water quality (Osungbemiro *et al*., 2014). The antibiotic of choice is tetracycline which is mainly in hatcheries for prophylaxis in fingerlings. This was also affirmed by the fisheries officers interviewed. Tetracycline is known to be one of the most commonly used antibiotics in fish farms especially in hatcheries (Guglielmetti *et al*., 2009; Dietze *et al*., 2005).

Antibiotics may be administered through feed and hence uningested fish feed may leach into the environment and accumulate resulting in resistance (Dietze *et al*., 2005). From

the study, 47.6% of farmers use both commercially manufactured feed and food residues such as groundnut husks and rice bran. The commercially manufactured feed presumably had no antibiotics added as stated on the labels by manufacturers. As reported by Gabriel *et al*. (2007), most sub-Saharan African fish farmers depend largely on imported fish feed or on non-conventional feed such as kitchen waste and plant sources as few are produced locally. With the exception of the hatchery farmers who add tetracycline to the fish feed for fingerlings, the farmers do not add antibiotics to their fish feed.

With the rapid increase in freshwater fish farming, there is the need for enforcement of regulations on use of antibiotics in fish farms and in animal production in Ghana as this may control the development of antibiotic resistance in these farms. Regulations on antibiotic use in aquaculture have been enforced in some countries in North America and Europe (Heuer *et al.*, 2009). Surveillance on antibiotic resistant isolates from the studied farms is important to control the spread of antibiotic resistance.

Pond effluents may be a source of microbiological pollutants to receiving water bodies (Boyd, 2003). From this study, 25.4% of farmers interviewed dispose of effluent from their fish ponds into rivers or streams whilst 58.7% dispose effluents through drains. The disposal of water from ponds into nearby water bodies may contribute to the transfer of antibiotic resistant microorganisms into receiving water bodies. Though this study did not investigate the levels of bacteria at the receiving water bodies, the high numbers of resistant bacteria isolated from the fish pond environment could be transferred to receiving water bodies as observed in a study of water from fish ponds and receiving streams from selected farms in the Ashanti Region, significant levels of bacteria upstream, downstream and reference locations of the streams receiving effluents from the fish ponds (Ansah *et al*., 2013).

S. aureus, E.coli, S. typhi, Shigella spp and Pseudomonas aeruginosa were isolated from the selected fish ponds. These species have been isolated from fishes raised in fresh and brackish water as well as pond water (Uddin and Al-Harbi, 2012; Osungbemiro *et al*., 2014).

The presence of these enteric bacteria in both water and fish samples is an indication of faecal contamination as these pathogens are normally found in warm-blooded animals and are rarely part of the normal fish flora (Elsaidy *et al*., 2015; Koonse *et al*., 2005). In a study of fertilized ponds in Ghana, Ampofo and Clerk (2010) isolated *Pseudomonas spp, Salmonella spp, E. coli, S. aureus* and *Shigella spp* from tilapia in fertilized fish ponds which corroborate our findings. *Pseudomonas spp* have also been identified as fish pathogens and can remain present in tilapia even when processed (Newaj Fyzul *et al*., 2008; Najiah *et al*., 2009). This can pose a threat to public health as they can be transferred to humans and a possible transfer of resistant bacterial strains to humans

The use of organic manure by farmers may contribute to antibiotic resistance on the farms by transfer of antibiotic residues and resistant bacteria to fish farms if the commercial farms from which the manure is sourced use antibiotics (Elsaidy *et al*., 2015). From the survey, 93.6% of respondents use poultry manure for fertilizing the ponds. This could be a possible source of enteric bacteria in both the pond water and fish samples as observed in studies of integrated fish farms in Vietnam where tetracycline resistant *Enterococcus faecium, Enterococcus faecalis,* and other *Enterococcus spp*. in the water-sediment and manure samples isolated in the ponds were found to have originated mainly from the pig manure (Dang *et al*., 2011). A study in Egypt by Elsaidy *et al*. (2015) reported higher incidence of both *E. coli* and

Salmonella spp in water and fish raised in ponds receiving unfermented chicken manure than in those receiving fermented chicken manure. The study recommended the use of fermented chicken manure as a bacteriologically safe fish pond fertilizer. Isolates of *S. aureus, E.coli, S. typhi* and *Shigella spp* showed 100% resistance to ampicillin. This finding is comparable to report by Newaj-Fyzul *et al.* (2008), where 92.0% of five genera of bacteria isolated from tilapia and cohosalmon hatcheries were ampicillin resistant and in which various resistant phenotypes to penicillin, vancomycin, chloramphenicol, tetracycline, sulphamethoxazole/trimethoprim and gentamicin were found in 20 to 100% of isolates. Even though antibiotics were not previously used in the hatcheries prior to their study. Karki *et al.* (2013) also reported ampicillin resistance in bacteria isolated from the hatcheries in the USA and recommended further study to determine the source of antibiotic resistance in the hatcheries. Su *et al*. (2011) observed that enterobacteria isolated from integrated fish farms in China showed high antibiotic resistance to ampicillin (80%), tetracycline (52%) and trimethoprim (50%) and indicated high multiple antibiotic resistance in isolates from animal manure on the farms. *E. coli* isolates showed least resistance to gentamicin (1.7%) and ciprofloxacin (19%). In a study of cultured catfish in Malaysia, *E. coli* isolates were 100% susceptible to norfloxacin, sulphamethoxazole/trimethoprim and chloramphenicol but showed resistance of 35.3%, 23.5% and 11.8% to ampicillin, tetracycline and nitrofurantoin respectively (Samuel *et al*., 2011).

Generally, resistance of isolates from the hatcheries to antibiotics was slightly lower than that of isolates from the main farms. However, there was higher resistance to tetracycline and erythromycin in isolates from hatcheries than from main farms. There was 100% resistance in isolates from both hatcheries and main farms to ampicillin, penicillin and flucloxacillin (Table 4.5). The differences in resistance to the antibiotics

from the main farms and hatcheries were however, not significant (p>0.05). Both hatcheries use tetracycline for prophylaxis in the fingerlings and this could account for the high resistance of 90.80% in isolates from the hatcheries. This differs from report by Karki *et al.* (2013) where bacterial isolates from hatcheryraised tilapia and cohosalmon were all resistant to ampicillin and penicillin but were sensitive to gentamicin. The isolates also showed varying resistance to chloramphenicol, tetracycline, vancomycin and streptomycin. However, the hatcheries in that study used no antibiotics. Seyfried *et al.* (2010), suggested that aquatic environments may harbour tetracycline resistant genes regardless of the use of tetracycline or not and the source of water for the hatchery tanks (well and tap water) may also contribute to the resistance of isolates to antibiotics as bacteria flora in the fish gut may be acquired from the water or feed. The resistant bacteria though they may not be pathogenic, can transfer resistance to other bacteria.

From the survey, tetracycline was stated as the antibiotic used in fish hatcheries mainly to prevent diseases in the fingerlings. This could account for why isolates from both hatcheries and main fish farms showed high resistance of 90% and 84%, respectively, to tetracycline. There have been reports of tetracycline resistant determinants in isolates of *Salmonella spp* from tilapia in South African fish ponds where there had not been recent use of tetracycline (Chenia and Vietze, 2012). Tetracycline resistant determinants may accumulate and persist in aquatic environments (Seyfried *et al.*, 2010).

Though most farmers (73%) reported not to have used antibiotics on the main farms, high resistance of isolates to antibiotics studied was recorded. This is similar to a study by Shah *et al.* (2012), where bacteria isolated from water, pond sediment and fish from fish ponds in Pakistan and Tanzania with no recorded history of antibiotic use showed

resistance to tetracycline, sulphamethoxazole/trimethoprim, amoxicillin and chloramphenicol and they hypothesized the contribution of integrated fish farming practices using domestic farm waste as a likely source of resistance genes in the aquaculture environment. Schmidt *et al.* (2001) also reported the presence of resistant genes in aeromonads isolated from a fish farm with no history of recent antibiotic use for therapeutic purposes nor in feed and suggested the resistance was due to the persistence of previously acquired resistance genes in the pond environment.

There was no significant difference (p>0.05) in resistance to antibiotics in isolates from water compared to isolates from catfish and tilapia except in tetracycline (p=0.033). There was statistically significant (p=0.027) higher levels of tetracycline resistant isolates from pond water compared to tilapia but not catfish. The increase in tetracycline resistant isolates in pond water may be attributed to the excretion of tetracycline resistant isolates into the water which subsequently donate resistant genes to other bacteria in the pond water. The presence of tetracycline residues in water may also lead to selective pressure (Petersen *et al*., 2002).

There was high level of resistance to ampicillin (100%), tetracycline (84.5-91.5%), cefuroxime (59.2-96.6%), sulphamethoxazole/trimethoprim (61.2-89.4%) and chloramphenicol (87.9-95.7%) in the enteric bacteria (*E.coli, S. typhi* and *Shigella spp*), which ordinarily are not part of fish flora but an indication of faecal contamination as shown in other studies where various antibiotic resistant pathogenic bacteria such as *E.coli, S. typhi, Shigella spp* have been isolated from animal manure (Su *et al*., 2011; Dang *et al*., 2011; Petersen *et al*., 2002). The presence of these enteric bacteria in this study may be due to the use of organic manure from commercial farms, especially poultry as most farmers interviewed use poultry manure to fertilize the ponds. Donkor *et al*. (2012) and Turkson *et al*. (2008) showed that different classes of antibiotics

including penicillins, tetracyclines and sulphonamides among others are used in livestock and poultry farming in Ghana and may contribute to antibiotic resistance. The use of manure on the farms may have contributed to high antibiotic resistance in the bacteria isolates from the main farms.

The presence of these highly resistant pathogens which are also pathogens of humans is a public health threat as these resistant bacteria may cause diseases in humans. These include gastroenteritis, diarrhoea, shigellosis and salmonellosis (Novotny *et al*., 2004). The resistant bacteria may transfer resistance directly to humans or indirectly by transferring resistant determinants to other pathogenic bacteria of humans. These multi drug resistant bacteria may be a problem as therapeutic failure may occur in the event of an outbreak of fish diseases in the fish on these farms as well as diseases caused by these resistant bacteria in humans.

These resistant bacteria may also be transferred into other water bodies considering the mode of disposal of water from the farms. All the farms studied are situated by streams or rivers and water is discarded mainly through outlets into the river or stream. Multidrug resistant bacteria from fish farms have been found in water environments in various rports (Zhang *et al*., 2009; Baquero *et al*., 2008; Stachowiak *et al*., 2010) and are a call for concern**.**

The emergence of multidrug resistance (MDR) bacteria is of major concern globally. This is due to the fact that MDR bacteria are difficult to treat in aquaculture, livestock and in humans. MDR was observed in all but *Pseudomonas spp* with most isolates of *S. aureus, S. typhi, and E.coli* and *Shigella spp* showing MDR. Though there are reports of multidrug resistance in *Pseudomonas spp,* because only two antibiotics were tested against *Psuedomonas spp* in this study, multidrug resistance cannot be concluded. The

use of more than one antibiotic on fish farms may result in selective pressure leading to multiple antibiotic resistant isolates on fish farms as reported by Sarter *et al.* (2007) where bacteria of the genus *enterobacteriacae*, *pseudomonads* and *vibrionaceae* showed multiple resistance to antibiotics including oxytetracycline, chloramphenicol, trimethoprim-sulphamethoxazole, nitrofurantoin, nalidixic acid, and ampicillin. Infections caused by multidrug resistant organisms may be difficult to manage or can lead to high mortality since these organisms have multiple resistance mechanisms which enables them to inactivate antibiotics. Hence, the observed trend of multidrug resistant strains poses a major public health concern globally (Magiorakos *et al*., 2012).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

From the survey of fish farmers in the Ashanti Region, 73% of respondents do not use antibiotics on main fish farms. Tetracycline is used for prophylaxis in fish hatcheries. Other practices such as organic manure use may contribute to antibiotic resistance and disposal of water into nearby water bodies may contribute to the spread of antibiotic resistance.

Isolates of *S. aureus, E.coli, Shigella spp, S. typhi and P. aeruginosa* from pond water and guts of catfish and tilapia from fish farms showed multidrug resistance to test antibiotics even though all the farms studied had no history of use of antibiotics. Isolates from hatcheries also showed multidrug resistance even though only tetracycline is used for prophylaxis in fingerlings. There was no significant difference in the resistance of bacteria isolates to antibiotics from the pond water, tilapia and catfish except in tetracycline resistant isolates from water compared to catfish. There was also no significant difference in resistance of bacteria isolates from hatcheries and main farms to antibiotics.

6.2 RECOMMENDATIONS

- It is recommended that the mechanisms of resistance in bacteria isolates from fish farms be studied to determine if there is any correlation between resistant genes in bacteria isolates from fish farms and that in clinical samples.

- Education on the implications of antibiotic use in fish farms should be continued by Fisheries officers to prevent an increase in antibiotic resistance on fish farms.
- It is also recommended that the Food and Drugs Authority (FDA) regulates the use of antibiotics in fish farming in Ghana.
- It is also recommended that studies should be done to determine the source of antibiotic resistant genes such as Tet genes responsible for tetracycline resistance and Extended spectrum β-lactamases (ESBLs) for β -lactam resistance in bacteria isolates from fish farms in the Ashanti Region.
- Farms should have effluent treatment systems in place to reduce risk of biological pollution of receiving waters and environment.

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APPENDICES

APPENDIX I

QUESTIONNAIRE

ANTIBIOTIC USE IN FISH FARMING IN THE ASHANTI REGION

LOCATION……

1. How long has the farm been operating?

[a]<2 years [b] 2-5 years [c] 5-10 years [d] >10years

2. Is aqua farming your only occupation? [a] YES [b] NO **3. How many ponds do you have?** …… 4. **How many types of fishes do you deal with? (Name them)**

……

5. Which antibiotics do you use in farming?

[a] Tetracycline [b] Chloramphenicol [c] Amoxycillin [d] None

[e] Others……

6. **Where do you get them from?**

[a] Pharmacy [b]chemical sellers [c]veterinary shops [d] other……

7. **What do you use antibiotics for?**

[a] Disease prevention [b] disease treatment [c] growth promotion [d] Other……

8. How do you use the antibiotics?

……

9. What is the source of water for the pond?

[a] River [b] Stream [c] Pipe borne water [d] Other……

10. **How often do you change the water in the pond?**

[a] 1-3 months [b]4-6 months [c]7-12months [d]>12 months

11. **How do you discard water from the pond?**

……**1**

12. **What disease do you most commonly find in the fish?**

……

13. **How do you identify diseased fish?**

……

14. **How do you treat diseased fish?**

……

15. **How many types of fishes do you deal with within a particular pond?**

……

16. **Do the fishes suffer similar diseases?**

[a]YES [b] NO

17. **Has there ever been an outbreak of a dangerous disease on the farm?**

[a] YES [b] NO

If YES, What disease was it? ……

18. How do you prevent disease outbreak on the fish farm?

……

19. What feed do you give the fish?

[a] Formulated feed from shops [b] Self-manufactured feed [c] rice bran [d]others

20. Which antibiotics do you add to the feed?

[a]Tetracycline [b] Chloramphenicol [c] Amoxycillin [d] None [e] Others……

21. Do you add manure to the ponds?

[a] YES [b] NO

22. Which manures do you add to the fish ponds?

[a] Poultry droppings [b] pig droppings [c] Cow dung [d] Others ……

23. Why do you use manure?

[a] To fertilize the pond [b] To feed the fish [c] Others……

24. How many types of fishes do you deal with within a particular pond?

……

25. Do the fishes suffer similar diseases?

[a]YES [b] NO

26. What other purposes do you use antibiotics for around the ponds?

……

27. How do you dispose of waste from around the pond?

……

……

QUESTIONNAIRE (FISHERIES OFFICERS)

ANTIBIOTIC USE IN FISH FARMING IN THE ASHANTI REGION

1. How long have you worked with the fisheries commission or as a veterinary officer?

[a]<2 years [b] 2-5 years [c] 5-10 years [d] >10years

2. Which antibiotics are commonly used in fish farming?

[a] Tetracycline [b] Chloramphenicol [c] Amoxicillin [d] Others……

3. What dosage forms of antibiotics are recommended for use in fish farming?

[a] Tablets [b] liquid [c] Powders [d] Others

4. **What is the source of these?**

[a] Pharmacy [b]chemical sellers [c]veterinary shops [d] other……

5. **What are the antibiotics used for?**

[a] Disease prevention [b] disease treatment [c] growth promotion [d] Other……

6. When do you recommend use of antibiotics in fish farms?

[a] hatcheries [b] main ponds [c] others

7. How are the antibiotics used?

……
……

8. How often should the water in the pond be changed?

[a] 1-3 months [b]4-6 months [c]7-12months [d]>12 months

9. What infectious disease do you find in the fish?

[a]Catfish……

[b]Tilapia……

[c] Others……

10. How do you identify diseased fish?

……
……

11. How do you treat infections in fish?

……
……

12. Do the fishes (catfish and tilapia) suffer similar diseases?

[a]YES [b] NO

13. Has there ever been an outbreak of a dangerous disease on any of the fish farms?

[a] YES [b] NO

If YES, What disease was it? …….

14. How is disease outbreak prevented on fish farms?

……
…….

……
…….

15. Which manure is recommended for use in fish ponds?

[a] Poultry droppings [b] pig droppings [c] Cow dung [d] Others
……..

16. Why should manure be used?

[a] To fertilize the pond [b] To feed the fish [c] Others……

APPENDIX 2

RESULTS FROM SURVEY OF FISH FARMERS AND FISHERIES OFFICERS

Table A.1 DEMOGRAPHICS OF FISH FARMERS

YEARS OF OPERATION			**OCCUPATION OF FARMER**			**NUMBER OF PONDS**			**TYPE OF FISH FARMED**		
	f	Valid %		f	Valid %	Number of ponds	f	valid %		F	Valid %
< 2 years	2	3.2	**Fish farming only**	10	16.1	**1-5**	53.0	85.50	**Catfish**	4	6.6
2-5 years	23	36.5	**Other**	52	83.9	**6-10**	6.0	9.7	**Tilapia**	5	8.2
5-10 years	28	44.4				**11-15**	1.0	1.6	**Catfish and Tilapia**	52	85.2
> 10 years	10	15.9				**16-20**	2.0	3.2			

Table A.2: DISEASES OF FISH ON FARMS

DISEASES OBSERVED IN FISH				INCIDENCE OF OUTBREAK ON FARM				HOW FISH DISEASES ARE PREVENTED ON FARMS			
	Frequency	%	Valid %		Frequency	%	Valid %		Frequency	%	Valid %
None	17	27.0	27.0	**Yes**	7	11.1	17.1	**Hygienic/ best management practices**	17	27.0	85.0
tail rot	1	1.6	1.6	**No**	34	54.0	82.9	**Removal of diseased fish**	1	1.6	5.0
tail rot, pop eye	1	1.6	1.6					**no idea**	2	3.2	10.0

Table A.3: USE OF MANURE ON FISH FARMS

USE OF MANURE ON FARMS	REASON FOR USE OF MANURE

	Frequency	%	Valid %		Frequency	%	Valid %
Yes	45	71.4	72.6	**To fertilize pond**	44	69.8	93.6
No	17	27.0	27.4	**Feed fish**	1	1.6	2.1
Total	**62**	**98.4**	**100.0**	**To fertilize pond and to feed fish**	2	3.2	4.3

Table A.4: OTHER USES OF ANTIBOTICS ON FISH FARM

OTHER USE OF ANTIBIOTIC AROUND THE POND			
	Frequency	Percent	Valid Percent
Livestock farming	7	11.1	36.8
None	12	19.0	63.2

Table A.5: YEARS OF EXPERIENCE AS FISHERIES OFFICER

NUMBER OF YEARS WORKED AS FISHERIES OFFICER			
	Frequency	Percent	Valid Percent

< 2 YEARS	1	11.1	11.1
2-5 YEARS	3	33.3	33.3
6-10 YEARS	2	22.2	22.2
>10 YEARS	3	33.3	33.3
Total	9	100.0	100.0

Table A.6: RECOMMENDED TIMES FOR CHANGE OF POND WATER BY FISHERIES OFFICERS

How often should water for ponds be changed			
	Frequency	Percent	Valid Percent
3 months for concrete ponds, 2 years for earthen ponds	3	33.3	42.9
2 years	1	11.1	14.3
everyday for hatcheries	1	11.1	14.3
when necessary	2	22.2	28.6

Table A.7: DISEASES COMMONLY OBSERVED IN FISHES BY FISHERIES OFFICERS

Which diseases are commonly observed in catfish				Which diseases are commonly observed in tilapia				How are fish diseases identified			
	Frequency	%	Valid %		Frequency	%	Valid %		Frequency	%	Valid %
Head crack	2	22.2	50.0	**Tail rot**	2	22.2	66.7	**Change in movement pattern**	2	22.2	28.6
Others	2	22.2	50.0	**Tail rot and pop eye effect**	1	11.1	33.3	**colouration of skin**	2	22.2	28.6

								Change in feeding and movement	3	33.3	42.9

Table A.8: MANURE RECOMMENDED FOR USE IN FISH FARMING BY FISHERIES OFFICERS

Which manure do you recommend for use in fish farming?				Why should manure be used			
	Frequency	Percent	Valid Percent		Frequency	Percent	Valid Percent
Poultry manure	6	66.7	85.7	**To fertilize pond**	6	66.7	100.0
Pig manure	1	11.1	14.3				

APPENDIX II (PREPARATION OF MICROBIOLOGICAL MEDIA)

A.MANNITOL SALT AGAR

Code: CM0085

A selective medium for the isolation of presumptive pathogenic staphylococci. Most other bacteria are inhibited, with the exception of a few halophilic species.

Formula	**gm/litre**
`Lab-Lemco' powder	1.0
Peptone	10.0
Mannitol	10.0
Sodium chloride	75.0
Phenol red	0.025
Agar	15.0
pH 7.5 ± 0.2 at 25°C	

Directions

111 g of mannitol salt agar was suspended in 1 litre of distilled water, brought to boil completely and sterilized by autoclaving at 121°C for 15 minutes.

B.PSEUDOMONAS CETRIMIDE AGAR (USP, EP)

Code: CM0579

Pseudomonas Cetrimide Agar is used for the selective isolation and identification of Pseudomonas aeruginosa.

Formula	**gm/litre**
Gelatin peptone	20.0
Magnesium Chloride	1.4
Potassium Sulphate	10.0
Cetrimide	0.3
Agar	13.6

Final pH 7.2 ± 0.2 at 25°C

Directions

45.3 g of Pseudomonas Cetrimide agar was suspended in 1 litre of sterile distilled water and brought to boil completely. It was sterilized by autoclaving at 121°C for 15 minutes, cooled to approximately 50°C and poured into sterile Petri dishes.

C. SALMONELLA SHIGELLA AGAR (SS AGAR)

Code: CM0099

A differential selective medium for the isolation of Salmonella and some Shigella species from clinical specimens, foods etc.

Formula	**gm/litre**
`Lab-Lemco' powder	5.0
Peptone	5.0
Lactose	10.0
Bile salts	8.5
Sodium citrate	10.0
Sodium thiosulphate	8.5
Ferric citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	15.0

pH 7.0 ± 0.2 at 25°C

Directions

63 g of Salmonella Shigella Agar was suspended in 1 litre of distilled water brought to the boil with frequent agitation and allowed to simmer gently to dissolve the agar. It was cooled to about 50°C, mixed and poured into sterile Petri dishes.

D. EOSIN METHYLENE BLUE AGAR (MODIFIED) LEVINE

Code: CM0069

An isolation medium for the differentiation of the Enterobacteriaceae.

Formula	**gm/litre**
Peptone	10.0
Lactose	10.0
Dipotassium hydrogen phosphate	2.0
Eosin Y	0.4
Methylene blue	0.065
Agar	15.0
pH 6.8 ± 0.2	

Directions

37.5 g of Eosin Methylene Blue agar powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely. It was sterilized by autoclaving at 121°C for 15 minutes, cooled to 60°C shaken gently and poured into sterile petri dishes.

E. TRIPLE SUGAR IRON AGAR

Code: CM0277

A composite medium for the differentiation of Enterobacteriaceae by three sugar fermentations and hydrogen sulphide production

Formula	**gm/litre**
`Lab-Lemco' powder	3.0
Yeast extract	3.0
Peptone	20.0
Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0

Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	0.024
Agar	12.0
pH 7.4 ± 0.2 at 25°C	

Directions

65 g of Triple sugar Iron agar was suspended in 1 litre of distilled water and brought to the boil to dissolve completely. It was mixed well and distributed into tubes and sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to set in sloped form with a deep butt.

F. BLOOD AGAR BASE NO.2

Code: CM0271

An improved Blood Agar Base possessing enhanced nutritional properties suitable for the cultivation of fastidious pathogens and other micro-organisms.

Formula	**gm/litre**
Proteose peptone	15.0
Liver digest	2.5
Yeast extract	5.0
Sodium chloride	5.0
Agar	12.0
pH 7.4 ± 0.2 at 25°C	

Directions

40 g of Blood agar base was suspended in1 litre of distilled water brought to the boil to dissolve completely and sterilized by autoclaving at 121°C for 15 minutes. It was cooled to 50°C,7% of sterile blood was added , mixed gently with rotation and poured into sterile petri dishes.

G. BAIRD-PARKER AGAR BASE

Code: CM0275

A selective and diagnostic medium for the isolation and enumeration of

Staphylococcus aureus	*in foods*
Formula gm/litre	
Tryptone	10.0
`Lab-Lemco' powder	5.0
Yeast extract	1.0
Sodium pyruvate	10.0
Glycine	12.0
Lithium chloride	5.0
Agar	20.0

pH 6.8 ± 0.2 at 25°C

Directions

63 g of Baird parker agar base was suspended in one litre of distilled water and boiled to dissolve the medium and sterilized by autoclaving at 121°C for 15 minutes. It was cooled to 50°C and 50ml of Egg Yolk Tellurite Emulsion aseptically added, mixed well and poured into sterile petri dishes.

H. MUELLER-HINTON AGAR Code:

CM0337

An antimicrobial susceptibility testing medium which may be used in internationally recognized standard procedures.

Formula	**gm/litre**
Beef, dehydrated infusion 300.0 from	
Casein hydrolysate	17.5
Starch 1.5 Agar 17.0 pH 7.3 ±	
0.1 at 25°C	
Directions	

38 g of Mueller Hinton agar was added to 1 litre of distilled water and brought to the boil to dissolve the medium completely. It was sterilized by autoclaving at 121°C for 15 minutes.

I.TRYPTONE SOYA BROTH (Casein soya bean digest medium) EP/USP/JP/BP Code: CM0129

Formula	**gm/litre**
Pancreatic digest of casein	17.0
Enzymatic digest of soya bean*	3.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.5
Glucose	2.5
pH 7.3 ± 0.2 at 25°C	

Directions

30 g of Tryptone Soya Broth powder was added to 1 litre of distilled water, mixed well and distributed into final tubes. It was sterilized by autoclaving at 121°C for 15 minutes.

J. NUTRIENT BROTH

Code: CM0001

A general purpose fluid medium for the cultivation of micro-organisms not exacting in their nutritional requirements. Blood, serum, sugars, etc., may be added as required for special purposes.

Formula	**gm/litre**
`Lab-Lemco' powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
pH 7.4 ± 0.2 at 25°C	

Directions

13 g of nutrient broth powder was added to 1 litre of distilled water, mixed well and distributed into final containers. It was sterilized by autoclaving at 121°C for 15 minutes.

K.KOSER CITRATE MEDIUM M069

Formula gm/litre

Sodium ammonium phosphate 1.500
Monopotassium phosphate 1.000

Magnesium sulphate 0.200

Sodiumcitrate3.000

Final pH (at 25°C) 6.7±0.2

Directions

5.7 g of Koser Citrate medium was suspended in 1000 ml distilled water. Dispense into tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes

L.PEPTONE WATER

Code: CM0009

A basal medium to which carbohydrates and indicator may be added for fermentation studies.

Formula	**gm/litre**
Peptone	10.0
Sodium chloride 5.0	pH 7.2
± 0.2	

Directions

15 g of peptone water powder was dissolved in 1 litre of distilled water and distribute into tubes. Final tubes were sterilized by autoclaving at 121°C for 15 minutes.

APPENDIX III (GROWTH CHARACTERISTICS AND BIOCHEMICAL TESTS)

Table 0.A.1: Morphological and biochemical characteristics of *S.aureus*

ISOLATE	SOURCE	COLONY CHARACTERISTICS ON AGAR			BIOCHEMICAL TESTS		INFERENCE
		MANNITOL SALT AGAR	BAIRD-PARKER	BLOOD AGAR	CATALASE	COAGULASE	
1ASa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
1ASa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
2A1Sa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
2A1Sa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
2A2Sa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
2A2Sa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
2A3Sa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
2A3Sa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
3ASa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
3ASa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
5ASa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
5ASa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
6ASa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
6ASa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
7ASa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
8ASa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
8ASa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*

Y/H- Yellow colonies with haemolysis

Table B.1: Morphological and biochemical characteristics of *S.aureus*

ISOLATE	SOURCE	COLONY CHARACTERISTICS ON AGAR			BIOCHEMICAL TESTS		INFERENCE
		MANNITOL SALT AGAR	BAIRD-PARKER AGAR	BLOOD AGAR	CATALASE	COAGULASE	
10ASa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
10ASa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
11ASa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
11ASa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
12A1Sa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
12A1Sa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
12A2Sa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
12A2Sa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
12A3Sa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
12A3Sa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
12A4Sa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
12A4Sa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
13A1Sa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*

13A1Sa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
13A2Sa1	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
13A2Sa2	Water	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
1BSa1	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
1BSa2	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
2B2Sa1	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
2B2Sa2	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
2B4Sa1	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*

Y/H- Yellow with haemolysis

ISOLATE		GROWTH CHARATERISTICS ON AGAR		BIOCHEMICAL TESTS			INFERENCE
		MANNITOL SALT AGAR	BAIRD-PARKER AGAR	BLOOD AGAR	CATALASE	COAGULASE	
2B4Sa2	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
3BSa1	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
4BSa2	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
5BSa1	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*

5BSa2	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
6BSa1	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
6BSa2	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
7BSa1	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
8BSa1	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
8BSa2	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
10BSa1	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
10BSa2	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
11BSa1	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
11BSa2	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
12BSa1	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
12BSa2	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
13B1Sa1	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
13B1Sa2	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
13B2Sa1	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
13B2Sa2	Tilapia	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
2C1Sa1	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*

Table B.1: Morphological and biochemical characteristics of *S.aureus*

Y/H- Yellow with haemolysis

Table B.1: Morphological and biochemical characteristics of *S. aureus*

ISOLATE	SOURCE	GROWTH CHARACTERISTICS ON AGAR			BIOCHEMICAL TESTS		INFERENCE
		MANNITOL SALT AGAR	BAIRD-PARKER AGAR	BLOOD AGAR	CATALASE	COAGULASE	
2C1Sa2	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
2C4Sa1	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
4CSa1	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
4CSa2	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
6CSa2	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
6CSa2	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
6CSa3	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
8CSa1	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
8CSa2	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
9CSa1	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
9CSa2	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
10CSa1	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*
10CSa2	Catfish	Bright yellow colonies	shiny grey-black colonies	Y/H	+	+	*S. aureus*

YY/H- Yellow with haemolysis

Table B.2: Morphological and biochemical characteristics of *E. coli* isolates from fish farms

ISOLATE	SOURCE	COLONY CHARACTERISTICS ON AGAR	BIOCHEMICAL TESTS									INFERENCE
		EOSIN METHYLENE BLUE AGAR	INDOLE	CITRATE	MR	VP	OXIDASE	TSI				
								SLANT	BUTT	H2S	GAS	
1AEc1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
2A1Ec1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
2A1Ec2	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
2A2Ec1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
2A2Ec2	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
2A3Ec1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
2A3Ec2	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
3AEc1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
6AEc1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
7AEc1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
7AEc2	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
10AEc1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
10AEc2	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
11AEc1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
11AEc2	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
12A1Ec1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
12A1Ec2	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
12A2Ec1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*

12A2Ec2	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*

GMS/DPC-Green metallic sheen with dark purple center

Table Morphological and biochemical characteristics of isolates from fish farms

B.2: *E. coli*

ISOLATE	SOURCE	GROWTH CHARACTERISTICS ON AGAR	BIOCHEMICAL TESTS									INFERENCE
			INDOLE	CITRATE	MR	VP	OXIDASE	TRIPLE SUGAR IRON				
		EOSIN METHYLENE BLUE AGAR						SLANT	BUTT	H2S	GAS	
12A3Ec1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
12A3Ec2	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
12A4Ec1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
12A4Ec2	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
13A1Ec1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
13A1Ec2	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
13A2Ec1	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
13A2Ec2	Water	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
1BEc2	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
2B2Ec1	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
2B2Ec2	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
2B3Ec1	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
2B3Ec2	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*

3BEc1	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
3BEc2	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
4BEc1	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
4BEc2	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
5BEc2	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
6BEc2	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
9BEc1	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
11BEc1	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*

GMS/DPC- green metallic sheen with dark purple center

Table B.2: Morphological and biochemical characteristics of *E. coli* isolates from fish farms

ISOLATE	SOURCE	GROWTH CHARACTERISTICS ON AGAR	BIOCHEMICAL TESTS									INFERENCE
			INDOLE	CITRATE	MR	VP	OXIDASE	TRIPLE SUGAR IRON				
		EOSIN METHYLENE BLUE AGAR						SLANT	BUTT	H2S	GAS	
11BEc2	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
12BEc1	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
12BEc2	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
13B1Ec1	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
13B1Ec2	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
13B2Ec1	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*

Table B.3: Morphological and biochemical characteristics of *S.typhi* isolates from fish farms

13B2Ec2	Tilapia	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
2C1Ec1	Catfish	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
2C1Ec2	Catfish	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
2C4Ec1	Catfish	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
2C4Ec2	Catfish	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
4CEc1	Catfish	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
6CEc1	Catfish	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
10CEc1	Catfish	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
10CEc2	Catfish	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
12CEc1	Catfish	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*
12CEc2	Catfish	GMS/DPC	+	–	+	–	–	yellow	yellow	–	+	*E. coli*

GMS/DPC-Green metallic sheen with dark purple center

ISOLATE	SOURCE	COLONY CHARACTERISTICS	BIOCHEMICAL TESTS									INFERENCE
			INDOLE	CITRATE	MR	VP	OXIDASE	TSI				
		SALMONELLA SHIGELLA AGAR						SLANT	BUTT	H2S	GAS	
1ASt1	Water	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
2A1St1	Water	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
2A2St1	Water	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
2A2St2	Water	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
2A3St1	Water	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
3ASt1	Water	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*

3ASt2	Water	Colourless with black center	-	+	+	-	-	Red	Yellow	+	-	*S.typhi*
6ASt1	Water	Colourless with black center	-	+	+	-	-	Red	Yellow	+	-	*S.typhi*
7ASt1	Water	Colourless with black center	-	+	+	-	-	Red	Yellow	+	-	*S.typhi*
10ASt1	Water	Colourless with black center	-	+	+	-	-	Red	Yellow	+	-	*S.typhi*
10ASt2	Water	Colourless with black center	-	+	+	-	-	Red	Yellow	+	-	*S.typhi*
11ASt1	Water	Colourless with black center	-	+	+	-	-	Red	Yellow	+	-	*S.typhi*
11ASt2	Water	Colourless with black center	-	+	+	-	-	Red	Yellow	+	-	*S.typhi*
12A1St1	Water	Colourless with black center	-	+	+	-	-	Red	Yellow	+	-	*S.typhi*
12A1St2	Water	Colourless with black center	-	+	+	-	-	Red	Yellow	+	-	*S.typhi*
12A2St1	Water	Colourless with black center	-	+	+	-	-	Red	Yellow	+	-	*S.typhi*
12A2St2	Water	Colourless with black center	-	+	+	-	-	Red	Yellow	+	-	*S.typhi*
12A3St1	Water	Colourless with black center	-	+	+	-	-	Red	Yellow	+	-	*S.typhi*
12A3St2	Water	Colourless with black center	-	+	+	-	-	Red	Yellow	+	-	*S.typhi*
12A4St1	Water	Colourless with black center	-	+	+	-	-	Red	Yellow	+	-	*S.typhi*

-/ Negative +/ positive

Table B.3: Morphological and biochemical characteristics of *S.typhi* isolates from fish farms

ISOLATE	SOURCE	GROWTH CHARACTERISTICS ON AGAR	BIOCHEMICAL TESTS									INFERENCE
		SALMONELLA-SHIGELLA AGAR	ISOLATE	CITRATE	MR	VP	OXIDASE	TRIPLE SUGAR IRON				
								SLANT	BUTT	H2S	GAS	
13A1St1	Water	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
13A1St2	Water	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
13A2St1	Water	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
13A2St2	Water	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
3BSt1	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
4BSt1	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
4BSt2	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
5Bst2	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
6BSt1	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
7BSt2	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
9BSt1	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
11BSt1	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
11BSt2	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
12BSt1	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
12BSt2	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
13B1St1	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
13B1St2	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
13B2St1	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
13B2St2	Tilapia	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
2C4St1	Catfish	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*

Table B.4: Morphological and biochemical characteristics of *Shigella spp* isolates from fish farms

-/ Negative +/ positive

Table B.3: Morphological and biochemical characteristics of *S.typhi* isolates from fish farms

ISOLATE	SOURCE	GROWTH CHARACTERISTICS ON AGAR	BIOCHEMICAL TESTS									INFERENCE
		SALMONELLA-SHIGELLA AGAR	INDOLE	CITRATE	MR	VP	OXIDASE	TRIPLE SUGAR IRON				
								SLANT	BUTT	H2S	GAS	
2C4St2	Catfish	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
4CSt1	Catfish	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
6CSt1	Catfish	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
6CSt2	Catfish	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
8CSt1	Catfish	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
8CSt2	Catfish	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
9CSt1	Catfish	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
10CSt1	Catfish	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*
10CSt2	Catfish	Colourless with black center	–	+	+	–	–	Red	Yellow	+	–	*S.typhi*

-/ Negative +/ positive

ISOLATE	SOURCE	COLONY CHARACTERISTICS	BIOCHEMICAL TESTS									INFERENCE
		SSA	INDOLE	CITRATE	MR	VP	OXIDASE	TSI				
								SLANT	BUTT	H2S	GAS	

Table B.4: Morphological and biochemical characteristics of *Shigella spp* isolates from fish farms

1ASs1	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
2A1Ss1	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
2A1Ss2	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
2A2Ss2	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
2A3Ss1	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
2A3Ss2	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
3ASs1	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
3ASs2	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
7ASs1	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
7ASs2	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
8ASs1	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
8ASs2	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
11ASs1	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
11ASs2	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
12A1Ss1	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
12A1Ss2	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
12A2Ss1	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
12A2Ss2	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
12A3Ss1	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*
12A3Ss2	Water	Colourless	+	-	+	-	-	Red	Yellow	-	-	*Shigella spp*

-/ Negative +/ positive

Table B.4: Morphological and biochemical characteristics of *Shigella spp* isolates from fish farms

ISOLATE	SOURCE	GROWTH CHARACTERISTICS ON AGAR	BIOCHEMICAL TESTS									INFERENCE
		SALMONELLA-SHIGELLA AGAR	INDOLE	CITRATE	MR	VP	OXIDASE	TRIPLE SUGAR IRON				
								SLANT	BUTT	H2S	GAS	
12A4Ss1	Water	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
12A4Ss2	Water	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
13A1Ss1	Water	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
13A1Ss2	Water	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
13A2Ss1	Water	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
13A2Ss2	Water	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
1BSs1	Tilapia	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
1BSs2	Tilapia	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
2B2Ss1	Tilapia	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
6BSs2	Tilapia	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
11BSs1	Tilapia	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
11BSs2	Tilapia	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
12BSs1	Tilapia	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
12BSs2	Tilapia	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
13B1Ss1	Tilapia	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
13B1Ss2	Tilapia	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
13B2Ss1	Tilapia	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
13B2Ss2	Tilapia	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
2C4Ss2	Catfish	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*

Table B.4: Morphological and biochemical characteristics of *Shigella spp* isolates from fish farms

6CSs1	Catfish	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
6CSs2	Catfish	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*

-/ Negative +/ positive

ISOLATE	SOURCE	GROWTH CHARACTERISTICS ON AGAR	BOCHEMICAL TESTS									INFERENCE
		SALMONELLA SHIGELLA AGAR	INDOLE	CITRATE	MR	VP	OXIDASE	TRIPLE SUGAR IRON				
								SLANT	BUTT	H2S	GAS	
8CSs1	Catfish	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
9CSs2	Catfish	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
10CSs1	Catfish	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
10CSs2	Catfish	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
12CSs1	Catfish	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*
12CSs2	Catfish	Colourless	+	–	+	–	–	Red	Yellow	–	–	*Shigella spp*

-/ Negative +/ positive

ISOLATE	SOURCE	COLONY CHARACTERISTICS ON AGAR	BIOCHEMICAL TEST			INFERENCE
		CETRIMIDE AGAR	CATALASE	OXIDASE	CITRATE	
1APa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
1APa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
2A1Pa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
2A1Pa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*

Table B.5: Morphological and biochemical characteristics of *P. aeruginosa* isolates from fish farms

2A2Pa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
2A2Pa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
2A3Pa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
2A3Pa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
2A4Pa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
2A4Pa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
6APa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
6APa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
7APa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
7APa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
8APa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
8APa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
10APa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
10APa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
11APa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
11APa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
12A2Pa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
12A2Pa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
12A4Pa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*

-/ Negative +/ positive

Table B.5: Morphological and biochemical characteristics of *P. aeruginosa* isolates from fish farms

ISOLATE	SOURCE	GROWTH CHARACTERISTISTICS ON AGAR	BIOCHEMICAL TESTS			INFERENCE
		PSEUDOMONAS CETRIMIDE AGAR	CATALSE	OXIDASE	CITRATE	
12A4Pa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
12A1Pa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
12A1Pa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
12A3Pa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
12A3Pa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
13A1Pa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
13A1Pa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
13A2Pa1	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
13A2Pa2	Water	Yellow-green colonies	+	+	+	*P. aeruginosa*
1BPa1	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
1BPa2	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
2B2Pa1	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
2B2Pa2	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
2B3Pa1	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
2B3Pa2	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
3BPa1	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
3BPa2	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
4BPa1	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
4BPa2	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
6BPa2	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
6BPa2	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
9BPa1	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*

Table B.5: Morphological and biochemical characteristics of *P. aeruginosa* isolates from fish farms

9BPa2	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*

-/ Negative +/ positive

ISOLATE	SOURCE	GROWTH CHARACTERISTICS ON AGAR	BIOCHEMICAL TESTS			
		PSEUDOMONAS CETRIMIDE AGAR	CATALASE	OXIDASE	CITRATE	INFERENCE
10BPa1	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
10BPa2	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
12BPa1	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
12BPa2	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
13B1Pa1	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
13B1Pa2	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
13B2Pa1	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
13B2Pa2	Tilapia	Yellow-green colonies	+	+	+	*P. aeruginosa*
2C3Pa1	Catfish	Yellow-green colonies	+	+	+	*P. aeruginosa*
2C3Pa2	Catfish	Yellow-green colonies	+	+	+	*P. aeruginosa*
2C4Pa1	Catfish	Yellow-green colonies	+	+	+	*P. aeruginosa*
2C4Pa2	Catfish	Yellow-green colonies	+	+	+	*P. aeruginosa*
10CPa1	Catfish	Yellow-green colonies	+	+	+	*P. aeruginosa*
10CPa2	Catfish	Yellow-green colonies	+	+	+	*P. aeruginosa*
12CPa1	Catfish	Yellow-green colonies	+	+	+	*P. aeruginosa*
12CPa2	Catfish	Yellow-green colonies	+	+	+	*P. aeruginosa*

-/ Negative +/ positive APPENDIX IV (ANTIMICROBIAL SUSCEPTIBILITY TESTING)

Table C.1: CLSI 2014 zone diameter (mm) interpretative criteria for *S. aureus*

CLSI Zone diameter(mm) interpretative criteria			
	S	**I**	**R**
Penicillin	≥29		≤28
Erythromycin	≥23	14-22	≤13
Tetracycline	≥19	15-18	≤14
Ciprofloxacin	≥21	16-20	≤15
Cotrimoxazole	≥16	11 to 15	≤10
Gentamicin	≥15	13-14	≤12
Cefuroxime	≥18	15-17	≤14

S- Sensitive I-Intermediate R- Resistant

Table C.2: Antimicrobial sensitivity profiles of *S. aureus* isolates from fish farms

ISOLATE	**PEN**			**AMP**			**FLX**			**ERY**			**TET**		
	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**
1ASa1	0	0	**R**	8	0	**R**	0	0	**R**	0	0	**R**	0	0	**R**
1ASa2	0	0	**R**	16	1.63	**R**	0	0	**R**	0	0	**R**	11.3	0.94	**R**
2A1Sa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
2A1Sa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	20.7	0.9	**I**	0.0	0.0	**R**
2A2Sa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	20.0	0.0	**I**	11.3	0.9	**R**
2A2Sa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
2A3Sa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
2A3Sa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	9.3	0.9	**R**
3ASa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
3ASa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
5ASa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	30.7	0.9	**S**	18.0	1.6	**I**
5ASa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	20.7	0.9	**S**	11.3	0.9	**R**
6ASa1	0.0	0.0	**R**	14.7	**0.9**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
6ASa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	19.3	0.9	**I**	0.0	0.0	**R**
7ASa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
8ASa1	0.0	0.0	**R**	12.0	**1.6**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	8.7	0.9	**R**
8ASa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	8.0	0.0	**R**	10.0	0.0	**R**
10ASa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	10.7	0.9	**R**
10ASa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
11ASa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**

Table C.2: Antimicrobial sensitivity profiles of *S. aureus* isolates from fish farms

11ASa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12A1Sa1	12.7	0.9	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**

SD- Standard deviation IN- Interpretation S- Sensitive I- Intermediate R- Resistant PEN- Penicillin AMP-Ampicillin FLX- Flucloxacillin

ERY- Erythromycin TET- Tetracycline

ISOLATE	**PEN**			**AMP**			**FLX**			**ERY**			**TET**		
	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**
12A1Sa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12A2Sa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12A2Sa2	0.0	0.0	**R**	0.0	**0.0**	**R**	22.7	1.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12A3Sa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12A3Sa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	28.7	0.9	**S**	14.0	1.6	**R**
12A4Sa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12A4Sa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
13A1Sa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	10.0	0.0	**R**
13A1Sa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	10.0	1.6	**R**
13A2Sa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
13A2Sa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
1BSa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
1BSa2	0.0	0.0	**R**	9.3	**0.9**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
2B2Sa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
2B2Sa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**

Table C.2: Antimicrobial sensitivity profiles of *S. aureus* isolates from fish farms

2B4Sa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
2B4Sa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
3BSa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
4BSa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
5BSa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
5BSa2	0.0	0.0	**R**	11.3	**0.9**	**R**	0.0	0.0	**R**	27.3	0.9	**S**	22.0	1.6	**S**
6BSa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	10.0	0.0	**R**	12.7	0.9	**R**

SD- Standard deviation IN- Interpretation S- Sensitive I- Intermediate R- Resistant PEN- Penicillin AMP Ampicillin FLX- Flucloxacillin

ERY- Erythromycin TET- Tetracycline

ISOLATE	**PEN**			**AMP**			**FLX**			**ERY**			**TET**		
	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**
6BSa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
7BSa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
8BSa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
8BSa2	0.0	0.0	**R**	20.7	**0.9**	**R**	9.3	0.9	**R**	29.3	0.9	**S**	21.3	0.9	**S**
10BSa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	24.7	0.9	**S**	15.3	0.9	**I**
10BSa2	0.0	0.0	**R**	16.0	**1.6**	**R**	0.0	0.0	**R**	29.3	0.9	**S**	21.3	0.9	**S**
11BSa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	10.0	0.0	**R**
11BSa2	14.7	0.9	**R**	6.7	**0.9**	**R**	11.3	0.9	**R**	14.7	1.9	**I**	20.0	1.6	**S**
12BSa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12BSa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
13B1Sa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**

Table C.2: Antimicrobial sensitivity profiles of *S. aureus* isolates from fish farms

13B1Sa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
13B2Sa1	0.0	0.0	**R**	11.3	**0.9**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	8.0	0.0	**R**
13B2Sa2	0.0	0.0	**R**	12.7	**0.9**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
2C1Sa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
2C1Sa2	0.0	0.0	**R**	15.3	**1.9**	**R**	0.0	0.0	**R**	9.3	1.9	**R**	12.0	1.6	**R**
2C4Sa1	0.0	0.0	**R**	12.7	**0.9**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
4CSa1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
4CSa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
6CSa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
6CSa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
6CSa3	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
8CSa1	22.0	5.9	**R**	21.3	**0.0**	**R**	19.3	2.5	**R**	18.7	2.5	**I**	28.7	0.9	**S**

SD- Standard deviation IN- Interpretation S- Sensitive I- Intermediate R- Resistant PEN- Penicillin AMP-Ampicillin FLX- Flucloxacillin

ERY- Erythromycin TET- Tetracycline

Table C.2: Antimicrobial sensitivity profiles of *S.aureus* isolates from fish farms

ISOLATE	**PEN**			**AMP**			**FLX**			**ERY**			**TET**		
	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**
8CSa2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
9CSa1	0.0	0.0	**R**	25.3	**0.9**	**R**	18.7	0.9	**R**	24.7	0.9	**S**	22.0	0.0	**S**
9CSa2	0.0	0.0	**R**	10.0	**0.0**	**R**	9.3	0.9	**R**	0.0	0.0	**R**	15.3	0.9	**I**
10CSa1	0.0	0.0	**R**	14.0	**1.6**	**R**	0.0	0.0	**R**	36.0	1.6	**S**	20.0	1.6	**S**
10CSa2	0.0	0.0	**R**	10.0	**0.0**	**R**	0.0	0.0	**R**	30.0	0.0	**S**	12.7	0.9	**R**

SD- Standard deviation IN- Interpretation S- Sensitive I- Intermediate R- Resistant PEN- Penicillin AMP-Ampicillin FLX- Flucloxacillin ERY-Erythromycin TET- Tetracycline

Table C.2: Antimicrobial sensitivity profiles of *S. aureus* isolates from fish farms

ISOLATE	**COT**			**CRX**			**GEN**			**CPR**		
	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN(mm)	SD	**IN**
1ASa1	20.7	0.9	**S**	16	1.6	I	18.7	0.9	**S**	24.7	0.9	**S**
1ASa2	0	0	**R**	22	1.6	S	22.7	2.5	**S**	26.7	2.5	**S**
2A1Sa1	0.0	0.0	**R**	0.0	0.0	R	25.3	1.9	**S**	17.3	1.9	**I**
2A1Sa2	0.0	0.0	**R**	0.0	0.0	R	24.0	1.6	**S**	0.0	0.0	**R**
2A2Sa1	0.0	0.0	**R**	0.0	0.0	R	22.0	0.0	**S**	24.0	1.6	**S**
2A2Sa2	22.7	1.9	**S**	0.0	0.0	R	17.3	0.9	**S**	30.0	0.0	**S**

Table C.2: Antimicrobial sensitivity profiles of *S.aureus* isolates from fish farms

2A3Sa1	0.0	0.0	**R**	0.0	0.0	R	25.3	0.9	**S**	23.3	0.9	**S**

COT-Trimethoprim/sulphamethoxazole, CRX-Cefuroxime GEN-Gentamicin CPR- Ciprofloxacin SD-Standard deviation IN-Interpretation

S-Sensitive I-Intermediate R-Resistant

	COT			CRX			GEN			CPR		
ISOLATE	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**
2A3Sa2	18.7	3.4	**S**	9.3	0.9	**R**	24.7	0.9	**S**	28.7	0.9	**S**
3ASa1	23.3	2.5	**S**	0.0	0.0	**R**	28.0	1.6	**S**	0.0	0.0	**R**
3ASa2	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
5ASa1	0.0	0.0	**R**	0.0	0.0	**R**	30.0	0.0	**S**	38.7	0.9	**S**
5ASa2	0.0	0.0	**R**	0.0	0.0	**R**	24.7	1.9	**S**	26.0	1.6	**S**
6ASa1	0.0	0.0	**R**	0.0	0.0	**R**	27.3	0.9	**S**	26.7	0.9	**S**
6ASa2	0.0	0.0	**R**	0.0	0.0	**R**	24.7	0.9	**S**	0.0	0.0	**R**
7ASa1	0.0	0.0	**R**	0.0	0.0	**R**	16.0	0.0	**S**	11.3	0.0	**R**
8ASa1	14.7	0.9	**I**	19.3	0.9	**S**	26.0	1.6	**S**	24.7	0.9	**S**
8ASa2	0.0	0.0	**R**	0.0	0.0	**R**	22.7	2.5	**S**	18.0	0.0	**I**
10ASa1	30.7	0.9	**S**	20.7	0.9	**S**	24.0	0.0	**S**	22.7	1.6	**S**
10ASa2	0.0	0.0	**R**	18.0	0.0	**S**	27.3	0.9	**S**	20.0	0.0	**I**
11ASa1	0.0	0.0	**R**	0.0	0.0	**R**	21.3	0.9	**S**	14.0	0.0	**R**
11ASa2	24.7	0.9	**S**	0.0	0.0	**R**	21.3	0.9	**S**	34.7	0.9	**S**
12A1Sa1	0.0	0.0	**R**	0.0	0.0	**R**	17.3	0.9	**S**	26.0	0.9	**S**
12A1Sa2	0.0	0.0	**R**	0.0	0.0	**R**	26.0	1.6	**S**	12.7	0.0	**R**
12A2Sa1	13.3	0.9	**I**	0.0	0.0	**R**	30.0	0.0	**S**	27.3	1.9	**S**

Table C.2: Antimicrobial sensitivity profiles of *S.aureus* isolates from fish farms

12A2Sa2	0.0	0.0	**R**	0.0	0.0	**R**	27.3	1.9	**S**	31.3	2.8	**S**
12A3Sa1	0.0	0.0	**R**	0.0	0.0	**R**	28.7	2.5	**S**	20.0	1.9	**I**
12A3Sa2	0.0	0.0	**R**	0.0	0.0	**R**	30.0	0.0	**S**	22.0	1.9	**S**
12A4Sa1	0.0	0.0	**R**	0.0	0.0	**R**	24.0	0.0	**S**	0.0	0.9	**R**
12A4Sa2	0.0	0.0	**R**	0.0	0.0	**R**	20.0	0.0	**S**	20.7	0.0	**I**
13A1Sa1	0.0	0.0	**R**	20.7	0.9	**S**	25.3	0.9	**S**	0.0	0.0	**R**

COT-Trimetoprim/sulphamethoxazole, CRX-Cefuroxime GEN-Gentamicin CPR- Ciprofloxacin SD-Standard deviation IN-Interpretation S-Sensitive I-Intermediate R-Resistant

ISOLATE	COT MEAN(mm)	SD	**IN**	CRX MEAN(mm)	SD	**IN**	GEN MEAN(mm)	SD	**IN**	CPR MEAN(mm)	SD	**IN**
13A1Sa2	19.3	0.9	**S**	15.3	0.9	**I**	26.0	0.0	**S**	26.7	0.9	**S**
13A2Sa1	27.3	1.9	**S**	0.0	0.0	**R**	17.3	0.9	**S**	21.3	0.0	**S**
13A2Sa2	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**	20.0	0.0	**I**
1BSa1	0.0	0.0	**R**	12.7	0.9	**R**	17.3	0.9	**S**	24.7	0.9	**S**
1BSa2	18.7	0.9	**S**	10.7	0.9	**R**	12.7	0.9	**R**	16.0	2.8	**I**
2B2Sa1	0.0	0.0	**R**	0.0	0.0	**R**	21.3	2.5	**S**	0.0	0.0	**R**
2B2Sa2	17.3	0.9	**S**	8.0	1.6	**R**	22.7	1.9	**S**	0.0	0.0	**R**
2B4Sa1	0.0	0.0	**R**	12.0	0.0	**R**	24.7	0.9	**S**	26.0	0.0	**S**
2B4Sa2	0.0	0.0	**R**	16.0	0.0	**I**	29.3	0.9	**S**	28.0	1.9	**S**
3BSa1	0.0	0.0	**R**	0.0	0.0	**R**	25.3	1.9	**S**	12.7	0.9	**R**
4BSa2	0.0	0.0	**R**	0.0	0.0	**R**	31.3	0.9	**S**	22.7	2.5	**S**
5BSa1	0.0	0.0	**R**	0.0	0.0	**R**	25.3	3.4	**S**	0.0	0.0	**R**
5BSa2	24.7	0.9	**S**	23.3	0.9	**S**	25.3	0.9	**S**	30.7	4.1	**S**

Table C.2: Antimicrobial sensitivity profiles of *S.aureus* isolates from fish farms

6BSa1	12.7	0.9	**I**	0.0	0.0	**R**	24.7	0.9	**S**	9.3	0.9	**R**
6BSa2	0.0	0.0	**R**	14.7	0.9	**R**	25.3	0.9	**S**	6.7	0.9	**R**
7BSa1	0.0	0.0	**R**	17.3	0.9	**I**	24.7	0.9	**S**	30.7	0.9	**S**
8BSa1	0.0	0.0	**R**	28.0	1.6	**S**	22.0	1.6	**S**	29.3	1.6	**S**
8BSa2	35.3	2.5	**S**	11.3	3.4	**R**	27.3	0.9	**S**	34.7	1.6	**S**
10BSa1	15.3	0.9	**I**	13.3	0.9	**R**	26.7	0.9	**S**	11.3	0.0	**R**
10BSa2	29.3	0.9	**S**	8.7	0.9	**R**	23.3	3.4	**S**	0.0	1.6	**R**
11BSa1	0.0	0.0	**R**	0.0	0.0	**R**	28.7	0.9	**S**	0.0	0.0	**R**
11BSa2	10.0	1.6	**R**	12.7	0.9	**R**	32.7	0.9	**S**	28.7	1.6	**S**
12BSa1	0.0	0.0	**R**	0.0	0.0	**R**	14.0	0.0	**I**	22.7	0.0	**S**

COT-Trimethoprim/sulphamethoxazole, CRX-Cefuroxime GEN-Gentamicin CPR- Ciprofloxacin SD-Standard deviation IN-Interpretation

S-Sensitive I-Intermediate R-Resistant

	COT			CRX			GEN			CPR		
ISOLATE	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**
12BSa2	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**	16.0	0.0	**I**
13B1Sa1	15.3	0.9	**I**	0.0	0.0	**R**	21.3	0.9	**S**	29.3	0.9	**S**
13B1Sa2	0.0	0.0	**R**	0.0	0.0	**R**	20.0	0.0	**S**	29.3	0.9	**S**
13B2Sa1	20.0	0.0	**S**	18.7	0.9	**S**	26.7	0.9	**S**	30.0	0.0	**S**
13B2Sa2	24.0	1.6	**S**	24.0	0.0	**S**	21.3	0.9	**S**	30.7	0.9	**S**
2C1Sa1	0.0	0.0	**R**	0.0	0.0	**R**	29.3	3.8	**S**	27.3	0.9	**S**
2C1Sa2	21.3	0.9	**S**	0.0	0.0	**R**	32.0	1.6	**S**	28.0	1.9	**S**
2C4Sa1	0.0	0.0	**R**	14.0	0.0	**R**	24.0	0.0	**S**	26.0	0.0	**S**
4CSa1	0.0	0.0	**R**	12.0	0.0	**R**	30.0	0.0	**S**	32.0	0.0	**S**
4CSa2	0.0	0.0	**R**	0.0	0.0	**R**	25.3	6.6	**S**	0.0	0.0	**R**

Table C.2: Antimicrobial sensitivity profiles of *S.aureus* isolates from fish farms

6CSa2	25.3	0.9	**S**	20.0	0.0	**R**	25.3	0.9	**S**	20.0	0.9	**I**
6CSa2	0.0	0.0	**R**	16.0	0.0	**I**	24.7	0.9	**S**	16.7	0.0	**I**
6CSa3	0.0	0.0	**R**	15.3	0.9	**I**	28.0	1.6	**S**	0.0	0.9	**R**
8CSa1	18.0	4.3	**S**	19.3	3.8	**S**	40.0	1.6	**S**	30.0	1.9	**S**
8CSa2	0.0	0.0	**R**	0.0	0.0	**R**	21.3	0.9	**S**	29.3	0.9	**S**
9CSa1	34.0	1.6	**S**	27.3	2.5	**S**	25.3	0.9	**S**	28.7	0.9	**S**
9CSa2	19.3	0.9	**S**	24.7	0.9	**S**	24.0	0.0	**S**	26.0	0.9	**S**
10CSa1	31.3	2.5	**S**	9.3	0.9	**R**	27.3	0.9	**S**	26.0	4.1	**S**
10CSa2	29.3	0.9	**S**	0.0	0.0	**R**	29.3	0.9	**S**	0.0	0.9	**R**

COT-Trimethoprim/sulphamethoxazole, CRX-Cefuroxime GEN-Gentamicin CPR- Ciprofloxacin SD-Standard deviation IN-Inference

SSensitive I-Intermediate R-Resistant

Table

D.1: CLSI 2014 zone diameter interpretative criteria, *E.coli*

CLSI ZONE DIAMETER(MM) INTERPRETATIVE CRITERIA FOR E.COLI			
	S	**I**	**R**
AMPICILLIN	≥17	14-16	≤13
CEFUROXIME	≥23	15-22	≤14
GENTAMICIN	≥15	13-14	≤12
TETRACYCLINE	≥15	12- 14	≤11
CIPROFLOXACIN	≥21	16-20	≤15
COTRIMOXAZOLE	≥16	11-15	≤10
CHLORAMPHENICOL	≥18	13-17	≤12

S= SUSCEPTIBLE, I=INTERMEDIATE, R=RESISTANT

Table D.2: Antimicrobial susceptibility profiles of *E.coli* isolates from fish farms

	TET			**COT**			**CRX**			**GEN**			**CPR**			**CHL**			**AMP**		
ISOLATE	MEAN (mm)	SD	**IN**	MEAN (mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**
1AEc1	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	20.0	0.0	**S**	14.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
2A1Ec1	14.7	**0.9**	**I**	20.0	0.0	**S**	0.0	0.0	**R**	14.7	0.9	**I**	34.7	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
2A1Ec2	0.0	**0.0**	**R**	23.3	0.9	**S**	14.0	0.0	**R**	38.7	0.9	**S**	36.7	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
2A2Ec1	0.0	**0.0**	**R**	22.7	0.9	**S**	12.0	0.0	**R**	23.3	0.9	**S**	25.3	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
2A2Ec2	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	22.0	0.0	**S**	28.7	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
2A3Ec1	0.0	**0.0**	**R**	21.3	0.9	**S**	0.0	0.0	**R**	27.3	0.9	**S**	40.0	0.0	**S**	0.0	0.0	**R**	0.0	0.0	**R**
2A3Ec2	0.0	**0.0**	**R**	24.0	0.0	**S**	12.0	0.0	**R**	22.0	0.0	**S**	28.7	1.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
3AEc1	12.0	**0.0**	**I**	10.7	0.9	**R**	0.0	0.0	**R**	23.3	0.9	**S**	30.7	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**

SD-Standard deviation IN- Interpretation S-Sensitive I-Intermediate R- Resistant TET-Tetracycline COT- Trimethoprim/Sulphamethoxazole CHL- Chloramphenicol AMP-Ampicillin

Table D.2: Antimicrobial sensitivity profiles of *E.coli* isolates from fish farms

	TET			COT			CRX			GEN			CPR			CHL			AMP		
ISOLATE	MEAN (mm)	**SD**	**IN**	MEAN (mm)	SD	**IN**	MEAN (mm)	SD	**IN**	MEAN (mm)	SD	**IN**	MEAN (mm)	SD	**IN**	MEAN (mm)	SD	**IN**	MEAN (mm)	**SD**	**IN**
6AEc1	0.0	**0.0**	**R**	0.0	0.0	**S**	0.0	0.0	**R**	25.3	0.9	**S**	30.7	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
7AEc1	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**	17.3	0.9	**I**	0.0	0.0	**R**	0.0	0.0	**R**
7AEc2	12.7	**0.0**	**I**	0.0	0.0	**R**	0.0	0.0	**R**	25.3	0.9	**S**	26.0	0.0	**S**	0.0	0.0	**R**	0.0	0.0	**R**
10AEc1	0.0	**0.0**	**R**	0.0	0.0	**R**	12.0	0.0	**R**	23.3	0.9	**S**	20.0	0.0	**I**	0.0	0.0	**R**	0.0	0.0	**R**
10AEc2	11.3	**0.9**	**R**	24.0	0.0	**S**	17.3	1.9	**I**	24.0	0.0	**S**	24.0	0.0	**S**	12.0	0.0	**I**	0.0	0.0	**R**
11AEc1	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	24.7	0.9	**S**	31.3	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
11AEc2	16.0	**0.0**	**S**	0.0	0.0	**R**	12.0	0.0	**R**	24.0	0.0	**S**	33.3	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
12A1Ec1	0.0	**0.0**	**R**	0.0	0.0	**R**	10.0	0.0	**R**	18.7	0.9	**S**	22.0	0.0	**S**	0.0	0.0	**R**	0.0	0.0	**R**
12A1Ec2	15.3	**0.9**	**S**	0.0	0.0	**R**	0.0	0.0	**R**	22.7	0.9	**S**	11.3	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12A2Ec1	0.0	**0.0**	**R**	13.3	0.9	**I**	10.7	0.9	**R**	20.0	0.0	**S**	20.7	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12A2Ec2	0.0	**0.0**	**R**	0.0	0.0	**R**	11.3	0.9	**R**	18.0	0.0	**S**	25.3	0.9	**S**	9.3	0.9	**R**	0.0	0.0	**R**
12A3Ec1	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	30.0	3.3	**S**	12.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12A3Ec2	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	12.7	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12A4Ec1	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	24.7	0.9	**S**	12.7	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12A4Ec2	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	24.7	0.9	**S**	20.0	0.0	**I**	0.0	0.0	**R**	0.0	0.0	**R**

Table D.2: *E.coli* isolates from fish farms

13A1Ec1	0.0	**0.0**	**R**	20.7	0.9	**S**	0.0	0.0	**R**	27.3	0.9	**S**	32.7	0.9	**S**	12.7	0.9	**I**	0.0	0.0	**R**
13A1Ec2	8.0	**0.0**	**R**	18.0	0.0	**S**	0.0	0.0	**R**	22.0	0.0	**S**	22.7	0.9	**S**	12.0	0.0	**I**	0.0	0.0	**R**
13A2Ec1	0.0	**0.0**	**R**	20.0	0.0	**S**	0.0	0.0	**R**	22.0	0.0	**S**	31.3	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
13A2Ec2	0.0	**0.0**	**R**	16.0	0.0	**S**	0.0	0.0	**R**	26.7	0.9	**S**	26.0	1.6	**S**	14.0	0.0	**I**	0.0	0.0	**R**
1BEc2	0.0	**0.0**	**R**	20.0	0.0	**S**	11.3	0.9	**R**	30.7	1.9	**S**	36.7	2.5	**S**	0.0	0.0	**R**	0.0	0.0	**R**
2B2Ec1	13.3	**0.9**	**I**	23.3	0.9	**S**	12.7	0.9	**R**	30.7	1.9	**S**	42.0	1.6	**S**	17.3	1.9	**I**	0.0	0.0	**R**

SD-Standard deviation IN- Interpretation S-Sensitive I-Intermediate R- Resistant TET-Tetracycline COT- Trimetoprim/Sulphamethoxazole CHL- Chloramphenicol AMP-Ampicillin

Antimicrobial sensitivity profiles of

	TET			COT			CRX			GEN			CPR			CHL			AMP		
ISOLATE	MEAN(mm)	**SD**	**IN**	MEAN (mm)	SD	**IN**	MEAN (mm)	SD	**IN**	MEAN (mm)	SD	**IN**	MEAN (mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	**SD**	**IN**
2B2Ec2	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	29.3	0.9	**S**	22.0	0.0	**S**	0.0	0.0	**R**	0.0	0.0	**R**
2B3Ec1	0.0	**0.0**	**R**	0.0	0.0	**R**	12.7	0.9	**R**	22.0	0.0	**S**	26.7	0.9	**S**	10.0	0.0	**R**	0.0	0.0	**R**
2B3Ec2	10.7	**0.9**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	24.0	0.0	**S**	21.3	1.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
3BEc1	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	40.0	0.0	**S**	30.7	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
3BEc2	13.3	**0.9**	**I**	0.0	0.0	**R**	0.0	0.0	**R**	32.7	0.9	**S**	32.7	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
4BEc1	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	24.0	0.0	**S**	20.7	1.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
4BEc2	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	26.0	1.6	**S**	14.7	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**
5BEc2	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	28.0	0.0	**S**	28.7	0.0	**S**	0.0	0.0	**R**	0.0	0.0	**R**
6BEc2	0.0	**0.0**	**R**	18.0	0.0	**R**	0.0	0.0	**R**	20.0	0.0	**S**	19.3	0.9	**I**	0.0	0.0	**R**	0.0	0.0	**R**
9BEc1	0.0	**0.0**	**R**	28.0	1.6	**S**	14.0	0.0	**R**	24.7	0.9	**S**	34.0	0.0	**S**	0.0	0.0	**R**	0.0	0.0	**R**

Table D.2: Antimicrobial sensitivity profiles of *E.coli* isolates from fish farms

11BEc1	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	28.7	0.9	**S**	10.7	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**
11BEc2	22.0	**0.0**	**S**	32.0	0.0	**S**	0.0	0.0	**R**	27.3	0.9	**S**	32.0	0.0	**S**	0.0	0.0	**R**	0.0	0.0	**R**
12BEc1	13.3	**0.9**	**I**	0.0	0.0	**R**	0.0	0.0	**R**	24.0	0.0	**S**	20.7	0.9	**I**	0.0	0.0	**R**	0.0	0.0	**R**
12BEc2	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	25.3	0.9	**S**	23.3	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
13B1Ec1	0.0	**0.0**	**R**	16.0	0.0	**S**	0.0	0.0	**R**	18.0	0.0	**S**	28.0	0.0	**S**	14.0	1.6	**I**	0.0	0.0	**R**
13B1Ec2	0.0	**0.0**	**R**	16.0	0.0	**S**	0.0	0.0	**R**	20.7	0.9	**S**	33.3	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
13B2Ec1	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	22.7	9.9	**S**	26.0	0.0	**S**	0.0	0.0	**R**	0.0	0.0	**R**
13B2Ec2	0.0	**0.0**	**R**	12.0	0.0	**I**	0.0	0.0	**R**	20.0	0.0	**S**	25.3	0.9	**S**	12.0	0.0	**I**	0.0	0.0	**R**
2C1Ec1	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	30.0	1.6	**S**	20.7	0.9	**I**	0.0	0.0	**R**	0.0	0.0	**R**
2C1Ec2	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	24.7	0.9	**S**	13.3	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**
2C4Ec1	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	25.3	0.9	**S**	31.3	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**

SD-Standard deviation IN- Interpretation S-Sensitive I-Intermediate R- Resistant TET-Tetracycline COT- Trimetoprim/Sulphamethoxazole CHL- Chloramphenicol AMPAmpicillin

	TET			COT			CRX			GEN			CPR			CHL			AMP		
ISOLATE	MEAN (mm)	**SD**	**IN**	MEAN(mm)	SD	**IN**	MEA N(m m)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	**SD**	**IN**
2C4Ec2	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	19.3	0.9	**S**	21.3	1.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
4CEc1	0.0	**0.0**	**R**	24.7	0.9	**S**	12.7	0.9	**R**	22.0	0.0	**S**	12.7	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**
6CEc1	0.0	**0.0**	**R**	22.0	0.0	**S**	0.0	0.0	**R**	27.3	0.9	**S**	30.7	0.9	**S**	10.0	0.0	**R**	0.0	0.0	**R**
10CEc1	10.0	**0.0**	**R**	27.3	1.9	**S**	18.0	0.0	**I**	26.0	1.6	**S**	37.3	1.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
10CEc2	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	27.3	0.9	**S**	22.7	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
12CEc1	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	25.3	0.9	**S**	14.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12CEc2	0.0	**0.0**	**R**	0.0	0.0	**R**	0.0	0.0	**R**	29.3	0.9	**S**	23.3	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**

Table D.2: *E.coli* isolates from fish farms

SD-Standard deviation IN- Interpretation S-Sensitive I-Intermediate R- Resistant TET-Tetracycline COT- Trimetoprim/Sulphamethoxazole CHL- Chloramphenicol AMP-Ampicillin

Table

E.1: CLSI zone diameter interpretative criteria, 2014 *P. aeruginosa*

CLSI ZONE DIAMETER (MM) IN PSEUDOMONAS AERUGINOSA	INTERPRETATIVE CRITERIA FOR		
	S	**I**	**R**
GENTAMICIN	≥15	13-14	≤12
CIPROFLOXACIN	≥21	16-20	≤15

S= SUSCEPTIBLE, I=INTERMEDIATE, R=RESISTANT

Table E.2: Antimicrobial susceptibility profiles of *P. aeruginosa* isolates from fish farms

	GEN			**CPR**		
ISOLATE	**MEAN(mm)**	**SD**	**IN**	**MEAN(mm)**	**SD**	**IN**
1APa1	18.0	**1.6**	**S**	18.0	**1.6**	**I**
1APa2	16.0	**2.8**	**S**	12.0	**1.6**	**R**
2A1Pa1	14.0	**1.6**	**I**	19.3	**0.9**	**I**
2A1Pa2	18.7	**0.9**	**S**	18.7	**1.9**	**I**
2A2Pa1	12.7	**1.9**	**R**	0.0	**0.0**	**R**

Table E.2: Antimicrobial

2A2Pa2	13.3	**0.9**	**I**	16.7	**2.5**	**I**

SD-Standard deviation IN-Interpretation S-Sensitive I- Intermediate R-Resistant GEN- Gentamicin CPR- Ciprofloxacin

P. aeruginosa isolates from fish farms

	GEN			CPR		
ISOLATE	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**
2A3Pa1	16.7	**0.9**	**S**	20.7	**0.9**	**S**
2A3Pa2	14.7	**2.5**	**I**	19.3	**0.9**	**I**
2A4Pa1	15.3	**1.9**	**S**	18.7	**2.5**	**I**
2A4Pa2	12.7	**0.9**	**R**	18.7	**0.9**	**I**
6APa1	8.0	**0.0**	**R**	0.0	**0.0**	**R**
6APa2	9.3	**1.9**	**R**	0.0	**0.0**	**R**
7APa1	7.3	**0.9**	**R**	19.3	**0.9**	**I**
7APa2	10.7	**0.9**	**R**	20.0	**1.6**	**I**
8APa1	16.0	**2.8**	**S**	18.7	**1.9**	**I**
8APa2	18.0	**0.0**	**S**	12.7	**1.9**	**R**
10APa1	9.3	**0.9**	**R**	18.0	**1.6**	**I**
10APa2	10.7	**0.9**	**R**	15.3	**3.8**	**R**
11APa1	13.3	**2.5**	**I**	0.0	**0.0**	**R**
11APa2	12.7	**0.9**	**R**	18.0	**1.6**	**I**
12A2Pa1	19.3	**0.9**	**S**	14.7	**0.9**	**R**
12A2Pa2	18.7	**0.9**	**S**	17.3	**0.9**	**I**
12A4Pa1	12.0	**0.0**	**R**	0.0	**0.0**	**R**

Table E.2: Antimicrobial sensitivity profiles of

12A4Pa2	16.0	**1.6**	**S**	17.3	**0.9**	**I**
12A1Pa1	16.7	**0.9**	**S**	0.0	**0.0**	**R**
12A1Pa2	16.0	**2.8**	**S**	20.0	**1.6**	**I**
12A3Pa1	0.0	**0.0**	**R**	0.0	**0.0**	**R**
12A3Pa2	20.7	**0.9**	**S**	0.0	**0.0**	**R**
13A1Pa1	16.7	**0.9**	**S**	24.7	**0.9**	**S**
13A1Pa2	24.7	**0.9**	**S**	24.0	**0.0**	**S**

SD-Standard deviation IN-Interpretation S-Sensitive I- Intermediate R-Resistant GEN- Gentamicin CPR- Ciprofloxacin susceptibility profiles of *P. aeruginosa* isolates from fish farms

	GEN			CPR		
ISOLATE	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**
13A2Pa1	22.0	**0.0**	**S**	34.0	**0.0**	**S**
13A2Pa2	14.0	**0.0**	**I**	30.7	**0.9**	**S**
1BPa1	15.3	**1.9**	**S**	0.0	**0.0**	**R**
1BPa2	17.3	**0.9**	**S**	0.0	**0.0**	**R**
2B2Pa1	15.3	**0.9**	**S**	0.0	**0.0**	**R**
2B2Pa2	12.7	**0.9**	**R**	0.0	**0.0**	**R**
2B3Pa1	10.7	**0.9**	**R**	0.0	**0.0**	**R**
2B3Pa2	10.7	**0.9**	**R**	0.0	**0.0**	**R**
3BPa1	19.3	**0.9**	**S**	0.0	**0.0**	**R**
3BPa2	12.7	**0.9**	**R**	0.0	**0.0**	**R**
4BPa1	17.3	**2.5**	**S**	0.0	**0.0**	**R**
4BPa2	20.0	**1.6**	**S**	18.7	**0.9**	**I**
6BPa2	19.3	**0.9**	**S**	18.7	**0.9**	**I**

Table E.2: Antimicrobial

6BPa2	20.0	**0.0**	**S**	20.7	**0.9**	**I**
9BPa1	9.3	**0.9**	**R**	8.7	**0.9**	**R**
9BPa2	10.7	**0.9**	**R**	0.0	**0.0**	**R**
10BPa1	19.3	**0.9**	**S**	0.0	**0.0**	**R**
10BPa2	15.3	**0.9**	**S**	0.0	**0.0**	**R**
12BPa1	21.3	**0.9**	**S**	16.7	**0.9**	**I**
12BPa2	20.7	**0.9**	**S**	16.7	**0.9**	**I**
13B1Pa1	24.7	**0.9**	**S**	28.7	**0.9**	**S**
13B1Pa2	22.7	**1.9**	**S**	30.0	**0.0**	**S**

SD-Standard deviation IN-Interpretation S-Sensitive I- Intermediate R-Resistant GEN- Gentamicin CPR- Ciprofloxacin

P. aeruginosa isolates from fish farms

	GEN			CPR		
ISOLATE	MEAN(mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**
13B2Pa1	30.0	**0.0**	**S**	32.0	**0.0**	**S**
13B2Pa2	16.0	**0.0**	**S**	30.0	**0.0**	**S**
2C3Pa1	18.7	**1.9**	**S**	0.0	**0.0**	**R**
2C3Pa2	18.0	**1.6**	**S**	0.0	**0.0**	**R**
2C4Pa1	14.7	**1.9**	**I**	19.3	**0.9**	**I**
2C4Pa2	19.3	**1.9**	**S**	0.0	**0.0**	**R**
10CPa1	0.0	**0.0**	**R**	0.0	**0.0**	**R**
10CPa2	18.0	**1.6**	**S**	0.0	**0.0**	**R**
12CPa1	15.3	**0.9**	**S**	0.0	**0.0**	**R**
12CPa2	20.7	**0.9**	**S**	8.0	**0.0**	**R**

SD-Standard deviation IN-Interpretation S-Sensitive I- Intermediate R-Resistant GEN- Gentamicin CPR- Ciprofloxacin

Table E.2: Antimicrobial sensitivity profiles of

Table F.1: CLSI zone diameter interpretative criteria 2014, *Shigella spp*

CLSI ZONE DIAMETER INTERPRETATIVE GUIDELINES, SHIGELLA SPP			
	S	**I**	**R**
AMPICILLIN	≥17	14-16	≤13
CIPROFLOXACIN	≥21	16-20	≤15
COTRIMOXAZOLE	≥16	11- 15	≤10
CEFUROXIME	≥23	15-22	≤14
CHLORAMPHENICOL	≥18	13-17	≤12
GENTAMICIN	≥15	13-14	≤12
TETRACYCLINE	≥15	12 to 14	≤11

S= SUSCEPTIBLE, I=INTERMEDIATE, R=RESISTANT

Table F.2: Antimicrobial sensitivity profiles of *Shigella spp* isolates from fish farms

	TET			COT			CRX			GEN			CPR			CHL			AMP		
ID	**MEAN (mm)**	**SD**	**IN**	**MEAN (mm)**	SD	**IN**	**MEAN (mm)**	**SD**	**IN**	**MEAN (mm)**	**SD**	**IN**	**MEAN (mm)**	**SD**	**IN**	**MEAN(mm)**	**SD**	**IN**	**MEAN(mm)**	**SD**	**IN**
1ASs1	0.0	0.0	R	0.0	0	**R**	0.0	0.0	**R**	20.7	0.9	**S**	27.3	0.9	**S**	0.0	0	**R**	0.0	0.0	**R**
2A1Ss1	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	22.0	0.0	**S**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
2A1Ss2	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	20.7	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
2A2Ss2	**0.0**	**0.0**	**R**	0.0	0	**R**	15.3	0.9	**I**	16.7	0.9	**S**	9.3	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**
2A3Ss1	**10**	**0.0**	**R**	0.0	0	**R**	15.3	0.9	**I**	20.0	0.0	**S**	18.0	0.0	**I**	0.0	0.0	**R**	0.0	0.0	**R**
2A3Ss2	0.0	0.0	R	0.0	0	**R**	0.0	0.0	**R**	23.3	0.9	**S**	24.0	0.0	**S**	0.0	0.0	**R**	0.0	0.0	**R**
3ASs1	0.0	0.0	**R**	0.0	0	**R**	14.7	0.9	**R**	20.0	1.6	**S**	19.3	0.9	**I**	0.0	0	**R**	0.0	0.0	**R**

SD- Standard deviation IN- Interpretation S-Sensitive I-Intermediate R-Resistant TET- Tetracycline COT- Trimethoprim/ Sulphamethoxazole CRX- Cefuroxime GEN- Gentamicin CPR- Ciprofloxacin CHL- Chloramphenicol AMP- Ampicillin

F.2: *Shigella spp* isolates from fish farms

	TET			COT			CRX			GEN			CPR			CHL			AMP		
ISOLATE	MEAN (mm)	SD	**IN**	MEAN (mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN (mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**
3ASs2	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	20.0	0.0	**S**	18.7	0.9	**I**	0.0	0	**R**	0.0	0.0	**R**
7ASs1	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	24.0	1.6	**S**	20.7	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
7ASs2	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	26.7	0.9	**S**	15.3	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**
8ASs1	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	27.3	0.9	**S**	13.3	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**

Table Antimicrobial sensitivity profiles of

8ASs2	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	25.3	0.9	**S**	13.3	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**
11ASs1	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	27.3	0.9	**S**	16.0	1.6	**I**	0.0	0.0	**R**	0.0	0.0	**R**
11ASs2	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	25.3	0.9	**S**	17.3	0.9	**I**	0.0	0.0	**R**	0.0	0.0	**R**
12A1Ss1	0.0	0.0	**R**	0.0	0	**R**	10.0	0.0	**R**	22.7	0.9	**S**	17.3	0.9	**I**	0.0	0.0	**R**	0.0	0.0	**R**
12A1Ss2	0.0	0.0	**R**	0.0	0	**R**	12.0	0.0	**R**	25.3	0.9	**S**	22.7	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
12A2Ss1	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	16.7	0.9	**S**	18.0	0.0	**I**	0.0	0.0	**R**	0.0	0.0	**R**
12A2Ss2	0.0	0.0	**R**	0.0	0	**R**	16.7	0.9	**I**	20.7	0.9	**S**	0.0	0.0	**I**	0.0	0.0	**R**	0.0	0.0	**R**
12A3Ss1	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	21.3	0.9	**S**	13.3	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12A3Ss2	**22**	**0**	**S**	0.0	0	**R**	0.0	0.0	**R**	21.3	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12A4Ss1	0.0	0.0	R	0.0	0	**R**	18.0	0.0	**I**	25.3	0.9	**S**	20.7	0.9	**I**	0.0	0.0	**R**	0.0	0.0	**R**
12A4Ss2	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	27.3	0.9	**S**	20.7	0.9	**I**	0.0	0.0	**R**	0.0	0.0	**R**
13A1Ss1	0.0	0.0	**R**	0.0	0	**R**	18.7	0.9	**I**	29.3	0.9	**S**	22.7	1.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
13A1Ss2	0.0	0.0	**R**	0.0	0	**R**	20.7	0.9	**I**	27.3	0.9	**S**	18.7	0.9	**I**	15.3	0.9	**I**	0.0	0.0	**R**
13A2Ss1	10.0	0.0	**R**	0.0	0	**R**	18.0	0.0	**I**	27.3	1.9	**S**	27.3	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
13A2Ss2	0.0	0.0	**R**	24.7	0.9	**S**	11.3	0.9	**R**	24.7	0.9	**S**	30.7	0.9	**S**	11.3	0.9	**R**	0.0	0.0	**R**
1BSs1	0.0	**0**	**R**	0.0	0	**R**	0.0	0.0	**R**	18.0	0.0	**S**	16.0	0.0	**I**	0.0	0.0	**R**	0.0	0.0	**R**
1BSs2	0.0	**0**	**R**	0.0	0	**R**	0.0	0.0	**R**	21.3	0.9	**S**	17.3	0.9	**I**	0.0	0.0	**R**	0.0	0.0	**R**
2B2Ss1	0.0	**0**	**R**	0.0	0	**R**	0.0	0.0	**R**	27.3	0.9	**S**	19.3	0.9	**I**	0.0	0	**R**	0.0	0.0	**R**

SD- Standard deviation IN- Interpretation S-Sensitive I-Intermediate R-Resistant TET- Tetracycline COT- Trimethoprim/ Sulphamethoxazole CRX- Cefuroxime GEN- Gentamicin CPR- Ciprofloxacin CHL- Chloramphenicol AMP- Ampicillin

F.2: Antimicrobial sensitivity profiles of *Shigella spp* isolates from fish farms

	TET			COT			CRX			GEN			CPR			CHL			AMP		
ISOLATE	**MEAN (mm)**	**SD**	**IN**	MEAN (mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN (mm)	SD	**IN**	MEAN (mm)	SD	**IN**	MEAN(mm)	SD	**IN**	MEAN(mm)	SD	**IN**
6BSs2	**0**	**0**	**R**	0.0	0	**R**	0.0	0.0	**R**	28.0	1.6	**S**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**

Table

11BSs1	**0**	**0**	**R**	0.0	0	**R**	0.0	0.0	**R**	27.3	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
11BSs2	**8**	**0**	**R**	0.0	0	**R**	0.0	0.0	**R**	27.3	0.9	**S**	13.3	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12BSs1	**0**	**0**	**R**	0.0	0	**R**	0.0	0.0	**R**	24.0	0.0	**S**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
12BSs2	**0**	**0**	**R**	0.0	0	**R**	0.0	0.0	**R**	20.7	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
13B1Ss1	11.3	0.9	**R**	19.3	3.8	**S**	18.7	0.9	**I**	21.3	0.9	**S**	18.7	0.9	**I**	10.7	0.0	**R**	0.0	0.0	**R**
13B1Ss2	14.0	0.0	**I**	0.0	0.0	**R**	18.0	0.0	**I**	27.3	0.9	**S**	20.7	0.9	**I**	14.7	0.9	**I**	0.0	0.0	**R**
13B2Ss1	17.3	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**	24.7	0.9	**S**	26.0	1.6	**S**	0.0	0.0	**R**	0.0	0.0	**R**
13B2Ss2	12.7	0.9	**I**	21.3	0.9	**S**	20.0	0.0	**I**	24.0	0.0	**S**	15.3	0.9	**R**	0.0	0.0	**R**	0.0	0.0	**R**
2C4Ss2	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	22.7	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**
6CSs1	0.0	0.0	**R**	16.0	0	**S**	0.0	0.0	**R**	14.0	0.0	**I**	17.3	0.9	**I**	10.7	0.9	**R**	0.0	0.0	**R**
6CSs2	0.0	0.0	**R**	14.7	0.9	**I**	12.0	0.0	**R**	16.0	0.0	**S**	15.3	0.9	**R**	8.0	0.0	**R**	0.0	0.0	**R**
8CSs1	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	21.3	0.9	**S**	24.7	0.9	**S**	0.0	0.0	**R**	0.0	0.0	**R**
9CSs2	0.0	0.0	**R**	0.0	0	**R**	0.0	0.0	**R**	20.0	0.0	**S**	22.0	1.6	**S**	0.0	0.0	**R**	0.0	0.0	**R**
10CSs1	**16.7**	**9.3**	**S**	0.0	0	**R**	0.0	0.0	**R**	18.7	0.9	**S**	18.7	0.9	**I**	0.0	0.0	**R**	0.0	0.0	**R**
10CSs2	0.0	0	**R**	0.0	0	**R**	0.0	0.0	**R**	23.3	0.9	**S**	19.3	0.9	**I**	0.0	0.0	**R**	0.0	0.0	**R**
12CSs1	0.0	0	**R**	0.0	0	**R**	0.0	0.0	**R**	22.7	0.9	**S**	16.0	1.6	**I**	0.0	0.0	**R**	0.0	0.0	**R**
12CSs2	0.0	0	**R**	0.0	0	**R**	0.0	0.0	**R**	10.0	0.0	**S**	0.0	0.0	**R**	0.0	0.0	**R**	0.0	0.0	**R**

SD- Standard deviation IN- Interpretation S-Sensitive I-Intermediate R-Resistant TET- Tetracycline COT- Trimethoprim/ Sulphamethoxazole CRX- Cefuroxime GEN- Gentamicin CPR- Ciprofloxacin CHL- Chloramphenicol AMP- Ampicillin

Table

G.1: CLSI zone diameter interpretative criteria 2014, *S. typhi*

CLSI ZONE DIAMETER(MM) ITERPRETATIVE GUIDELINES-SALMONELLA SPP			
	S	I	**R**
AMPICILLIN	≥17	14-16	≤13
CIPROFLOXACIN	≥31	21-30	≤20
COTRIMOXAZOLE	≥16	11-15	≤10
CEFUROXIME	≥23	15-22	≤14
CHLORAMPHENICOL	≥18	13-17	≤12
GENTAMICIN	≥15	13-14	≤12
TETRACYCLINE	≥15	12 to 14	≤11

S= SUSCEPTIBLE, I=INTERMEDIATE, R=RESISTANT

Table G.2: Antimicrobial sensitivity profiles of *S.typhi* isolates from fish farms

	TET			**COT**			**CRX**			**GEN**			**CPR**			**CHL**			**AMP**		
ISOLATE	MEAN (mm)	SD	**IN**	MEAN(mm)	**SD**	**IN**	MEAN (mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN (mm)	**SD**	IN	MEAN (mm)	SD	IN	MEAN (mm)	SD	IN
1ASt1	0.0	0.0	**R**	0.0	**0.0**	**R**	20.7	**0.9**	**I**	24.7	**0.9**	**S**	29.3	**0.9**	**I**	11.3	**0.9**	**R**	0.0	**0**	**R**
2A1St1	0.0	0.0	**R**	10.0	**0.0**	**R**	18.7	**1.9**	**I**	23.3	**0.9**	**S**	29.3	**0.9**	**I**	12.0	**0.0**	**R**	0.0	**0**	**R**
2A2St1	0.0	0.0	**R**	18.0	**0.0**	**S**	14.0	**0.0**	**R**	20.0	**0.0**	**S**	26.0	**0.0**	**I**	8.0	**0.0**	**R**	12.0	**0**	**R**
2A2St2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	0.0	**0**	**R**
2A3St1	13.3	0.9	**I**	20.7	**0.9**	**S**	18.7	**0.9**	**I**	24.7	**0.9**	**S**	31.3	**0.9**	**S**	16.0	**0.0**	**I**	0.0	**0**	**R**
3ASt1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	21.3	**0.9**	**S**	23.3	**0.9**	**I**	0.0	**0.0**	**R**	0.0	**0**	**R**
3ASt2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	18.7	**0.9**	**S**	35.3	**3.8**	**S**	0.0	**0.0**	**R**	0.0	**0**	**R**

Table

SD- Standard deviation IN- Interpretation S-Sensitive I-Intermediate R-Resistant TET- Tetracycline COT- Trimethoprim/ Sulphamethoxazole CRX- Cefuroxime GEN- Gentamicin CPR- Ciprofloxacin CHL- Chloramphenicol AMP- Ampicillin

G.2: Antimicrobial sensitivity profiles of *S. typhi* isolates from fish farms

	TET			COT			CRX			GEN			CPR			CHL			AMP		
ISOLATE	MEAN (mm)	SD	**IN**	MEAN (mm)	**SD**	**IN**	MEAN (mm)	**SD**	**IN**	MEAN (mm)	**SD**	**IN**	MEAN (mm)	**SD**	**IN**	MEAN (mm)	**SD**	**IN**	MEAN (mm)	**SD**	**IN**
6ASt1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	34.0	**2.8**	**S**	20.7	**0.9**	**R**	0.0	**0.0**	**R**	0.0	**0**	**R**
7ASt1	0.0	0.0	**R**	0.0	**0.0**	**R**	15.3	**0.9**	**I**	26.0	**0.0**	**S**	26.7	**0.9**	**I**	0.0	**0.0**	**R**	0.0	**0**	**R**
10ASt1	10.0	0.0	**R**	10.0	**0.0**	**R**	0.0	**0.0**	**R**	24.7	**0.9**	**S**	37.3	**1.9**	**S**	0.0	**0.0**	**R**	0.0	**0**	**R**
10ASt2	10.0	0.0	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	37.3	**1.9**	**S**	48.0	**1.6**	**S**	0.0	**0.0**	**R**	0.0	**0**	**R**
11ASt1	0.0	0.0	**R**	21.3	**0.9**	**S**	20.0	**0.0**	**I**	23.3	**0.9**	**S**	27.3	**0.9**	**I**	0.0	**0.0**	**R**	0.0	**0**	**R**
11ASt2	0.0	0.0	**R**	19.3	**0.9**	**S**	20.7	**0.9**	**I**	20.7	**0.9**	**S**	27.3	**0.9**	**I**	0.0	**0.0**	**R**	0.0	**0**	**R**
12A1St1	10.0	0.0	**R**	0.0	**0.0**	**R**	20.0	**0.0**	**I**	20.7	**0.9**	**S**	33.3	**0.9**	**S**	0.0	**0.0**	**R**	0.0	**0**	**R**
12A1St2	14.0	0.0	**I**	0.0	**0.0**	**R**	20.0	**1.6**	**I**	20.7	**0.9**	**S**	26.0	**0.0**	**I**	0.0	**0.0**	**R**	0.0	**0**	**R**
12A2St1	0.0	0.0	**R**	0.0	**0.0**	**R**	20.0	**0.0**	**I**	22.7	**0.9**	**S**	32.0	**0.0**	**S**	0.0	**0.0**	**R**	10.7	**0.9**	**R**
12A2St2	0.0	0.0	**R**	0.0	**0.0**	**R**	22.0	**0.0**	**I**	26.7	**0.9**	**S**	32.0	**0.0**	**S**	0.0	**0.0**	**R**	12.7	**0.9**	**R**
12A3St1	0.0	0.0	**R**	25.3	**0.9**	**S**	15.3	**0.9**	**I**	25.3	**0.9**	**S**	30.7	**0.9**	**I**	13.3	**0.9**	**I**	0.0	**0**	**R**
12A3St2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	29.3	**0.9**	**S**	35.3	**0.9**	**S**	0.0	**0.0**	**R**	0.0	**0**	**R**
12A4St1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	12.0	**0.0**	**R**	8.0	**0.0**	**R**	0.0	**0.0**	**R**	0.0	**0**	**R**

Table

13A1St1	0.0	0.0	**R**	28.7	**0.9**	**S**	0.0	**0.0**	**R**	30.0	**1.6**	**S**	31.3	**0.9**	**S**	0.0	**0.0**	**R**	0.0	**0**	**R**
13A1St2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	24.0	**0.0**	**S**	31.3	**0.9**	**S**	0.0	**0.0**	**R**	0.0	**0**	**R**
13A2St1	12.0	0.0	**I**	25.3	**0.9**	**S**	17.3	**0.9**	**I**	28.7	**0.9**	**S**	25.3	**0.9**	**I**	0.0	**0.0**	**R**	0.0	**0**	**R**
13A2St2	0.0	0.0	**R**	20.0	**0.0**	**S**	16.7	**0.9**	**I**	26.0	**0.0**	**S**	25.3	**0.9**	**I**	0.0	**0.0**	**R**	0.0	**0**	**R**
3BSt1	10.0	0.0	**R**	12.0	**0.0**	**I**	0.0	**0.0**	**R**	23.3	**0.9**	**S**	14.0	**0.0**	**R**	0.0	**0.0**	**R**	0.0	**0**	**R**
4BSt1	0.0	0.0	**R**	10.7	**0.9**	**R**	11.3	**0.9**	**R**	23.3	**10.4**	**S**	10.7	**0.9**	**R**	0.0	**0.0**	**R**	0.0	**0**	**R**
4BSt2	0.0	0.0	**R**	0.0	**0.0**	**R**	14.7	**0.9**	**R**	26.7	**0.9**	**S**	14.7	**0.9**	**R**	10.0	**0.0**	**R**	0.0	**0**	**R**
5Bst2	19.3	0.9	**S**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	24.7	**0.9**	**S**	22.7	**0.9**	**I**	0.0	**0.0**	**R**	0.0	**0**	**R**
6BSt1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	26.7	**0.0**	**S**	31.3	**0.0**	**S**	14.0	**0.0**	**R**	0.0	**0**	**R**
7BSt2	14.0	0.0	**I**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	30.7	**0.9**	**S**	30.0	**0.0**	**I**	19.3	**1.9**	**S**	0.0	**0**	**R**

SD- Standard deviation IN- Interpretation S-Sensitive I-Intermediate R-Resistant TET- Tetracycline COT- Trimethoprim/ Sulphamethoxazole

CRX- Cefuroxime GEN- Gentamicin CPR- Ciprofloxacin CHL- Chloramphenicol AMP- Ampicillin

G.2: Antimicrobial sensitivity profiles of *S. typhi* isolates from fish farms

	TET			COT			CRX			GEN			CPR			CHL			AMP		
ISOLATE	MEAN (mm)	SD	**IN**	MEAN (mm)	**SD**	**IN**	MEAN (mm)	**SD**	**IN**	MEAN (mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**	MEAN (mm)	**SD**	**IN**	MEAN(mm)	**SD**	**IN**
9BSt1	0.0	0.0	**R**	24.7	**0.9**	**S**	12.0	**0.0**	**R**	21.3	**0.9**	**S**	28.7	**0.9**	**I**	10.0	**0.0**	**R**	0.0	**0**	**R**
11BSt1	0.0	0.0	**R**	0.0	**0.0**	**R**	18.0	**0.0**	**I**	22.0	**0.0**	**S**	30.0	**1.6**	**I**	0.0	**0.0**	**R**	0.0	**0**	**R**
11BSt2	0.0	0.0	**R**	20.0	**0.0**	**S**	18.7	**0.9**	**I**	22.7	**0.9**	**S**	28.7	**0.9**	**I**	12.7	**0.9**	**R**	12.0	**0**	**R**
12BSt1	0.0	0.0	**R**	0.0	**0.0**	**R**	16.0	**0.0**	**I**	24.7	**0.9**	**S**	25.3	**0.9**	**I**	10.0	**0.0**	**R**	0.0	**0**	**R**
12BSt2	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	24.7	**0.9**	**S**	12.0	**0.0**	**R**	0.0	**0.0**	**R**	0.0	**0**	**R**

Table

13B1St1	0.0	0.0	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	14.0	**0.0**	**I**	17.3	**0.9**	**R**	9.3	**0.9**	**R**	0.0	**0**	**R**
13B1St2	0.0	0.0	**R**	22.7	**0.9**	**S**	0.0	**0.0**	**R**	24.0	**0.0**	**S**	26.7	**0.9**	**I**	11.3	**0.9**	**R**	0.0	**0**	**R**
13B2St1	0.0	0.0	**R**	24.7	**0.9**	**S**	0.0	**0.0**	**R**	23.3	**0.9**	**S**	30.7	**0.9**	**I**	12.7	**0.9**	**R**	0.0	**0**	**R**
13B2St2	0.0	0.0	**R**	23.3	**0.9**	**S**	0.0	**0.0**	**R**	23.3	**0.9**	**S**	28.7	**0.9**	**I**	0.0	**0.0**	**R**	0.0	**0**	**R**
2C4St1	0.0	0	**R**	0.0	**0.0**	**R**	22.0	**0.0**	**I**	35.3	**0.9**	**S**	40.0	**0.0**	**S**	17.3	**0.9**	**I**	0.0	**0**	**R**
2C4St2	0.0	0	**R**	20.0	**0.0**	**S**	0.0	**0.0**	**R**	38.0	**0.0**	**S**	31.3	**0.9**	**S**	0.0	**0.0**	**R**	0.0	**0**	**R**
4CSt1	0	0	R	0.0	**0.0**	**R**	0.0	**0.0**	**R**	22.0	**0.0**	**S**	28.0	**1.6**	**I**	0.0	**0.0**	**R**	0.0	**0**	**R**
6CSt1	0.0	0	**R**	20.0	**0.0**	**S**	17.3	**1.9**	**I**	22.7	**0.9**	**S**	26.0	**0.0**	**I**	10.7	**0.9**	**R**	0.0	**0**	**R**
6CSt2	16.0	0	**S**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	28.7	**0.9**	**S**	26.0	**0.0**	**S**	0.0	**0.0**	**R**	0.0	**0**	**R**
8CSt1	0.0	0	**R**	20.7	**0.9**	**S**	19.3	**0.9**	**I**	23.3	**0.9**	**S**	24.7	**0.9**	**I**	19.3	**0.9**	**S**	0.0	**0**	**R**
8CSt2	0.0	0	**R**	24.7	**0.9**	**S**	24.7	**0.9**	**S**	22.0	**1.6**	**S**	28.7	**0.9**	**I**	0.0	**0.0**	**R**	0.0	**0**	**R**
9CSt1	0.0	0	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	28.0	**1.6**	**S**	37.3	**1.9**	**S**	0.0	**0.0**	**R**	0.0	**0**	**R**
10CSt1	0.0	0	**R**	0.0	**0.0**	**R**	0.0	**0.0**	**R**	29.3	**0.9**	**S**	14.7	**0.9**	**R**	0.0	**0.0**	**R**	0.0	**0**	**R**
10CSt2	0.0	0	**R**	22.7	**0.9**	**S**	13.3	**0.9**	**R**	29.3	**0.9**	**S**	38.7	**0.9**	**S**	0.0	**0.0**	**R**	0.0	**0**	**R**

SD- Standard deviation IN- Interpretation S-Sensitive I-Intermediate R-Resistant TET- Tetracycline COT- Trimethoprim/ Sulphamethoxazole CRX-

Cefuroxime GEN- Gentamicin CPR- Ciprofloxacin CHL- Chloramphenicol AMP- Ampicillin

H.1: Number of antibiotics to which isolates are resistant

No. of antibiotics	ORGANISM				
	S. aureus	*E.coli*	*Shigella spp*	*S. typhi*	*P. aeruginosa*
	% isolates	% isolates	% isolates	% isolates	% isolates
1	0	0.0	0.0	6.1	30.6
2	0	3.4	6.4	14.3	29.0

Table

3	7.0	13.8	17.0	34.7	0
4	7.0	27.6	46.8	28.6	0
5	25.4	39.7	29.8	12.2	0
6	39.4	13.8	0.0	4.1	0
7	19.7	1.7	0	0	0
8	1.4	0	0	0	0