RADIATION SENSITIVITY AND MOLECULAR CHARACTERIZATION OF WATER- BORNE MULTIDRUG RESISTANT *ESCHERICHIA COLI*

by

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DECLARATION

I declare that this thesis on "**Radiation Sensitivity And Molecular Characterization of Water- Borne Multidrug Resistant *Escherichia Coli* "** carried out in the Dangme West District, in the Greater Accra Region of Ghana, and supervised by Prof. Kennedy Kwasi Addo of University of Ghana and Prof. O.R. Awofolu of University of South Africa, is my own work. The thesis has not previously been submitted in part or in whole to any other university for academic examination towards any qualification. That it is my own work in design and in execution and that all reference material contained therein has been duly acknowledged.

_____ May 30, 2014

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DEDICATION

To the glory of God, this thesis is dedicated to my wife, Carolyn and to my children: Stephanie - Ann Naa-Deide Odonkor, Stephen Nii-Korley Odonkor Jr. and Nicholas-Jason Nii-Tetteh Odonkor. To my parents: Beatrice Deide Armah and Alfred Tetteh Odonkor and to all my siblings.

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This is the doing of the Lord; it is marvellous in our eyes!! Therefore I conclude with the hymn below:

1. Captain of Israel's host, and Guide
Of all who seek the land above,
Beneath Thy shadow we abide,
The cloud of Thy protecting love;
Our strength, Thy grace; our rule, Thy Word;
Our end, the glory of the Lord.

2. By thine unerring Spirit led,
We shall not in the desert stray;
We shall not full direction need
Nor miss our providential way;
As far from danger as from fear,
While Love, almighty Love, is near.

Stephen Tawiah Odonkor

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ABSTRACT

Background: The spread of antibiotic-resistant microorganisms in the environment is recognized widely as an important public health issue, with concerns about future ability to treat infectious diseases. The main risk to public health is that the resistance genes are transferred from environmental bacteria to human pathogens. Safe water is one of the most important needs in public health in the twenty first century. The major health threat posed by drinking unsafe water is the transmission of infectious diseases, which are the leading causes of mortality and morbidity for children under the age of 5 and it is estimated to cause 1.5 million deaths annually in developing countries. In addition to the wide spread cases of water-borne diseases resulting from the contamination of water sources, concerns have been raised when these diseases fail to be cured due to development of resistance to most prescribed antibiotics by the contaminating microorganisms. It is now a well-established fact that *E. coli* is a significant cause of diarrheal illnesses both in infants and adults in many parts of the world. Data on clinical isolates is plenty while less attention has been given to environmental isolates of these enteric pathogens. Samples from the environment such as water may serve as probable reservoirs of these pathogens; this is compounded by the entry of functional compounds of antibiotics into waterways, through humans and animals that have ingested antibiotics. This is because antibiotics are not completely metabolized and may enter waterways through the waste products of these humans or animals. Studies on antimicrobial resistance is important in order to detect changes in patterns of resistance, implement control measures on the use of antimicrobial agents, and to prevent the spread of multidrug-resistant strains of bacteria. It also provides surveillance data for antibiotic resistances, necessary to define or update guidelines for empirical treatment, as well as a guide for appropriate antibiotic supplies.

Study objectives: The objectives of this research were: (i) to determine the total and faecal coliform status of drinking water sources, as an indication of quality; (ii) to determine the bacteriological profile of bacteria flora in the drinking water sources; (iii) to determine prevalence and susceptibility profiles of antibiotic resistant water-borne *E. coli;* (iv) to investigate the virulence genes associated with multiple antibiotic resistant *E. coli* isolates; (v) to compare three laboratory based techniques: PCR, API 20E, and Culture based methods used for detection of *E. coli* and (vi) to determine the association between multiple antibiotic resistance and radiation sensitivity (D_{10}).

Methodology: Four hundred and sixty four (464) water samples were collected for assessment between June 2011 and May 2012. The samples were collected from 57 sampling sites, from six different water sources including: boreholes (10), a canal (1), dams (15), hand-dug wells (15), a river (1), and streams (15). Total coliforms, faecal coliforms, and *E. coli* analysis were done by the MPN method. Bacteria isolation and identification were done using API 20E, conventional methods, and a PCR based DNA STRIP technology that allows simultaneous detection of virulence genes and confirmation of *E. coli* isolates. Antibiotic susceptibility testing was also conducted using the Kirby-bauer method. Radiation sensitivity was done using a cobalt 60 source.

Results: The results obtained indicated that all the water sources were of poor quality in terms of microbial distributions with total coliform and faecal coliform counts ranging between 0 to $2.4x10^3$ MPN/100ml. *E. coli* counts ranged between 10 to $7.9x10^1$MPN/100ml. Disease risk assessment of the various water sources indicated that dam water sources presented a high disease risk, while borehole water sources had a low disease risk. A total of five hundred and twenty bacterial isolates (520) were obtained during the period of study. Three hundred and five (305) isolates representing 58.65% of the total were obtained during the dry season, as against (205) representing 41.35% in the rainy season. The most commonly occurring bacteria in the water samples was *Klebsiella* spp constituting 20%. The next most occurring organism was *E. coli* (18.7%). This was followed by *Pseudomonas aeruginosa* (15.61%), *Enterobacter* spp. (15.4%), *Proteus vulgaris* (13.1%), and *Enterococcus faecalis* (9.2%). The least isolated bacteria were *Vibrio cholerae* (1.2%) and *Shigella* spp. (1.2%). The prevalence of multi drug resistance *E. coli* was 49.48 %. *E. coli* isolates showed a high resistance patterns to the tested antibiotics. They were most resistant to penicillin (32.99%), cefuroxime (28.87%), erythromycin (23.71%), and tetracycline (21.45%). In contrast, they were susceptible to nitrofurantoin (93.8%), cefotaxime and amikacin (91.75%), gentamicin (90.7%), nalidixic acid (89.65%), ciprofloxacin (74.2%), chloramphenicol (69.07%), pipemidic acid (65.97%) and cefuroxime (52.58%). Sixty-three percent (63%) of the multidrug resistant *E. coli* strains recorded a multiple antibiotic resistance (MAR) index value of >0.2. Six (6%) percent of he multiple antibiotic resistant were *eae* virulence genes producing however, none of the *E. coli* isolates produced the *stx1* and *stx2* virulent gene. The analytical profile index (API) recorded specificity and sensitivity of 99.7% and 98.50 % respectively for the detection of *E. coli*. The

culture/ biochemical based methods for detection of *E coli* recorded specificity of 81.82% and a sensitivity of 96.91%. There was no association (P> 0.05) between radiation sensitivity (D_{10}) and antibiotic resistances.

Conclusion: The study has confirmed that majority of the water sources used for drinking and domestic purposes in the study area are highly contaminated with high levels of faecal coliforms above the recommended standards. There were also presence of bacteria of public health importance in the water sources. Both animals and humans could be sources of faecal bacteria contamination of the drinking water sources. The study confirmed a high prevalence of multiple antibiotic resistances in *E. coli* isolates. The *eae* virulence gene was associated with some of the multiple resistant *E. coli* isolates. The study also concludes that API 20E has a high specificity and sensitivity close to that of the PCR. Lastly, There is no association between multiple antibiotic resistant indexes and radiation sensitivity (D_{10}) of antibiotic resistant *E. coli.*

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AMK	Amikacin
AMP	Ampicillin
AMR	Antimicrobial Resistance
APHA	American Public Health Association
API	Analytical Profile Index
ARG	Antibiotic Resistant Genes
ATTC	American Type Culture Collection
BCT	Biochemical Test
CHL	Chloramphenicol
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
COT	Co-trimoxazole
CXM	Cefuroxime
DAEC	Diffusely adherent *E. coli*
DNA	Deoxyribonucleic Acid
EAEC	Enteroaggregative *E. coli*
EC	Electrical Conductivity
EHEC	Enterohemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ERY	Erythromycin
ETEC	Enterotoxigenic *E. coli*
FC	Faecal Coliform
GEN	Gentamicin
HCG	Horizontal Transfer of Genes
HGT	Horizontal Gene Transfer
IMViC	Indole, Methyl Red, Voges Proskauer and Simmon Citrate
MAR	Multiple Antibiotic Resistance
MAX	Maximum

MDG	Millennium Development Goals
MDR	Multi Drug Resistance
mg	Milligram
mg/L	Milligram Per Liter
MIC	Minimum Inhibitory Concentration
min	Minimum
ml	Milliliter
mm	Millimeter
MPL	Maximum Permissible Levels
MPN	Most Probable Number
NAL	Nalidixic acid
NCCLS	National Committee for Clinical Laboratory Standards
NIT	Nitrofurantoin
NO_3	Nitrates
PA	Pipemidic Acid
PCR	Polymerase Chain Reaction
PEN	Penicillin
PFGE	Pulsed-Filed Gel Electrophoresis
STP	Sewage Treatment Plant
TC	Total Coliform
TET	Tetracycline
ug	Microgram
VNC	Viable but Non Culturable
VRE	Vancomycin Resistant Enterococci
WHO	World Health Organization
XDR	Extremely Drug Resistant

Published /Unpublished Articles And Presentations

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2. Stephen T Odonkor and Joseph A Ampofo. *Escherichia coli, as an Indicator of Bacteriological Quality of water: An overview*. Microbiology Research (2013), 4(2), 5-8.
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CHAPTER ONE
1.0 INTRODUCTION

1.1 Water quality and contamination

Water is essential for survival. It has been stated that our existence is "intimately connected with the quality of water available to us. (Routledge & Stewart, 1998). An adequate supply of safe drinking water is one of the major prerequisites for a healthy life. Waterborne disease is still a major cause of death in many parts of the world, particularly in children, and it is also a significant economic constraint in many subsistence economies. Waterborne diseases are as a result of contaminations: the presence of elevated concentrations of substances in the environment above the natural background level for the area and for the organism (Fawell & Nieuwenhuijsen, 2003)

Drinking water is derived from two basic sources: surface water, such as rivers and reservoirs, and groundwater such as wells and boreholes. All water contains natural contaminants, particularly inorganic contaminants that arise from the geological strata through which the water flows and, to a varying extent, anthropogenic pollution by both microorganisms and chemicals. In general, groundwater is less vulnerable to pollution than surface waters (Fawell & Standfield, 2001). There are a number of possible sources of man-made contaminants, some of which are more important than others. These fall into the categories of point and diffuse sources. Discharges from industrial premises and sewage treatment works are point sources and as such are more readily identifiable and controlled; run off from agricultural land and from hard surfaces, such as roads, are not so obvious, or easily controlled. Such sources can give rise to a significant variation in the contaminant load over time.

There is also the possibility of spills of chemicals from industry and agriculture and slurries from intensive farm units that can contain pathogens. Badly sited latrines and septic tanks are a significant source of contamination, especially of wells (Odonkor & Ampofo, 2013).

Local industries can also give rise to contamination of water sources, particularly when chemicals are handled and disposed of without proper care. The run-off or leaching of nutrients into slow flowing or still surface waters can result in excessive growth of cyanobacteria or blue-green algae (Chorus & Bartram, 1999). Many species give rise to nuisance chemicals that can cause taste and odour and interfere with drinking water treatment. However, they frequently produce toxins, which are of concern for health, particularly if there is only limited treatment.

The basis on which drinking water safety is judged is national standards or international guidelines. The most important of these are the WHO Guidelines for Drinking-Water Quality (WHO, 2010). These are revised on a regular basis and are supported by a range of detailed documents describing many of the aspects of water safety. The Guidelines are now based on Water Safety Plans that encompass a much more proactive approach to safety from source-to-tap.

1.2 Biological contaminants of water

The most common and deadly pollutants in drinking water in developing countries are of biological origin. WHO (2000) states that the "infectious diseases caused by pathogenic bacteria, viruses and protozoa or by parasites are the most common and widespread health risk associated with drinking water". One study (Walsh, 1990) using 1986 data estimated that 10 major waterborne diseases are responsible for over 28 billion disease episodes annually in developing countries, of these, diarrhoeal diseases are the big killers. Esrey *et al.*, (1990) surveyed 142 studies on 6 of the major waterborne diseases and estimated that in developing countries (excluding China), there were 875 million cases of diarrhoea and 4.6 million deaths annually in the mid-1980s.

According to the World Bank estimate, more than 3 million children below age 5 die annually from diarrhoeal diseases contracted through drinking water in the developing world (World Bank, 1992). Table 1.1 shows orally transmitted pathogens in water.

Table 1.1 Orally transmitted pathogens in drinking water

Pathogens	Health significance	Persistence in water supplies [a]	Relative infective dose [b]
BACTERIA			
Campylobacter, *C. coli*	High	Moderate	Moderate
Pathogenic *Escherichia coli*	High	Moderate	High [c]
Salmonella typhi			
Other Salmonellae	High	Long	High
Shigella spp.	High	Short	Moderate
Vibro cholerae	High	Short	Moderate
Yersinia enterocolitica	High	Long	High (?)
Pseudomonas aeruginosa	Moderate	May multiply	High (?)
Aeromonas spp.	Moderate	May multiply	High (?)
VIRUSES			
Adenoviruses	High	?	Low
Enterroviruses	High	Long	Low
Hepatitis A	High	?	Low
Enterically transmitted non-A, non-B hepatitis virus, hepatitis E	High	?	low
Norwalk virus	High	?	Low
Rotavirus	High	?	Moderate
Small round viruses	Moderate	?	Low (?)
PROTOZOA			
Entamoeba histolytica	High	Moderate	Low
Giardia intestinalis	High	Moderate	Low
HELMINTHES			
Dracunculus medinensis	High	Moderate	Low

Source: World Health Organization (1993)
? – Not Known or unclear

The WHO estimate of the toll is more than 5 million deaths annually (of these, about 4 million children are under age 5) from unsafe drinking water (WHO, 1996). Although the quality of data on mortality and morbidity from unsafe drinking water is inconclusive (the estimates made by different experts of annual global child deaths vary by almost a factor of 2, from 2 to 4 million),

the magnitudes of the mortality and morbidity from waterborne diarrhoeal diseases unquestionably make them the world's biggest environmental health threat to populations. In addition, the primary cause for stunted growth for millions of children in the developing world is poor nutrition resulting in part from frequent bouts of diarrhoea. Repeated bouts of diarrhoea inhibit the ability of the body to absorb nutrients for a much longer period than the duration of the actual diarrhoeal episodes. Thus, children who survive the risk of dying from diarrhoeal diseases are at risk of stunting from malnutrition.

The use of such water for drinking, cooking, contact with it during washing, bathing, or even inhalation of its fine droplets as aerosols, may then result in infection. The minimum infectious dose (the smallest number of ingested pathogens necessary to cause disease) for the average healthy adult varies widely for various microorganism

This dose ranges from just a few organisms of *Salmonella typhi* to produce typhoid, several hundred organisms of *Shigella flexneri* to cause dysentery, several million cells of *Salmonella* serotype to cause gastroenteritis, to as many as a hundred million cells of *Vibrio cholerae* to produce cholera. The minimum infectious dose also varies by the age, health, nutritional and immunological status of the exposed individual. As WHO notes, "Those at greatest risk of waterborne disease are infants and young children, people who are debilitated or living under insanitary conditions, the sick, and the elderly. For these people, infective doses are significantly lower than for the general adult population" (WHO, 1993). The size of the minimum infectious dose does not directly translate into ease of prevention of the relevant disease (since concentrations of the pathogens in the water are variable, too). However, it does point to the reasonableness of the approach to minimize disease risk by defining a maximum allowable concentration of an indicator organism in drinking water.

1.3 Antibiotics in water

Antibiotic resistance genes conferring resistance to a wide variety of microorganism have been identified in a large range of water environments including wastewater, sewage, surface water, ground water and drinking water in both developed and developing countries. The spread of these antibiotic-resistant microorganisms in the environment is recognized widely as an important public health issue with physicians concerned about their future ability to treat

infectious diseases (Schmidt, 2002; Silra *et al*., 2010). The useful life span of an antibiotic is limited by the emergence and spread of resistant bacteria with early concerns about resistant bacteria focused primarily on clinical settings and nosocomial infections.

Over the last several decades, the focus of research has expanded with the realization that, resistant organisms are widespread in the environment. Antibiotic resistance has been observed in various aquatic environments including rivers, coastal areas, domestic sewage, surface waters, sediments, lakes, polluted ocean water by sewage and drinking water (Mezrioui & Baleux, 1994). Increased introduction of antimicrobial agents into these environments via medical therapy, agriculture and animal husbandry has resulted in new selective pressures on natural bacterial populations (Col & O'Connor, 1987; Dupont & Steele, 1987). It was reported that this has exacerbated the problem of controlling microbes in a disease setting and has caused a resurgence of bacterial diseases worldwide due to the acquisition and transfer of antibiotic resistance genes and virulence factors (Tomasz, 1994).

The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogens. The ability of the resistant bacteria and resistance genes to move from one ecosystem to another is documented by the various cases in which transmission of resistant bacteria has been demonstrated between animals and humans. The inclusion of certain growth promoters in animal feed has been recognized as a cause for the selection of the resistance genes in the commensal microflora of animals. They are transmitted to humans via the food chain (Kruse, 1999). Similarly, drinking and bathing water could represent a source for the acquisition of resistant bacteria in humans. However, further studies are necessary to validate this hypothesis. *E. coli* has been generally accepted as the predominant vehicle for the dissemination of resistance genes and vectors due to its abundance in such environments (Tauxe *et al*., 1997; Chen *et al*., 2010).

Although direct evidence surrounding the transfer of antibiotic resistant genes (ARGs) from the environment to humans is unavailable, some studies highlight the fact that antibiotic resistant genes can spread and be exchanged among environmental microorganisms of different genera (Agersø & Petersen, 2007). Antibiotic resistant genes can enter into aquatic environments by

direct discharging of untreated wastewater, or through sewage treatment plant (STP) effluents and discharged sludge (Auerbach *et al*., 2007). Many antibiotic resistant genes, such as, *vanA* and *vanB*, are not completely removed by the activated sludge process widely used in STPs, with genes detected in both influent and effluent water (Iversen *et al*., 2002; Caplin *et al*., 2008).

When antibiotic resistant genes enter into other water bodies with effluent water they can be transferred horizontally to the native bacteria in these aquatic environments (Schwartz *et al*., 2003). Applying real-time quantitative PCR (qPCR), Auerbach *et al.* (2007) investigated *tet* genes in Germany STPs and found that *tetQ* concentrations were highest in influent water while *tetG* concentrations were highest in activated sludge, and UV disinfection had no effect on the amount of detectable *tet* genes in wastewater effluent.

A number of physicochemical factors can influence the transfer of antibiotic resistant genes in aquatic environments. The first factor contributing to the horizontal transfer of antibiotic resistant genes is the selective pressure from increased antibiotic use and production for the treatment of disease and growth promotion. High selective pressure can facilitate the acquisition of antibiotic resistant genes, which may lead to increased fitness among certain bacteria, allowing for rapid emergence and dissemination (Enne *et al*., 2004; Luo *et al*., 2005). In addition, the presence of antibiotics at low inhibitory concentrations can accelerate horizontal transfer and dissemination of environmental antibiotic resistant genes (Kümmerer, 2004).

Genetic mechanisms involved in horizontal transfer of antibiotic resistant genes among environmental bacteria may include the following: (1) conjugative transfer by mobile genetic elements including plasmids, transposons, and integrons on plasmids or transposons; (2) transformation by naked DNA, in the case of a naturally competent state of some bacteria, or an environmentally induced competence such as the presence of calcium; and (3) transduction by bacteriophage (Zhang *et al*., 2009). Antibiotic resistance in most environmental bacteria is due to the acquisition of new genes, often associated with mobile elements. Studies have shown that even if cells carrying antibiotic resistant genes have been killed, the DNA released into the environment is able to persist, protected from DNAse, especially by certain soil/clay compositions, and can eventually be transformed into other cells (Blum *et al*., 1997; Hill & Top., 1998; Crecchio *et al*., 2005). Due to the broadening prevalence of resistant organisms,

resistance-conferring genes themselves are now considered emerging contaminants, especially genes selected in one location (e.g., the gut of an animal) and spread to the environment via surface and groundwater flow (Pruden *et al*., 2006; Koike *et al*., 2007).

Little information is available regarding the fate of antibiotics in the environment and their link to the emergence of resistant genotypes found there. Environmental reservoirs and pathways of antibiotic resistance are acquiring increased attention (Pruden *et al*., 2006; Martinez, 2008; Allen *et al*., 2010) as new strategies beyond the hospital setting are being explored to attenuate antibiotic resistance and prolong the lifespan of antibiotics. One promising approach towards better understanding the distribution and transport of antibiotic resistance in the environment is to consider antibiotic resistant genes as the principal contaminants of interest rather than their bacterial hosts (Pruden *et al*., 2006; McKinney *et al*., 2010).

1.4 Classification of antibiotics

Antibiotics are divided into two groups based on how they act: bacteriostatic and bactericidal. Bacteriostatic antibiotics prevent bacteria from growing or slow down their growth rate, effectively halt their reproduction. Upon removal of the bacteriostatic antibiotic, bacteria usually start to grow again. In contrast, bactericidal antibiotics kill bacteria. However, there is not always a clear distinction between bacteriostatic and bactericidal antibiotics: high concentrations of some bacteriostatic antibiotics are also bactericidal, whereas low concentrations of some bactericidal antibiotics are bacteriostatic. It remains a subject of debate as to whether one of the two groups causes more resistance than the other and if either is more prone to resistance gene evolution.

In addition to grouping antibiotics into bacteriostatic and bactericidal, antibiotics can be categorized into classes such as aminoglycosides, beta-lactams (includes cephalosporins, carbapenems and penicillins), glycopeptides, lincosamides, macrolides, quinolones, sulfonamides, and tetracyclines. All classes are used to treat gram-positive and gram-negative organisms, if not stated otherwise. Most classes are broad-spectrum antibiotics with a few examples that have a narrow spectrum such as the amino glycoside and streptomycin that targets staphylococci. Figure 1.1 shows the targets for antibiotics.

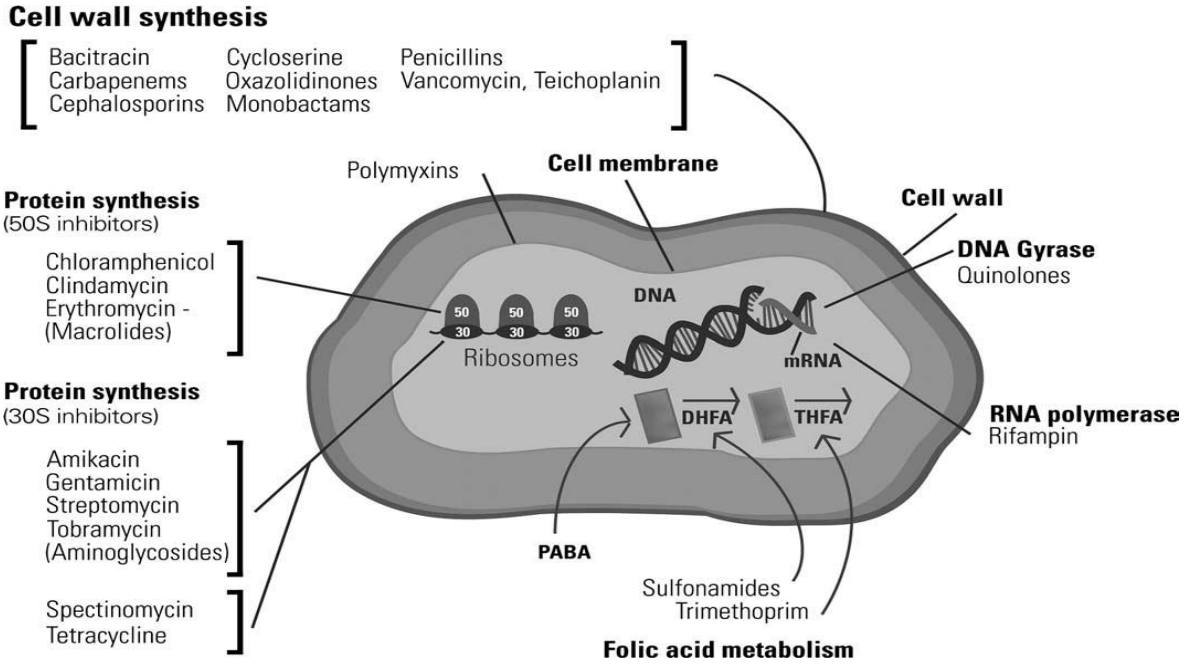

Figure 1.1 Bacterial targets for current antibiotics use in the clinics

1.5 Antibiotics and emerging resistances

The control of infectious diseases is seriously endangered by the rise in the number of microorganisms that are resistant to antimicrobial agents. This is because infections caused by resistant microorganisms often fail to respond to conventional treatment, resulting in prolonged illness leading to a greater risk of death. The World Health Organization (WHO) released a report focusing on antibiotic resistance as one of the most critical human health challenges of the next century, announcing the need for "a global strategy to contain resistance" (WHO/ UNICEF, 2000).

Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. The primary cause of antibiotic resistance is genetic mutation in bacteria (Laxminarayan, 2003). Inappropriate and irrational use of antimicrobial medicines provides favourable conditions for resistant microorganisms to emerge, spread and persist. The greater the duration of exposure of the antibiotic, the greater the risk of the development of resistance, irrespective of the severity of the need for the antibiotic. As resistance towards antibiotics becomes more common, a greater need for alternative treatments arises. However,

despite a push for new antibiotic therapies there has been a continued decline in the number of newly approved drugs (Dromigny & Perrier-Gros, 2003).

An antibiotic now refers to a substance produced by a microorganism, which in low concentrations inhibits the growth of other microorganisms (Giguère *et al.*, 2006, Szczepanowski *et al*., 2009). Antibiotics are one class of antimicrobials that are relatively harmless to the host. They are small molecules with a molecular weight less than 2000 units (Kaiser, 2009). When microbes become resistant to certain antibiotics, the effectiveness of the antibiotic to treatment are reduced, because patients remain infectious (Simon *et al*., 2009) over a long period and thus potentially spreading resistant microorganisms to others. Antimicrobial resistance threatens a return to the pre-antibiotic era (Choi *et al.*, 2007). Many infectious diseases risk becoming uncontrollable and could derail the progress made towards reaching the targets of the health-related United Nations Millennium Development Goals set for 2015, which propose that, by 2015, we halve the proportion of people without sustainable access to safe drinking water and basic sanitation (Sobsey & Bartram, 2003).

Antimicrobial resistance increases the costs of health care (Enne *et al*., 2001). When infections become resistant to first-line medicines, more therapies that are expensive must be used. The longer duration of illness and treatment, often in hospitals, increases health-care costs and the financial burden to families and societies. The achievements of modern medicine are put at risk by antimicrobial resistance. Without effective antimicrobials for care and prevention of infections, the success of treatments such as organ transplantation, cancer chemotherapy and major surgery would be compromised.

Antimicrobial resistance threatens health security, and damages trade and economies (Lipsitch *et al*., 2000). The growth of global trade and travel allows resistant microorganisms to spread rapidly to distant countries and continents. When a patient receives treatment with antibiotics, both the causative pathogen and the normal nonpathogenic microflora in the body will be affected (Todar, 2002). The indigenous microflora makes up a complex ecological system of great importance for human health. Besides being essential for the digestion of food and to metabolise drugs, they also produce essential vitamins and are important for the activation and

maintenance of the immune system in the gut. Ideally, antibiotics should effectively kill the pathogen responsible for infections and, simultaneously, cause as little disturbance as possible to the microflora of the individual.

The development of resistance is inevitable following the introduction of a new antibiotic. Initial rates of resistance to new drugs are normally on the order of 1% (Levine *et al.,* 2006). However, modern uses of antibiotics have caused a huge increase in the number of resistant bacteria. After a widespread use of an antibiotic within 8 - 12 years, strains become resistant to multiple drugs (Todar, 2002). Multiple drug resistant strains of some bacteria have reached the proportion that virtually no antibiotics are available for treatment. Bacteria may be inherently resistant to an antibiotic naturally (Godfrey, 1948). For example, an organism lacks a transport system for an antibiotic; or an organism lacks the target of the antibiotic molecule; or, as in the case of gram-negative bacteria, the cell wall is covered with an outer membrane that establishes a permeability barrier against the antibiotic. Several mechanisms are developed by bacteria in order to acquire resistance to antibiotics (Sabath, 1982).

Resistant organisms may become apparent because of the destruction of sensitive strains by the antibiotic, allowing naturally resistant strains to colonise the patient. For example, penicillin therapy destroys much of the normal mouth flora and the mouth becomes colonized by penicillin-resistant organisms previously present in small numbers. A genetic mutation may occur during treatment and becomes apparent when the sensitive organisms are destroyed. Mutation occurs more readily with some antimicrobial agents than with others, and especially with streptomycin, rifampicin, and nalidixic acid (Bosu & Afori-Adjei, 1997).

Certain organisms may acquire resistance because of the activity of phages (bacterial viruses) which incorporate a resistance present in one organism and when released carry the resistance over to an organism which was originally sensitive. When antibiotics are underused, overused or misused, the process of antibiotic resistance is increased (BPC, 1979) The indiscriminate use of antibiotics, which promotes antibiotic resistance, results from patients' non-compliance to recommended treatment, irrational use of antibiotics in humans, drug advertisement, antibiotic use in agriculture, poor quality antibiotics, inadequate surveillance and susceptibility testing.

1.6 Development of resistance

Bacteria can be unsusceptible or intrinsically resistant, which means that a whole species or genus is not susceptible to a certain antibiotic. Acquired resistance is the phenomenon whereby certain bacteria belonging to a susceptible species are not inhibited or killed any more by concentrations of an antibiotic that would normally do so. There are two ways to acquire resistance: by chromosomal mutation or by acquisition of exogenous genes.

The potential for antibiotic resistance by chromosomal mutation is intrinsic to all bacteria, because it occurs spontaneously in replicating bacteria due to the imperfect fidelity of DNA replication, and is therefore not influenced by the presence of antibiotics. Indeed, even before antibiotics became clinically available resistant bacteria could be found in soil, intestinal flora and clinical isolates, but only in very small numbers (Oatta & Hughes, 1983).

Resistance by gene acquisition generally refers to the prior existence of a resistance gene that is then acquired by another bacterium (Hooper, 2001) through transformation, conjugation, or transduction. Resistance genes can be found on the bacterial chromosome, or are carried on plasmids, that by conjugation can transfer itself to other bacteria, even of different species.

The resistance genes are found on plasmids, which are embedded, in small DNA units called transposons, that easily hop within one bacterium between its chromosome and plasmids present and even from bacteria to bacteria. Table 1.2 shows the emergence of resistance to various antimicrobial agents.

Table 1.2 Emergence of resistance after introduction of various antimicrobial agents

Antibiotic	Discovered	Clinically available	Observed resistance
Penicillin	1940	1943	1940
Methicillin		1961	1965
Streptomycin	1944	1947	1947, 1956
Tretracycline	1948	1952	1956
Erythromycin	1952	1955	1956
Vancomycin	1956	1972	1987
Nalidixic acid	1960	1962	1966
Gentamicin	1963	1967	1970
Third-generation Cephalosporins	1956	1980	1985
Fluroquinonlones	1978	1982	1985

Source: EMEA 1999

Integrons have been recognized as a prime agent for the development and spread of multidrug resistance in *enterobacteriaceae* (Hall & Collis, 1998; White *et al.,* 2001). This also includes human and animal *E. coli* isolates (Bass *et al.,* 1999; Zhao *et al.,* 2001; Schroeder *et al.,* 2002; Guerra *et al.,* 2003; Rijavec *et al.,* 2006). Yet it is not known the spectrum of antibiotics to which these integrons have influenced resistances in *E. coli.*

1.7 Mechanism of resistance

Intrinsic resistance

Some bacteria are intrinsically resistant to antimicrobial agents (Abraham & Chain, 1940) because they lack the target site for that drug action, the drug is unable to reach the site of action, or the organism contains a chromosomally encoded resistance mechanism (Kirby, 1944). For example, most gram-negative organisms are resistant to vancomycin because this large, hydrophobic drug cannot penetrate the organism's cell membrane (Neu, 1989). Thus, these organisms are intrinsically resistant due to a permeability factor. By contrast, *Klebsiella pneumoniae* strains are resistant to ampicillin and other penicillins because these organisms

contain a chromosomal β-lactamase that inactivates these and similar drugs (Bonnet, 2004). Thus, intrinsic resistance may be due to a number of different mechanisms.

Acquired resistance

Bacteria that are by nature susceptible to an antimicrobial agent may become resistant by chromosomal mutation or by the acquisition of new genetic material that encodes the proteins responsible for the resistance phenotype. Chromosomal mutations that occur within the genes that encode the target sites for antimicrobial agents may change the structure of the proteins enough to prevent binding of the drug. Streptomycin resistance in *M. tuberculosis* is a good example of this (Normark & Normark, 2002). Furthermore, the accumulation of point mutations in several different key genes in *M. tuberculosis* under the selective pressure of inadequate antimicrobial therapy (often a result of patients not consistently taking their medications) probably resulted in the multidrug-resistant strains of tuberculosis that caused outbreaks in several large cities in the United States in the early 1990s (Edlin *et al*., 1992). Similar reasons probably underlie the current prevalence of *M. tuberculosis* strains labeled XDR (extremely drug resistant) (CDC, 2007).

Molecular mechanisms

Understanding the acquisition of resistance by bacteria requires consideration of the general pathways for appearance or spread of resistant bacteria in health-care and community settings, and an examination of the molecular mechanisms at work. Several resistance mechanisms have had a major impact on the resistance of gram-positive hospital organisms including new dihydrofolate reductases, novel changes in penicillin-binding proteins, mutations in topoisomerase genes, variants of aminoglycoside modifying enzymes (which have become widely prevalent in *Enterococci*), and new enzymes that alter cell wall composition (Hawkey, 1998, John & Rice, 2000). These arise from duplication and diversification of existing genes, acquisition of new genes from other organisms, and mutation of acquired genes. The ability of pathogenic bacteria to acquire and disseminate these resistance determinants is orchestrated by a variety of plasmids, bacteriophages, transposons, and integrons (John & Rice, 2000).

1.8 Spread of antibiotic resistance

In enteric bacteria such as *E. coli*, resistance is mostly due to horizontal transfer of antibiotic resistance genes (HGT) residing on mobile genetic elements such as plasmids, transposons and integrons (Sherley *et al.*, 2004; Hochnut *et al.*, 2006; Carattoli, 2009). HGT is emerging as the primary means of spread of antibiotic resistance determinants, transferred singly or in clusters among bacterial populations (De la Cruz & Davies 2000; Carattoli, 2001; Carattoli, 2009). Through HGT, resistant bacteria donate resistance genes to other bacteria sharing the same ecological niche. A harmless but resistant bacterium can potentially donate its resistance determinants to a pathogen with serious consequences for disease management. The transfer of genetic material from one bacterial strain to another mostly involves the exchange of mobile extra-chromosomal DNA elements, such as transposons and plasmids. HGT may occur via conjugation, transformation or transduction and may take place both intraspecies and interspecies independent of antibiotic exposure. Conjugation involves both replication and transfer of DNA, allowing easier and faster means of proliferation of antibiotic resistance within bacterial populations.

1.9 Detection methods for waterborne pathogens and antibiotic resistance genes

A traditional approach in water and wastewater microbiology has been the monitoring of water quality by detection and enumeration of microbial indicator organisms, rather than of specific pathogens. The total-coliform group is a broad group with several members of faecal and non-faecal origin, and is conventionally used as the primary bacterial indicator of water suitability for domestic, industrial, or other uses (APHA, 1999). These indicators typically consist of coliform bacteria, faecal enterococci and *Escherichia coli* (NRC, 2004). Indicators of the presence of pathogens are predominantly utilized because of the inability to measure all microbial pathogens that may be present in environmental samples in a timely and cost-effective manner (Wade *et al*., 2003; Yates, 2007). Coliform group density has been recognized as an important criterion of the degree of pollution and sanitary quality of water and wastewater. The significance of the tests and the interpretation of results are well authenticated and have been used as a basis for standards of bacteriological quality of water supplies (APHA, 1999). However, more recently, a variety of reasons suggest that *E. coli* and other faecal indicator bacteria may not always be

effective indicators for the presence of bacterial, viral, or parasitic pathogens. These organisms vary significantly with respect to a variety of factors that may influence their fate and transport in the environment, including the size of the microorganism, abundance in faeces, environmental fitness, and nature of hydrological processes that transport organisms to and within the aquatic environment (Ogden *et al*., 2001; Sadeghi & Arnold, 2002; Olyphant *et al*., 2003; Anderson *et al*., 2005; Yates, 2007). For example, in still waters, *Cryptosporidium* oocyst and bacterial concentrations decline at different rates partially due to variable settling rates (Brookes *et al*., 2005).

Commensal *E. coli* may acclimatize and proliferate in secondary habitats, whereas some pathogens may not (Byappanahalli *et al*., 2006). Evidently, it can be expected that the relationships between indicator bacteria and pathogens in faecally contaminated waters will be complex and variable (Payment *et al*., 2003; Yates, 2007).

Although traditional or culture-based tests are relatively inexpensive and reproducible, they are known to underestimate the total number of bacteria by up to several orders of magnitude (Amann *et al*., 1995; Sartory & Watkins, 1999), even with prolonged incubation times and temperature variations (Elzanfaly *et al*., 1998). It has long been documented that artificial culture media lead to only a very small fraction (0.01–1%) of the total viable bacteria present in any given sample (Watkins & Xiangrong, 1997). Furthermore, introduced bacteria can increasingly deteriorate in aqueous environments, with some initially able to grow on selective media, then only on non-selective media (stressed cells), and finally becoming non-culturable (viable but non-culturable [VBNC]) if still capable of causing infection (McFeters, 1990; Colwell *et al*., 1996; Alexandrino *et al*., 2004). Therefore, even the application of selective agents in any culture-based method, including those for pathogens, is expected to produce a considerable underestimation of the actual or "true" numbers of potentially infective bacteria present (Ashbolt, 2005). Additionally, the cultivation and analysis of indicator microorganisms is labour-intensive, time consuming and difficult (Lemarchand *et al*., 2005). Consequently, rapid, accurate and culture-independent alternatives are being investigated to facilitate monitoring of pathogens in water and wastewater (Straub & Chandler, 2003).

Advances in molecular biology over the past 20 years have resulted in a number of new detection methods that depend on the recognition of specific gene sequences. Such methods have resolved some of the problems encountered using conventional methods, and are usually more rapid and tailored to detect specific strains or groups of organisms. A molecular method, such as the polymerase chain reaction (PCR) has high specificity, speed and sensitivity in pathogen detection, with a detection limit of <10 copies of a specific gene present in a mixed sample (Call *et al*., 2001). Despite this, there are problems with PCR-based detection, including low, quantification, differentiation between live and dead cells, and the decrease in sensitivity and specificity caused by post-PCR analysis (Shannon *et al*., 2007). Some of the difficulties associated with PCR-based pathogen detection have been eliminated by real-time quantitative PCR (qPCR) technology. This technology is sensitive, specific, and yields accurate quantitative results (Guy *et al*., 2003; MacKay, 2004; Shannon *et al*., 2007; Böckelmann *et al*., 2009) with rapid detection of microorganisms and antibiotic resistant genes in water samples. The amplified PCR product is quantified using lasers to detect a DNA-specific probe throughout the PCR cycling process, thereby eliminating any post-PCR processing, and providing a much lower detection which is ideal for quantification (Guy *et al*., 2003). In order to monitor the efficiency of alternative wastewater treatment systems and ensure adequate removal concentrations of indictor organisms and bacterial pathogens as well as antibiotic resistant genes from the final effluent to receiving water bodies, the number of associated pathogenic bacteria and antibiotic resistant genes should be measured during various stages of treatment.

1.10 Antimicrobial resistance among indicator organisms and environmental pathogens

Faecal coliform bacteria are indicators of faecal contamination and of the potential presence of pathogens associated with wastewater or sewage sludge. Indicator organisms are typically used to demonstrate the potential presence or absence of groups of pathogens (Kator & Rhodes, 2003). *E. coli* is a useful enteric bacterium for the study of waterborne transfer of antibiotic resistance. It is adapted to human and other warm-blooded animal gastrointestinal tracts, and is exposed to a variety of medical and veterinary antibiotic treatments (Edge & Hill, 2005). *E. coli* and *Enterococcus* spp. are two organisms that have been used as indicators of faecal contamination (Kator & Rhodes, 2003), yet some pathogenic *E. coli* strains are able to transfer or

receive genes from other organisms, enhancing their resistance (Garcia *et al.*, 2007). *Enterococcus* spp. have become a common cause of nosocomial infections and also represents an important factor in the emergence of vancomycin resistant strains (CDC NNIS System, 2003). Vancomycin-resistant *enterococci* (VRE) cause significant human infections, including those of the urinary tract, wounds, bloodstream, and endocardium (Moellering, 1992; Jett *et al*., 1994; Shepard & Gilmore, 2002). An important characteristic of *enterococci* is their intrinsic resistance or their ability to acquire vancomycin resistance genes (Panesso *et al*., 2002; Shaghaghi *et al.*, 2007), of which *vanA* and *vanB* are the most common. *Enterococci* are also considered intrinsically "rugged" bacteria, and as such are able to survive under unusually wide ranges of temperature, pH, and salinity, as well as resisting the bactericidal effects of detergents such as bile salts and sodium dodecyl sulfate (Flahaut *et al*., 1996). The most common agents of the vast majority of clinical enterococcal infections in humans are *Enterococcus faecalis* and *Enterococcus faecium* (Mundy *et al.*, 2000). The emergence of *E. faecalis* and *E. faecium* as leading problematic nosocomial pathogens has paralleled the appearance of strains within both species resistant to most antimicrobial drugs used to treat human infections. The range of antimicrobial agents to which *enterococci* have acquired resistance is quite broad and appears to be escalating at a rate that closely approximates the introduction of new agents to the pharmaceutical market (Gonzales *et al*., 2001; Shepard & Gilmore, 2002).

Enteric Salmonella infection is a global problem both in humans and animals, and is considered to be the most important bacterial etiology for enteric infections worldwide (McCormick *et al*., 1993). The ability of *Salmonella* spp. to survive in a variety of aquatic environments, including streams and rivers, further compounds the threat of *Salmonella* arising from untreated wastewater originating from agricultural operations and human population effluents (Spector, 1998). For example, the high prevalence of this pathogen in fresh poultry presents a potential threat to human health through contamination of water bodies from poorly treated or untreated poultry-processing water (Burr *et al*., 1998). *Salmonella* spp. are also commonly found in wastewater and urban sludge, and have been studied mainly using culture-based methods (Gantzer *et al*., 2001; Godfree & Farrell, 2005). Compared to other bacteria, *Salmonella* spp. have high survival rates in aquatic environments and are able to withstand a variety of stresses, including thermal and pH fluctuation, high osmolarity and low nutrient availability (Chao *et al*.,

1987; Winfield & Groisman, 2003). *Salmonella* spp. have also been described as being more resistant than *E. coli* to biotic factors (microbial predators or competing organisms) in sources of drinking water (Wright *et al.,* 2000), perhaps due to a difference in adhesion to protective particles (Winfield & Groisman, 2003). Wéry *et al*., (2008) studied the behaviour of pathogenic and indicator bacteria during urban wastewater treatment and found *Salmonella* spp. had a greater capacity to survive biological treatment, compared to that of *E. coli*. A possible explanation could be due to the specific survival strategies that pathogenic bacteria have developed to survive when outside hosts or, more generally, ability to survive under stress (Wéry *et al*., 2008).

Antimicrobial resistance (AMR) is recognized as a global problem in human and veterinary medicine. To aid in the estimation of the AMR crisis and to follow its evolution, surveillance programs have been established in many countries worldwide, (Tollefson *et al*., 2008) Most surveillance programs focus on isolates acquired from infected humans, farm-animals and related food-products (CDC, 2007), but do not include an assessment of environmentally derived isolates. Unfortunately, few surveillance programs include specific pathogens from animals, and most are dedicated to assessing resistance phenotypes only. Frequently, a particular resistance phenotype may originate from several different genotypes (Chopra & Roberts, 2001; Sköld , 2001). A more promising approach looks at assessing the diversity and distribution of resistance genes in bacterial populations, which represents a more detailed and potentially useful tool for improving our understanding of AMR epidemiology (Boerlin *et al.,* 2005).

Over the last several decades, the focus of research has expanded beyond clinical settings and nosocomial infections, following the realization that resistant organisms are widespread throughout the environment. Resistant bacteria have been isolated from rivers and streams worldwide (Rhodes *et al*., 2000, Ash *et al*., 2002; Gordon *et al*., 2007; Stachowiak *et al*., 2009).

In order to improve our understanding of the dissemination of antibiotic resistance and antibiotic resistant genes among indicator organisms and environmental pathogens within aquatic environments, the study of the occurrence, distribution, antibiotic resistant bacteria profiles, and frequency of conjugal transfer amongst waterborne isolates is needed.

1.11 Radiation sensitivity D_{10}

Radiation sensitivity D_{10} is defined as the radiation dose (kGy) required to reduce the number of that microorganism by 10-fold (one log cycle) or required to kill 90% of the total number (Whitby & Gelda, 1979). When a suspension of a microorganism is irradiated at incremental doses, the number of surviving cell forming colonies after each incremental dose may be used to construct a dose survival curve (see figure 1.2). The relative sensitivity of different microorganisms to ionizing radiation is based on their respective D_{10} values (which is the dose required to reduce the population by 90%). Lower D_{10} values indicating greater sensitivity of the organism in question may be explained by multiple targets and/or certain repair processes being operative at low doses. Microbial cells, whether pathogenic or comprising the normal microflora of foods, exhibit differences in their responses to ionizing radiation.

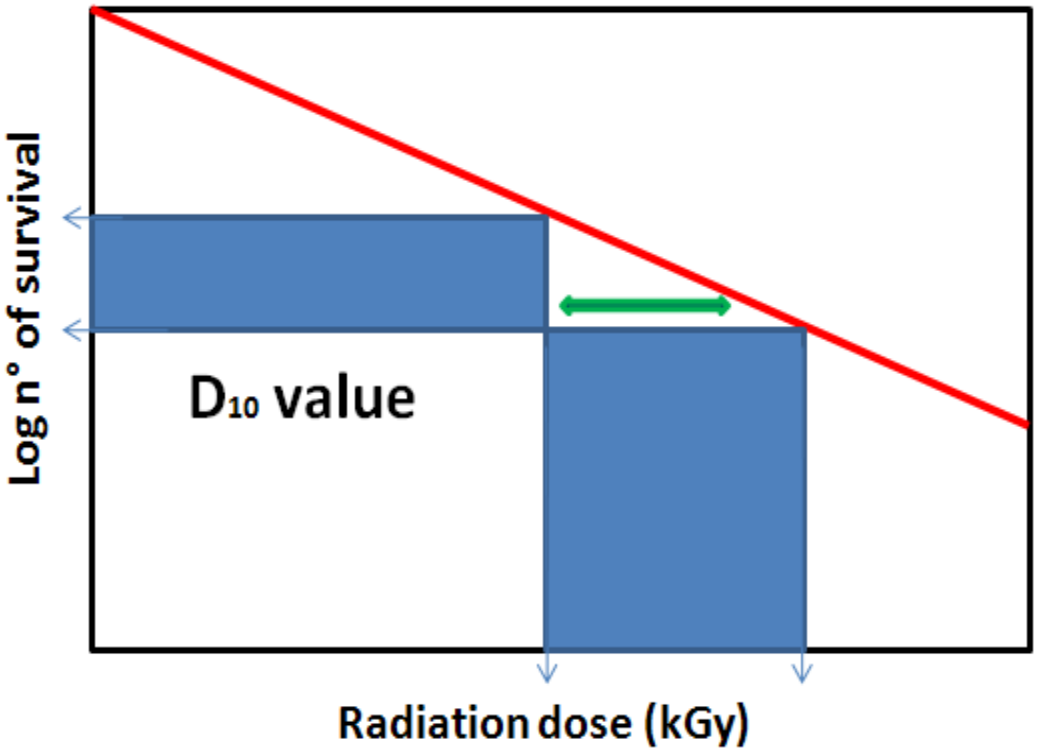

Figure. 1.2. Typical survival curve for a homogeneous microbial population

As ionizing radiation is a suitable method to control pathogenic bacteria in food, a large number of D_{10} values have yet been published (e.g. Buchanan & Doyle, 1997; Olson, 1998). Table 1.3 shows some published D_{10} values.

Table 1.3 Decimal reduction dose (D_{10}) of some microorganisms

Microorganism	D_{10} (kGy)	Disease	Reference
Salmonella typhimurim	0.30	Gastroenteritis	Borrely *et al.*, 1998
Mycobacterium tuberculosis	0.30	Tuberculosis	IAEA, 1975
Shigella dysenteriae	0.60	Dysentery	IAEA, 1975
Vibrio cholerae	0.48	Cholera	IAEA, 1975

1.12 The statement of problem, rationale, and motivation

Antibiotic resistance is a serious public health threat today. While the seriousness of the problem is now recognized, the complex web of resistance linking humans, and the environment is only beginning to be understood. Moreover stakeholders including physicians and government officials still know little about the problem, and the public remains in almost complete ignorance. Yet the progressive increase in antimicrobial resistance among enteric pathogens particularly *Shigella, Vibrio cholerae,* Enteropathogenic *E. coli, Salmonella typhi* and *Salmonella enteriditis* species are becoming a critical concern worldwide, particularly in the developing world where there are high rates of diarrhoeal diseases which are associated with mortality (Wright, *et al.*, 2009). This phenomenon is compounded by the fact that, data on resistance pathways and information are hard to find in Africa. Nevertheless, surveillance data are needed not only to define, update guidelines for empirical treatment, and as a guide for appropriate drug supplies but also for epidemiological analysis to inform health policy decisions.

The environmental health issues relating antibiotic resistances in Africa remains one of the least understood. Ironically, epidemics involving large numbers of individuals are prevalent in developing countries such as Ghana, due to a lack of potable water and flawed waste management, waterborne diseases hence, resistant waterborne infections.

A rigorous, coordinated surveillance effort, better models of transmission, rapid diagnostic tools, and standardized, easy-to-use susceptibility tests are all needed to help determine the patterns of

resistance in different parts of the world so that we may better treat patients and prevent new infections

Second, selection for antibiotic resistance is not confined to the human body or even to hospitals, clinics, and farms. Selection takes place anywhere an antibiotic is present, especially in natural environments, most notably sewage and surface water sediments, where antibiotics are likely to be coupled with high densities of various microorganisms. Although some work has been done on the total and faecal coliform status of selected water sources, the bacteriological profile of the bacteria populations associated with waters in terms of coliforms and their relations to the antibiotic resistance menace remains largely uninvestigated. However, antibiotic resistance in relations to members of the coliform groups found in water can be a local phenomenon, and in the absence of any reliable data, we cannot begin to address the problem.

Third, before any investigations can be carried out about the fate and transport of antibiotic resistant genes in water, it is necessary to characterize the occurrence, and identify major occurring bacteria in these water sources. The results of this will contribute to further our understanding of the impact of water treatment strategies on the expression of antibiotic resistance and pathogenic bacteria, more specifically among *E. coli* isolates.

Fourth, the emergence and spread of antibiotic-resistance threatens to make management of infections less effective and more expensive. Antibiotic resistance genes conferring resistance to a wide variety of antibiotics have been identified in a large range of water environments including wastewater, sewage, surface water, ground water and drinking water in both developed and developing countries. Water sources can act as a reservoir for antibiotic resistance – both in bacteria that normally reside in water sources as well as in faecal bacteria that are introduced into water sources due to human and animal use. Exposure to contaminated water sources (both direct consumption and contact) can lead to colonization and infection with resistant organisms. However, the relationship between resistance determinants in water sources and the classical clinical resistant microbes have not been studied in Ghana. However, this is essential for the institution of antimicrobial therapy, formulating strategies and policies to curb the antibiotic resistance challenge, and the implementation of infection control measures, thereby contributing

to the global efforts of controlling antibiotic resistance and to provide the foundation for a future study of role of the water sources in the transmission of antibiotic resistance in Ghana.

Fifth, considering the growing evidence that clinical resistance is closely associated with environmental antibiotic resistant bacteria (Tatavarthy *et al*., 2006; Prabhu *et al*., 2007; Abriouel *et al.*, 2008), additional research needs to be done to include nonpathogenic and environmental microorganisms. Further information is needed in respect of the transfer of bacterial resistance and environmental antibiotic resistant bacteria both within and between bacterial populations in water treatment systems. This information could lead to the modification or optimization of treatment processes, in order to target and enhance the removal efficiency of antibiotic resistant bacteria, resistance genes and associated virulence genes.

Sixth, One of the biggest obstacles to the judicious use of antibiotics in the developing world is identifying infectious organisms, since empiric therapy naturally results from the failure to pinpoint a pathogen and carry out susceptibility testing on it. Rapid and cheap methods that identify surrogate markers of bacterial and viral infections would be extremely helpful in prescribing antibiotics. Traditional methods of assaying water samples for the presence of pathogens rely on the culture of enteric bacteria (enterococci or coliforms) in conjunction with biochemical tests. These methods have several advantages: they are inexpensive, easy to perform, reproducible, and acceptable by those government agencies responsible for setting water quality standards. Molecular biology-based techniques such as PCR may offer distinct advantages in terms of sensitivity and specificity; however, one of the more critical factors in the use of PCR is the ability to provide a quality nucleic acid template free of any inhibitory substances (Wilson, 1997). However, not much research has been done in Ghana, comparing other detection methods with PCR to detect water borne pathogens such as *E. coli* let alone to characterize them. However there is an urgent need not only to characterize *E.coli* from water samples in order to compare them to worldwide clones, but also to recommend to stakeholders the specificity and sensitivity of the various detection methods to aid in decision making.

Finally, continual surveillance is necessary to understand the occurrence, fate, and mobility of antibiotic resistance among bacterial indicators of faecal contamination as well as pathogenic

bacteria within the various drinking water sources in Ghana. This information is essential for effective microbial source tracking and identification of public health risks and treatment options.

1.13 Research questions

Questions for the study are as follow:

a) What is the faecal and total coliform status of the drinking water sources in the Dangme West district?
b) What is the seasonal distribution of bacterial flora in the drinking water?
c) What are the prevalence and susceptibility profiles of antibiotic resistant water-borne *E. coli*?
d) What are the virulence genes associated with multiple *resistant E. coli* in the water sources ?
e) How does PCR, API 20E, and culture based laboratory methods compare in the detection of *E. coli?*
f) Is there an association between antibiotic resistance and radiation sensitivity (D_{10})?

1.14 Research objectives

1.14. 1 General objectives

The general aim of this study is to investigate the prevalence of multi antibiotic resistant *E. coli* and associated disease risk in the drinking water sources, and to determine the contamination pathways, aimed at identifying appropriate intervention methods to reduce peril of potential infections.

1.14.2 Specific objectives

To achieve the general objective, the following specific objectives were set as:

I. To determine the total and faecal coliform status of drinking water sources in the Dangme West District, as an indication of water quality.
II. To determine the bacteriological profile of bacteria flora in the drinking water sources.
III. To determine prevalence and susceptibility profiles of antibiotic resistant water-borne *E. coli*.

IV. To investigate the virulence genes associated multiple antibiotic resistant *E. coli* isolates, using a PCR based DNA STRIP technology.

V. To compare three laboratory based techniques (PCR, API 20E, and Culture based methods) used for detection of *E. coli.*

VI. To determine the association between multiple antibiotic resistance and radiation sensitivity (D_{10}).

1.15 The justification of the research

First, quality testing of drinking water is key to meeting water quality standards, guidelines, risk assessment, and management systems. The presence of microbial pathogens in polluted, untreated, and treated waters pose a considerable health risk to the public. (Feder *et al*., 2001; Stachowiak *et al*., 2010).

Second, Public health initiatives that address the ecological aspects of resistance, the environmental milieu, and patients' prior exposure to antibiotics is another key to preventing the selection and spread of resistance. However, these topics are not well understood and require a great deal of research such as this present study to further our understating on theses issues.

Third, surveillance should begin at the local level, collected, and compared at the national level. Data are currently lacking at the community level; continuous community sampling would be ideal. However to manage antibiotic resistance in particularly in developing countries such as Ghana, health officials need local profiles of resistance to the antibiotics that are available. The absence of which, we cannot begin to address the problem. The data emanating from this study will not only provide critical information needed in this regard, but also a better stratification of surveillance process.

Fourth, the need to provide baseline data describing the prevalence of resistance within bacterial populations and assess how this responds over time to changes in antibiotic use, are of great importance. In addition, changes due to interaction with other populations because of exchange of resistant bacteria or resistance genes between populations and the effects of interventions in terms of resistance epidemiology are needed to formulate effective strategies for treatment of

water-borne bacteria infections.

Fifth, the use of indicator bacteria such as *E. coli* is not only feasible to measure the selective pressure of antibiotic use in that population, but also to compare the prevalence of resistance in different populations and to detect transfer of resistance from one population to another.

Sixth, discovery of new routes of transmission, and the emerging waterborne pathogenic bacteria revealed a serious health hazard for both developed and developing countries. The use of molecular based technologies in microbial diagnostics has greatly enhanced the ability to detect and quantify pathogenic bacteria in water. Despite rapid diffusion of molecular tools in microbiology laboratories, there are still many drawbacks and obstacles concerning the use of these techniques in the developing world, let alone the challenge of specificity, reproducibility and reliability of nucleic acids and antibody based technologies for microbial detection.

Seventh, radiation processing has been used to improve the safety of food substances as well as in studies involving the use of pure isolates of bacteria. However, the effect of radiation on the antibiotic resistance of bacteria has not been extensively researched. This is particularly important when antibiotic resistance of inoculated bacteria is used as selective marker and to determine radiation doses at which multidrug resistant *E. coli* can be eliminated from drinking water.

Finally, there is the need to carry out messages about antibiotic resistance to the various stakeholders. In general, messages to the public need to include more science and less "noise" than they do now. Messages that patients and the public should hear about antibiotic resistance include: The risks to individuals. Without denying the benefits of antibiotic treatment in the right contexts, people should be made aware, for example, what the risks are from the antibiotic resistance patterns that are emerging. Outcomes of this study will provide information backed with reliable data to assist in the propagation of these messages.

1.16 Expected outcome

It is espected that the outcome of this research will provide useful information that could help inform policy towards effective management of rural water sources in Ghana. It will also provide essential information for the institution of antimicrobial therapy, formulating strategies and policies to curb the antibiotic resistance challenge, and the implementation of infection control measures, thereby contributing to the global efforts of controlling antibiotic resistance and to provide the foundation for future study of role of water sources in the transmission of antibiotic resistance in Ghana, sub-Saharan Africa and the world.

CHAPTER TWO
2.0 LITERATURE REVIEW AND THEORETICAL FRAMEWORK

2.1 Introduction

This chapter consists of the review of relevant literature to the thesis. The theoretical framework for the study is also presented in this chapter. Topical or thematic approach to literature review is adopted. Thus, the review is done in sections representing the categories or conceptual subjects for the topic under review.

In the preliminary work plan of this study a number of publications and data sources such as: PubMed, Africa Health line, UNISA E-data base, library catalogues and government reports were identified for review. The purpose of the review was to identify gaps in the subject area. Search terms such as: "antibiotics" "resistance" "water quality" "Africa" Sub-Saharan Africa" "Ghana" "antibiotics in water" etc. in different permutations was used. The second, task of the scope of the review included the compilation of a summary of published studies on the study topic and finally a discussion of the summarized review.

The literature search identified thousands of pertinent publications relevant to the current study. This was further narrowed down regionally, to Ghana. The breakdown of this literature is presented in table 2.1. The summary of the review (table 2.2) is presented in groups and in reverse chronological order, under the assumption that the more recent publications have greater relevancy to the study. Research publications reviewed were grouped into the following categories:

1) Bacteriological contamination of water and associated risk
2) Bacteria resistance to antibiotics
3) Bacteria detection methods and
4) Studies on radiation sensitivity (D_{10})

Subsequent analysis of the data obtained, resulted in the selection of twenty nine (29), published works considered most relevant to the current study. Theses published works were made up of

twelve (12) on studies on bacteriological contamination of water and associated risk. Eleven (11) on bacteria resistance to antibiotics, four (4) on methods of detection of antibiotic resistance bacteria and two (2) on studies on radiation sensitivity (D_{10}).

Table 2.1 Summary of published research materials on the subject under study

Demarcation	Number of published materials identified			
	Bacteria resistance to antibiotics	Bacteriological contamination of water and associated risk	Bacteria detection methods	Studies on radiation sensitivity (D_{10})
World wide	2882	2970	1600	300
Africa	800	750	87	10
Sub Saharan Africa	89	186	186	3
Ghana	18	35	12	2

There appear to be a scarcity of published materials of the subject matter in Ghana. Other sources such as health reports by Ministry of Health and other government reports had little or know information about the subject matter.

The theoretical framework for this study presented in section 2.6 of this chapter also reviewed a number of literature on antibiotic resistant modeling published from 1993 to 2013. This provided a basis and insight in conceptualizing the theoretical basis for this current study.

Table 2.2. Summary of publications on bacteriological contamination of water, antibiotic resistances, bacteria detection methods, and radiation sensitivity (D_{10}) in Ghana

Studies on bacteriological contamination of water and associated risk				
Author(s)	***Year***	***Title of study***	***Study objectives and methodology***	***Main findings/conclusions***
1. Odonkor & Ampofo	2013	*Escherichia coli* as an indicator of bacteriological quality of water: an overview	***Objective:*** To show that, *E. coli*, a member of the faecal coliform group, is a more specific indicator of faecal pollution than other faecal coliforms. ***Method:*** Review of the uses of indicators in water quality studies, to assess bacteriological contaminants in water.	The study concluded that *E. coli* appears to be the best indicator of bacteriological quality of water, primarily because of the, availability of affordable, fast, sensitive, specific and easier to perform detection methods for *E. coli*. However the fact remains that the life span of *E. coli* in water is short, thus it best determines, recent contaminations. It is therefore important that there is continuous monitoring for *E. coli* to determine the bacteriological quality of water.
2. Oyelude, *et al.*	2013	Quality of groundwater in Kassena-Nankana district, Ghana and its health Implications	***Objective***: To assessed the water quality of selected boreholes and hand-dug wells in the Kassena-Nankana District of Ghana. ***Method:*** Use of certified standard analytical methods to assess the potability of underground water from Kassena-Nankana District.	The water was hard with fluoride levels consistently lower than 0.5 mg/L. The mean physico-chemical quality of the water was average with borehole water slightly possessing better characteristics than water from hand-dug wells. Elevated levels of nitrite, nitrate, arsenic and lead were detected in few samples. The mean bacteriological quality of the underground water of the district was poor. The continuous consumption of the water without proper treatment may lead to health challenges such as: methaemoglobinaemia in infants, cardiovascular diseases, impaired renal function, impaired fertility, hypertension, and arsenic toxicity. It is therefore important for the water to be treated before consumption to prevent health challenges.
3. Machdara, *et al.*	2013	Application of Quantitative	***Objective:*** This study assessed in a densely populated area the risk from microbial	The major part of the burden of disease originated *from E. coli* O157:H7 (78%) and the least

	Microbial Risk Assessment to analyze the public health risk from poor drinking water quality in a low income area in Accra, Ghana	contamination of various sources of drinking water, ***Method:*** A Quantitative Microbiological Risk Assessment (QMRA) was conducted to estimate the risk of human health to microorganism exposure and dose–response relationships. Furthermore the cost-effectiveness in reducing the disease burden through targeted interventions was evaluated. Five risk pathways for drinking water were identified through a survey (110 families), namely household storage, private yard taps, communal taps, communal wells and water sachets. Samples from each source were analysed for *E. coli* and Ascaris contamination. Published ratios between *E. coli* and other pathogens were used for the QMRA and disease burden calculations.	important contributor was Cryptosporidium (0.01%). Other pathogens contributed 16% (Campylobacter), 5% (Rotavirus) and 0.3% (Ascaris). The sum of the disease burden of these pathogens was 0.5 DALYs per person per year, which is much higher than the WHO reference level. The major contamination pathway was found to be household storage. Disinfection of water at household level was the most cost-effective intervention (< 5 USD/DALY-averted) together with hygiene education. Water supply network improvements were significantly less cost-effective.
4. Tiimua & Adu Gyamfi	2013 Potable Quality Determination of Groundwater from Point Collection Sources in the Asante Mampong Municipality of Ashanti Region in Ghana	***Objective:*** To determine the potable quality of groundwater from point collection sources in the Asante Mampong Municipality of Ghana . ***Methodology:*** Groundwater was collected from 4 actively used hand pump boreholes and dugout wells for analyses using various standard laboratory methods (APHA, AWWA, WEF, 1998) to determine some physical, chemical and biological quality effects of the groundwater.	The study recorded average values of 5.95, 355.55 (µS/cm), 3.225 (NTU) and (7.565 and 0.0225) mg/L for pH, conductivity, turbidity, DO, TSS in the groundwater. The differences in levels of BOD5, TDS, NO3-N, Chloride and NO2-N were highly significant (P<0.000) even though Fe and ammonia did not show significant differences. Detection of these chemicals in drinking water has several implications, particularly in evaluating potential human health effects or ecological outcome; though the levels detected in their study were within the permissible limits proposed in the 2006 WHO guidelines for drinking water quality. Significant differences in total coliforms and

				Salmonella contamination were observed to have negative health implications. Stakeholder discussion is mandatory to partnering appropriate solutions to the groundwater quality problems.
5.	Omari & Yeboah-Manu	2012	The Study Of Bacterial Contamination Of Drinking Water Sources: A Case Study Of Mpraeso, Ghana	***Objective:*** The study aimed at determining the presence, type, count and causes of bacterial contamination of water used for drinking and other domestic purposes in Mpraeso. ***Method:*** Fifty-four (54) water samples (48 from 8 groundwater wells and 6 from a stream) were collected and analyzed for six months	The results showed that groundwater and surface water sources were polluted. The detection of bacterial cells in the water sources means that some forms of treatment needed to be done before consumption. The mean count of total coliform and faecal coliform ranged from 299 - 2267 MPN colonies/100 ml water sample and 111 – 1235 MPN colonies/100 ml water sample, respectively. For the groundwater sources, the *enterobacteriaceae* species detected were *Escherichia coli* (8 wells), *Enterococcus faecalis* (8 wells), *Klebsiella pneumoniae* (6 wells),*Enterobacter cloacae* (5), *Pseudomonas aeruginosa* (3), and *Proteus mirabilis* (3). All these bacteria species were detected in the surface water samples.
6.	Obiri-Danso &, Ephraim.	2011	Microbial quality of water in Barekese reservoir and feeder streams in Ghana	***Objective:*** To study the microbial quality of Barekese reservoir, the main drinking water reservoir for the city of Kumasi and it's environs (population = 2.5 million), and nine feeder streams in its catchment ***Method:*** Water samples were collected from 13 sampling sites and analysed for *Escherichia coli*, total and faecal coliforms. Furthermore, 18 faecal coliform isolates were randomly selected from all sampling sites and confirmed as *E. coli* using Analytical Profile Index 20E system. Escherichia coli, total and	Mean bacterial indicator numbers from all sampling sites ranged from 1.45×104 to 9.50×107 100 mL−1 for total coliforms, 1.60×103 to 9.00×105 100 mL−1 for faecal coliforms and 1.50×101 to 9.50×103 100 mL−1 for *E. coli.* Indicator numbers exceeded the World Health Organization (0.100 mL−1) for *E. coli* and Ghana Water Resources Commission-Target Water Quality Range of 5–100 100 mL−1 for total coliforms and 0.100 mL−1 for faecal coliforms in water used for domestic purposes. The identified isolates in Barekese Reservoir and its feeder streams belonged

			faecal coliforms were enumerated using the most probable number method.	to *Serratia*, *Enterobacter*, *Citrobacter*, *Salmonella* and *Klebsiella* genera. Bacterial numbers were significantly ($P < 0.05$) higher in the feeder streams, compared with Barekese Reservoir water. This finding indicates the feeder streams pose health risks to local communities that withdraw water from them. The results of this study highlight the urgent need to raise public awareness on the adverse effects of water-quality degradation through improper waste disposal methods in order to decrease the cost of treating the reservoir water.
7.Boamah, *et al.*	2011	Microbial Quality of Household Water Sources and Incidence of Diarrhoea in three Peri-Urban Communities In Kumasi, Ghana	***Objective:*** To assess the physicochemical and microbiological quality of the sources of household water supply and the prevalence of diarrhoea in three peri-urban communities in Kumasi, Ghana ***Method:*** Nine water sources, four from hand dug wells and five from boreholes, were identified, sampled and analysed for physicochemical (colour, turbidity, total dissolved solids, conductivity, and acidity) and microbiological characteristics.	The total dissolved solids and conductivity values determined were within the WHO limit but the turbidity and colour of two of the water sources exceeded the WHO limits. All the water sources were acidic and did not meet the WHO specification for pH for drinking water. Faecal coliforms and faecal streptococci were consistently present in the water sources at levels signifying human pollution. Diarrhoea cases among children < 5 years reported at the various health facilities within the studied communities increased during periods when high levels of microbial contamination in drinking water sources were recorded. Provision of potable household water should continue to be the priority of governments of developing countries, especially those in sub-Saharan Africa, to reduce the prevalence of water-borne diseases.
8. Anim, *et al.*	2010	Coliform status of water bodies from two districts in	***Objective:*** the study was undertaken to investigate the coliform status in water available to typical rural communities in	It was observed that, the total coliform values obtained ranged from 60–2,672 cfu, 0–680 cfu and 0–128 cfu for streams, hand-dug wells and

	Ghana, west Africa: implications for rural water resources management	Ghana, with a view to providing useful information that could help inform policy towards effective management of rural water resources. ***Method:*** Investigations were conducted on the total and faecal coliform status of streams, hand-dug wells and boreholes highly patronised by communities in the West Akim and Kwaebibirem Districts, Eastern Ghana.	boreholes, respectively. Faecal coliform values similarly range from 31–1,988 cfu, 0–136 cfu and 0–36 cfu for streams, hand-dugs and boreholes, respectively. The data indicates that samples from streams and hand-dug wells returned high coliform counts beyond the Maximum Permissible Levels (MPL) recommended by the World Health Organization (WHO). Some borehole water contained coliform counts above MPL. Because many rural people in the areas studied, and indeed in most areas in Ghana, depend heavily on these principal sources of water for drinking and other domestic purposes, a policy response that incorporates periodic assessment of water quality in rural communities may be appropriate.
9. Nkansah, *et al.* 2010	Assessment of the Quality of Water from Hand-Dug Wells in Ghana	***Objective:*** To assess the quality of water from hand-dug wells in the Kumasi metropolis, Ghana. ***Method:*** Investigations were conducted on the total and faecal coliform status of streams, hand-dug wells and boreholes highly patronised by communities in the West Akim and Kwaebibirem Districts, Eastern Ghana.	Total coliform values obtained range from 60–2,672 cfu, 0–680 cfu and 0–128 cfu for streams, hand-dug wells and boreholes, respectively. Faecal coliform values similarly range from 31–1,988 cfu, 0–136 cfu and 0–36 cfu for streams, hand dugs and boreholes, respectively. The data indicates that samples from streams and hand-dug wells returned high coliform counts beyond the Maximum Permissible Levels (MPL) recommended by the World Health Organization (WHO). Some borehole water contained coliform counts above MPL. Because many rural people in the areas studied, and indeed in most areas in Ghana, depend heavily on these principal sources of water for drinking and other domestic purposes, a policy response that incorporates periodic assessment of water quality in rural communities may be appropriate. In addition, it would seem that current

			government and development partner programmes in the water resources sector that emphasise provision of borehole water must be integrated with land use considerations, water quality monitoring and education on environmental awareness in local communities.
10. McGarvey, 2008 *et al.*	Community and household determinants of water quality in coastal Ghana	***Objective:*** To examine associations between social and demographic characteristics, water sources, sanitation factors and household drinking water quality in a representative sample of residents of the six coastal districts of Ghana's Central Region. ***Method:*** Thirty-six enumeration areas (EAs) were randomly chosen from a representative survey of 90 EAs in rural, semi-urban and urban residence strata. In each EA, 24 households were randomly chosen for water quality sampling and socio-demographic interview. Escherichia coli per 100 ml H_2O was quantified using the IDEXX ColilertR system and multi-stage regression models estimated cross-sectional associations between water sources, sanitation and socio-demographic factors.	It was observed that, almost three quarters, 74%, of the households have > 2 *E. coli* /100 ml H_2O. Tap water has significantly lower *E. coli* levels compared with surface or rainwater and well water had the highest levels. Households with a water closet toilet have significantly lower *E. coli* compared with those using pit latrines or no toilets. Household size is positively associated, and a possessions index is negatively associated, with *E. coli*. Variations in community and household socio-demographic and behavioral factors are key determinants of drinking water quality. These factors should be included in planning health education associated with investments in water systems.

11.Obiri Danso, *et al.*	2005	Aspects of health-related microbiology of the Subin, an urban river in Kumasi, Ghana.	***Objective:*** To assess the influence of urban waste, sewage and other human centred activities on the microbiological quality of the river Subin, this flows through the metropolis of Kumasi, Ghana, and serves as drinking water for communities downstream. ***Method:*** Three sites, Racecourse, Asafo and Asago, on the Subin were monitored over a year for total coliforms, faecal coliforms, enterococci and biochemical oxygen demand.	Bacterial indicator numbers (geometric mean 100ml−1) varied from 1.61×109 to 4.06×1013 for total coliforms, 9.75×108 to 8.98×1012 for faecal coliforms and 1.01×102 to 6.57×106 for enterococci. There was a consistent increase in bacterial loading as the river flows from the source (Racecourse) through Kumasi. Bacterial numbers were significantly ($p \leq 0.05$) higher during the rainy season compared with the dry (harmattan) season. The biochemical oxygen demand ranged from 8mgl−1 at the source of the river to 419mgl−1 at Asago; none of the sites achieved internationally accepted standards for water quality. The River Subin becomes grossly polluted as it flows through Kumasi and at Asago, a rural community downstream of Kumasi that abstracts water from the river for drinking; this probably contributes to the observed high levels of disease.
12. Ampofo, J.	1997	A survey of microbial pollution of rural domestic water supply in Ghana.	***Objective:*** To determine the occurrence and levels of microbial pollution in three major rivers in Ghana- the Birim river, Kakum river and the Nakwa river. ***Method:*** Routine bacteriological examinations were made to determine the levels of total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS). Results obtained showed that TC, FC and FS counts were high in all the three rivers thus making them unsafe to drink untreated water from those sources. Eight of the 10 stations sampled on the Birim river had their source of pollution from human waste while two stations had a	Maximum levels of 4448 TC/100 ml, 1848 FC/100 ml and 214 FS/100 ml were obtained in the Birim river. Two of the seven stations sampled on the Kakum river had their source of pollution from human waste with one station having a predominance of human waste in a mixed source of pollution. Two stations had their source of pollution from livestock and poultry and the other two from predominance of livestock and poultry in a mixed pollution. Livestock and poultry source dominate pollution in the Nakwa river, with two stations recording purely livestock and poultry source and two other stations showing mixed pollution with livestock and poultry source dominating. Two stations showed human source of

			livestock source of pollution.	contamination. There was no correlation between TC and FS levels on one hand, and FC and FS levels on the other hand, at 95% confidence limit. There was significant correlation, however, between TC and FC levels. High socio-anthropological activities in the basins of the three rivers were identified as a contributory factor to the later situation.
Studies on bacteria resistance to antibiotics.				
1.Gyansa-Lutterodt, M.	2013	Antibiotic resistance in Ghana	***Objective****:* To review the current antibiotic resistance in Ghana ***Method****:* Review of general issues on antibiotics in Ghana.	Monitoring of antibiotic use is poor, with nascent surveillance systems and scarce reporting of treatment failures. Weak regulatory systems generally allow free movement of goods within the Economic Community of West African States. In this setting, the potential negative effects of substandard, spurious, falsely labeled, or falsified counterfeit medical products cannot be over emphasized, but information about such products is generally difficult to obtain or almost unavailable. In Ghana the capacity to link results of laboratory diagnostic tests to selection of medicines is lacking, and immediate action is needed to ensure value for money.
2. Duredoh, *et al.*	2012	Antibiotic Resistance Patterns of *Escherichia coli* Isolates from Hospitals in Kumasi, Ghana	***Objective:*** To determine the antibiotic resistance patterns of *Escherichia coli* isolated from Kumasi-South, Tafo and Suntreso Hospitals, Kumasi, Ghana. ***Method:*** A Total of 600 swabs samples of the hospitals' floors, benches, beds, door handles, and waste water from drainages were collected between January and June, 2010. Microbes were cultured on MacConkey agar plates and using	Out of the total 97 *E. coli* isolates obtained from the hospitals. Beds in hospital wards had the highest number of *E. coli* strains (53.6%), followed by floors (20.6%) while drainages had the least isolates (3.1%). Majority of the *E. coli* isolates (90.7%) exhibited resistance to ampicillin while 6.2 and 3.1% showed intermediate and sensitive respectively. Co-trimoxazole, 78.4% of the isolates were resistant while 9.3 and 12.4% exhibited intermediate and sensitive responses respectively.

			morphological and biochemical means for identification, colonies of *E coli* were isolated and sub cultured. To confirm, further microscopic examinations and biochemical reactions including indole, oxidase, and arginine dehydrolase production, citrate utilization, nitrite reduction, fermentation of carbohydrates and methyl red-Voges Proskauer test, were carried out. Finally, antibiotic Sensitivity test was done using the Kirby-Bauer disc diffusion method	*E. coli* isolates (28.6 to 46.4%) were resistant to gentamicin, ciprofloxacin and ceftriaxone while 14.4 to 47.4% gave intermediate responses. Most isolates (80.4%) exhibited multi-drug resistance. There is a need to observe proper personal hygiene, use of effective disinfectants and proper disposal of contaminated/pathogenic materials in these hospitals to control nosocomial infections.
3. Tagoe, *et al.*	2011	A study of antibiotic susceptibility pattern of bacteria isolates in sachet drinking water sold in the Cape Coast metropolis of Ghana	***Objective:*** To determine the antibiotic susceptibility pattern of bacterial isolates in sachet water sold on the street of Cape Coast Metropolis of Ghana. ***Method:*** By Random sampling technique 11 different sachet waters were obtained from 11 different vendors in Cape Coast Metropolis of Ghana bi-monthly for six months. A volume of each sample was added to an equal volume of bacteriological peptone water, incubated for 24h at 37^{o}C and streaked onto Plate Count Agar. All pure isolates were sub-cultured aerobically onto blood and MacConkey agars for differential purposes. Kirby-Bauer modified disc diffusion method was used in determining antibiotic sensitivity pattern of brands with different bacterial isolates including *E.coli*, Coagulase negative *Staphylococcus*, *S. aureus*, *E. faecalis*, *K. aerogenes*, *M.*	The degree of resistance of isolates showed 100% resistance to ampicillin, flucloxacillin and penicillin, while none of them was resistant to gentamycin. The resistance to other antibiotics ranged from 93.3% for erythromycin and Cefuroxime, 60% for co-trimoxazole and 20% for tetracycline. The results indicate the presence of antibiotic resistant bacteria in sachet water consumed in the metropolis with its attendant potential health.

		catarrhalis, B. cereus, L. monocytogenes and *Enterobacter* sp. etc.	
4. Namboodiri, 2011 *et al.*	Quinolone resistance in *Escherichia coli* from Accra, Ghana	***Objective:*** to determine the prevalence of resistance to broad-spectrum antimicrobials with particular focus on the quinolones ***Method:*** *E. coli* isolates were recovered from stool specimens collected from individuals who presented for medical check-ups at the Korle-Bu Teaching Hospital and the Microbiology Department of the University of Ghana Medical School. isolate was tested for susceptibility to eight antimicrobials using the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) disc diffusion method. Minimum inhibitory concentrations (MICs) to nalidixic acid were measured using the agar dilution technique on Mueller-Hinton agar as recommended by the CLSI with *E. coli* ATCC 35218 as control. Mutational analysis of the Quinolone-Resistance Determining Regions of gyrA and parC DNA was extracted from each quinolone-resistant isolate, using the Promega Wizard genomic extraction kit. The QRDR of the gyrA and parC genes were amplified from DNA templates by PCR using Platinum PCR supermix (Invitrogen) and the primer pairs. Horizontally-acquired quinolone-resistance genes were identified by PCR and multi-locus sequence typing was	Forty (13.7%) of the 293 *E. coli* isolates evaluated were nalidixic acid-resistant. Thirteen (52%) of 2006 and 2007 isolates and 10 (66.7%) of 2008 isolates were also resistant to ciprofloxacin. All but one of the quinolone-resistant isolates was resistant to three or more other antimicrobial classes. Sequencing the quinolone-resistance determining regions of gyrA and parC, which encode quinolone targets, revealed that 28 quinolone-resistant *E. coli* harboured a substitution at position 83 of the gyrA gene product and 20 of these isolates had other gyrA and/or parC substitutions. Horizontally-acquired quinolone-resistance genes qnrB1, qnrB2, qnrS1 or qepA were detected in 12 of the isolates. In spite of considerable overall diversity among E. coli from Ghana, as evaluated by multilocus sequence typing, 15 quinolone-resistant *E. coli* belonged to sequence type complex 10. Five of these isolates carried qnrS1 alleles. In conclusion, Quinolone-resistant *E. coli* are commonly present in the faecal flora of Accra residents. The isolates have evolved resistance through multiple mechanisms and belong to very few lineages, suggesting clonal expansion. Containment strategies to limit the spread of quinolone-resistant *E. coli* need to be deployed to conserve quinolone effectiveness and promote alternatives to their use.

			carried out.	
5. Odonkor *et al*.	2011	Antimicrobial sensitivity patterns of urine isolates from a large Ghanaian hospital	***Objective:*** To investigate the pathogen prevalence and antimicrobial susceptibility patterns of the most prevalent pathogen isolated from urine samples of some patients at a large Ghanaian hospital. ***Method****:* Urine culture and sensitivity results were compiled and analyzed from laboratory record books of a large Ghanaian hospital from 2009 to 2010. Within this period, a total of 3774 pathogens were isolated comprising 3510 bacteria and 264 yeast isolates.	The most prevalent isolated pathogens were *Escherichia coli* (25.5%), *Klebsiella* (19.2%) and *Staphylococcus aureus* (18.6%). Other isolates included other *Coliform* spp (9.4%), *Streptococcus sp* (8.2%), *Pseudomonas* (7.1%), *Candida alibicans* (7%) and *Proteus* spp (4.9%). Only 9.3, 9.5 and 6.4% of *E.coli* was susceptible to ampicillin, pipemedic acid and cotrimoxazole respectively. Susceptibility of *E.coli* to nitrofuranton and cefuroxime was 18.5% and 18.3% respectively. Susceptibility to gentamycin, tetracycline, and nalidixic acid, were 15.4, 11.4 and 11.1% respectively.
6.Newman, *et al*.	2011	Resistance to antimicrobial drugs in Ghana	***Objective****:* To identify the agents of bacterial infection in Ghana, determine their antibiogram, and the possibility of setting up a surveillance program. ***Method****:* A prospective quantitative study set in various hospitals including two teaching hospitals, seven regional hospitals, and two district hospitals in Ghana. A total of 5099 bacterial isolates from various clinical specimens were collected over a period of 1 year, including data related to the patients. Susceptibility of the isolates was determined by the Kirby–Bauer method. In addition, the minimum inhibitory concentration (MIC) of multidrug-resistant isolates of epidemiological significance was also determined using the E-test.	A wide range of bacterial isolates were identified in both teaching and regional hospitals. High percentage of resistance was observed for tetracycline (82%), cotrimoxazole (73%), ampicillin (76%), and chloramphenicol (75%). Multidrug resistance was observed to a combination of ampicillin, tetracycline, chloramphenicol, and cotrimoxazole. On the other hand, a lower percentage of resistance was observed for ceftriaxone (6.3%), ciprofloxacin (11%), and amikacin (9.9%). Conclusively, the prevalence of multidrug resistance was widespread among the various isolates. Some multidrug-resistant strains of *Staphylococcus aureus*, *Salmonella typhi*, and non-typhoidal *Salmonella* (NTS) had high MIC to cefuroxime (.256), gentamicin (.256), and ciprofloxacin (.32).
7. Grob , *et al.*	2011	Bacteremia and	***Objective:*** To study antibiotic resistances	Although Ghana implemented several measures to

		Antimicrobial Drug Resistance over Time, Ghana	in Ghana over time. ***Method:*** A retrospective study in selected hospitals in Accra	control typhoid, the study found that, depending on the region, *S. enterica* serovar *typhi* remains the most prevalent bacterial species causing BBSI. This finding is in agreement with a recent study from the Ashanti region, where 12.4% of BBSI were caused by *S. enterica* serovar *Typhi*. In addition, emergent ciprofloxacin resistance has been described in Accra, the capital of Ghana. Therefore, the implementation of bacteriologic diagnosis should be considered even in smaller hospitals in a rural African setting to monitor pathogen distribution and resistance rates.
8.Eddoh & Alomatu.	2008	Comparison of antibiotic resistance patterns between Laboratories in Accra east, Ghana.	***Objective:*** To determines the distribution of antibiotic resistant microbes in two different laboratories and compares the results using statistical methods to ascertain if there is a variation due to human factors. ***Method:*** Patients attending two laboratories in east Accra were recruited and samples obtained from them were cultured for microbial growth. Microbes isolated were characterized and their sensitivity to different antibiotics tested. A total of 513 samples were collected from the patients who were mostly females; 68%. The samples that were mostly infected were urine (331), wound (116), HVS (78) and ear (26). There were few cases of throat, blood and uterus samples that were infected. spp.	Microbial isolates common in the different laboratories included *S. aureus* (96), *E. coli* (90), *Pseudomonas aeruginosa* and *Proteus*. Microbes isolated in the different laboratories were *Salmonella typhi*, *Shigella spp*. and *Streptococcus pneumoniae*. Microbial isolates from the two hospitals were found resistant (over 65%) to the antibiotics ampicillin (71% and 95%), cotrimoxazole (68% and 75%) and tetracycline (70% and 80%) and moderately resistant to gentamycin (29% and 23%), erythromycin (39% and 36%) and streptomycin, and sensitive to ceftazidine and minomycin. Statistically the results from the different laboratories were found to be similar hence having the same trend.
9.Djie-Maletz, *et al.*	2008	High rate of resistance to locally	***Objective:*** To determine the susceptibility of bacterial enteric pathogens and faecal	From the study, *salmonellae* showed an acceptable resistance pattern however, *E. coli* isolates and the

		used antibiotics among enteric bacteria from children in Northern Ghana	*Escherichia coli* isolates obtained from children in urban Tamale, Northern Ghana, to antibiotics widely used in the that area. ***Method:*** Five Shigella spp., 6 *Salmonella* spp. and 318 *E. coli* were isolated from stool specimens obtained from 367 children with or without acute diarrhoea. Isolates were differentiated using standard laboratory procedures and tested using a breakpoint micro broth dilution method for their susceptibility to 18 antimicrobials and by disc diffusion for their susceptibility to chloramphenicol.	closely related *shigellae* were highly resistant. About 91% and 81% of *E. coli* isolates from patients or controls, respectively, were resistant to ampicillin (MICs-8 mg/L), 88% and 76% to trimethoprim/sulfamethoxazole (MICs _ 80/4 mg/L) and 46% and 41% to chloramphenicol (inhibition zones -12 mm). Resistance to b-lactam antibiotics or chloramphenicol was observed more frequently among isolates obtained from infants when compared with older children (1–4 years of age). The conclusion to this study was that enteric bacteria from children in urban Northern Ghana are highly resistant to antibiotics used in that area. Therefore, new antibiotics should be introduced for the treatment of infections caused by these bacteria. Additionally, the establishment of a surveillance of the prevalence of the main bacterial infectious agents and their antimicrobial resistance is desirable.
10. Iruka, *et al*.	2007	Growing Problem of Multidrug-Resistant Enteric Pathogens in Africa	***Objective:*** To identify low-cost, high-impact interventions for resistance control ***Method:*** Review on antibiotic situations in Ghana and Africa as a whole	Interventions focused on relatively inexpensive and easily piloted resistance containment measures require safe water and sanitation, addressing the imbalance between antimicrobial drug supply and demand improvements in infectious disease control, access to and quality assurance of antimicrobial agents, as well as diagnostic facilities. Structural improvements along these lines will also enhance disease prevention and control as well as rational antimicrobial drug use. Additionally, more research is needed
11. Newman & Seidu.	2002	Carriage of antimicrobial resistant	***Objective:*** To determine the antibiotic resistance patterns of *Escherichia coli* isolated form intestinal flora	106 strains of *Escherichia coli* were examined, 68% of these were resistant to tetracycline, and 57% were resistant to ampicillin and cotrimoxazole

		Escherichia coli in adult intestinal flora.	***Method:*** Frequency of resistance to eleven different antimicrobial agents was examined in faecal flora of adults with no history of recent antimicrobial treatment using the disc diffusion sensitivity test.	respectively. There was no resistance to cefuroxime but resistance to ceftazidime was 13%. Fifty six out of the eighty eight (64%) isolates, which showed any resistance, were resistant to three or more antimicrobials. The most common resistant pattern was to three drugs tetracycline, ampicillin and cotrimoxazole. Six strains were susceptible to all antibiotics. One strain of *Escherichia coli* was resistant to eight antimicrobials. Thirty per cent of the *Escherichia coli* were resistant to gentamicin. This study reveals a high prevalence of resistant bacteria in faecal flora of healthy adults.
Studies on bacteria detection methods				
1. Hackman, *et al.*	2013	Phenotypic Characterization of AmpC beta-lactamase among Cefoxitin Resistant *Escherichia coli* and *Klebsiella pneumoniae* Isolates in Accra, Ghana	***Objective:*** To investigate the occurrence of AmpC beta-lactamases-producing phenotypes in *E. coli* and *K. pneumoniae* and their antimicrobial sensitivity profile. ***Method:*** *K. pneumonia* and *E. coli* were collected from the Central Laboratory of the Korle Bu Teaching Hospital (KBTH) and Advent Clinical Laboratories; both in the Accra Metropolis, Ghana. After which, identification of Bacterial Isolates, Determination of Minimal Inhibition Concentration (MIC) and Antibiotic Sensitivity were conducted.	After studying the bacterial isolates and AmpC beta-lactamases-producing phenotypes among the 400 bacterial isolates, 50 were resistant to cefoxitin of which 29 were *K. pneumonia* and 21 were *E.coli*
2. Dayie, *et al*.	2013	Penicillin resistance and serotype distribution of *Streptococcus pneumoniae* in Ghanaian children less than six years	***Objective:*** To determine the prevalence of nasopharyngeal carriage, serotype distribution, and penicillin resistance of *Streptococcus pneumoniae* in children ≤6 years of age in Ghana. ***Method:*** A cross-sectional study was carried out on a cluster-randomized sample	The overall prevalence of pneumococcal carriage among the children was 34% in Accra and 31% in Tamale. The predominant serotypes were 19F, 6B, 23F, and 6A with 23% of the isolates being non-typable in Accra and 12% in Tamale. Only two isolates (serotypes 19F and 6B) from Tamale had a MIC >2 μg/ml and were classified as fully

		of age	of children ≤6 years of age attending nurseries and kindergartens in Accra and Tamale, Ghana. Basic data on age, sex and exposure to antimicrobials in the previous month were collected on all study subjects. Nasopharyngeal swabs were obtained from participants and all pneumococcal isolates were characterized by serotyping and their penicillin resistance determined.	penicillin resistant with 45% of the isolates having intermediate resistance. These findings indicate that the 13-valent pneumococcal conjugate vaccine (PCV-13) recently introduced in Ghana will cover 48% and 51% of the serotypes identified in Accra and Tamale, respectively. The 23-valent pneumococcal polysaccharide vaccine (PPV-23) will cover 54% of all serotypes detected. The two penicillin resistant isolates (MIC 32 μg/ml) were serotypes included in both PCV-13 and PPV-23. A nationwide monitoring system of penicillin susceptibility patterns and pneumococcal serotypes is recommended.
3. Odonkor & Addo	2011	Evaluation of Three Methods For Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA)	**Objective**: To evaluate 3 methods for detection of Methicillin resistance in *S. aureus* and to determine β lactames production profile of MRSA using the MRSA screen assay as gold standard. **Method:** Two hundred and fifty (250) *S. aureus* isolates from routine microbiological specimen were collected from hospitals in Accra, Ghana between July and December 2010 for the study. The isolates were obtained from cultures of different specimens including wounds, sputum, blood, aspirates, urine, and cerebrospinal fluids. Methicillin disc diffusion (5μg),Oxacillin disc diffusion (5μg), β lactamase test (using nitrocefin disc) and MRSA screening assay test were performed on the isolates.	Out of the 250 isolates the methicillin disc diffusion test detected 54 representing 21.6% as MRSA positive and 196 representing 78.4% as negative. Oxacillin disc diffusion test detected 78 (31.26%) to be MRSA and 172(68.8%) as negative. The MRSA screen assay detected 84(33.6%) as MRSA and 166(66.4%) as negative. One hundred and fifty eight of the isolates produced β-lactamase. All phenotypic methods had significant differences in sensitivity and specificity for detection of MRSA. However, MRSA screen assay method in comparison to other methods had higher specificity and sensitivity for detection of MRSA in resource constraint settings.
4. Donkor, *et al.*	2008	Antimicrobial susceptibility of	**Objective**: To determine the antibiogram of *Salmonella typhi* and *Staphylococcus*	The prevalence of multiple drug resistance as determined on Mueller-Hinton agar was 83.3%

		Salmonella typhi and *Staphylococcus aureus* isolates and the effect of some media on susceptibility testing results	*aureus* isolates and to quantify the effect of Nutrient and Tryptone Soy agars on susceptibility testing results. **Method:** The Kirby Bauer method was used to evaluate the susceptibility of 30 isolates each of *Salmonella typhi* and *Staphylococcus aureus* to various antimicrobial agents on Mueller-Hinton agar (recommended medium). Subsequently, the susceptibility testing procedure was repeated on the isolates using Nutrient and Tryptone Soy agars which are commonly used in Ghana but not recommended for the Kirby Bauer method.	for *Salmonella typhi* and 80% for *Staphylococcus aureus*. For *Salmonella typhi*, resistance ranged from 6.7% (gentamicin and amikacin) to 83.3% (cotrimoxazole, ampicillin and chloramphenicol). In the case of *Staphylococcus aureus* resistance ranged from 16.7% (erythromycin and gentamicin) to 93.3% (penicillin).
Studies on radiation sensitivity (D_{10})				
1. Mahami *et al.*	2012	Radiation sensitivity of Listeria monocytogenes planktonic and biofilm-associated cells	**Objective**: to investigate the relative Susceptibilities of planktonic versus biofilm cells of *Listeria monocytogenes* on glass to gamma radiation.. **Method:** The planktonic and biofilm cultures were subjected to incremental gamma radiation doses (0, 0.5, 1.0, 1.5 , 2.0 kGy) from a Cobalt-60 source. The D_{10} values were calculated from the linear regression model for the logarithm of the surviving fraction and irradiation dose	The D_{10} value of L. monocytogenes planktonic cells (0.476KGy) was higher than that of biofilm-associated cells (0.379KGy) indicating biofilm cells were more sensitive to ionizing radiation than planktonic cells. The antimicrobial efficacy of ionizing radiation is therefore preserved or enhanced in the treatment of biofilm-associated bacteria.
2. Adu-Gyamfi *et al.*	2012	Microbiological Quality of Chicken Sold in Accra and	**Objective**: 1) To investigate the microbiological quality of chicken, at different retail outlets, namely	Mean total coliform counts for the supermarkets, local markets and farms were 3.80, 3.46 and 3.14 log10 cfu/g respectively and the mean *S. aureus*

	Determination of D_{10}-Value of *E. coli*	supermarkets, local markets and on farms in Accra. 2) To determine the D_{10}-value (decimal reduction dose) of *Escherichia coli* in refrigerated and frozen retailed chicken... **Method:** The microbiological quality of chicken was studied by analyzing 27 chicken thigh samples collected from the retail outlets. D10-value of *Escherichia coli* was determined by using a linear regression model after gamma irradiation of inoculated chicken samples with doses of 0, 150, 300, 450, 600, 750 and 900 Gy	counts were also 2.32, 2.28 and 2.70 log10 cfu/g respectively. There were no significant differences ($p > 0.05$) between the mean total viable count, total coliform counts and *S. aureus* count for the supermarkets, local markets and the farms. Mean counts of *E. coli* detected at the supermarket, local markets and farms were 1.27, 2.59 and 2.74 log10 cfu/g respec- tively. *Salmonella* spp. was detected in 2 out of the 27 samples. Fifty-two percent and 70% of samples respectively had total viable counts and total coliform counts within the microbial safety standards. Mean D_{10}-values of *E. coli* were 0.22 and 0.32 kGy in refrigerated and frozen chicken respectively

2.2 Bacteriological contamination of water and associated risk

Safe water is one of the most important felt needs in public health in the twenty first century (Sobsey & Bartram, 2003). At least 884 million people in the world still do not get their drinking water from improved sources; majority of these are from developing regions. Sub-Saharan Africa accounts for over a one third of that number, and is lagging behind in progress towards the MDG target, with only 60% of the population using improved sources of drinking-water (WHO, 2010). The major health threat posed by drinking unsafe water is infectious diseases. In fact, diseases are the leading causes of mortality and morbidity for children under the age of 5 and it is estimated to cause 1.5 million deaths annually in developing countries (WHO, 2010).

In Ghana, a number of studies have been carried out to determine the microbial contamination of water sources and the potential to cause disease. Oyelude *et al.,* (2013) and Timua & Adu (2013), assessed the ground water quality in the Kassena-Nankana district of Ghana and the Asante Mampong municipality of Ghana respectively. Their studies focused primarily on the bacteriological quality of ground water. Whereas the findings of the former was observations of significant differences in total coliforms and potential health implications of *Salmonella*, the later in their study concluded that the mean bacteriological quality of the underground water of the district was poor. Thus continuous consumption of the water without proper treatment may lead to health challenges. These studies like many others carried out in Ghana were done with emphasis on a single source of water in selected areas. However the potential exposures to risk of water borne diseases, especially to rural communities are not limited to ground water sources only (Shittu *et al.,* 2008). These findings though important do not give a holistic picture of the water borne threats faced by rural communities by which, policy can effectively be informed. Additionally, these studies were not carried out across the seasons in Ghana, that is dry season and rainy season, of whose importance cannot be over emphasized, in understanding the seasonal drift of infections and contamination pathways.

Machdara *et al.,* (2013) in their study entitled "Application of Quantitative Microbial Risk Assessment to analyse the public health risk from poor drinking water quality in a low income

area in Accra, Ghana" assessed in a densely populated area the risk from microbial contamination of various sources of drinking water. The sources were mainly treated with exception of water from community wells. The emphasis of their study was geared at the assessment of *Escherichia co*li and *Ascaris* contamination. Their study came to a conclusion that major part of the burden of disease originated from *E. coli* O157:H7 (78%) and the least important contributor was *Cryptosporidium* (0.01%). However, their study did not determine the virulence genes that, where associated with the major part of the diseases burden originating from the *E. coli* O157:H7. The importance of the use of *E. coli* as an indicator organism in their study is worth noting. Indeed a numbers of studies and reviews have recognized the preferences of *E. coli* as the best indicator of water quality (Odonkor & Ampofo, 2013).

However the fact remains that the life span of *E. coli* in water is short, thus it only best determines, recent contaminations (WHO, 2003; Le-Chavallier, & Au, 2004; Gerald, 2011; Odonkor & Ampofo, 2013). It is therefore important that there is continuous monitoring for *E. coli* to determine the bacteriological quality of water. Moreover, in order obtain, a complete, picture and fill the gaps from theses studies, a complete bacteriological profile of bacteria flora in water would therefore be essential. Second, just as in the majority of published studies reviewed subsequently, in this study, it is evident that little or no emphasis was placed on the identification of risk pathways, which is essential for water quality assessment and recommendations (Omari & Yeboah-Manu, 2012).

A number of studies (Ampofo, 1997, Obiri-Danso *et al.*, 2005, Nkansah *et al.*, 2010, Anim *et al.*, 2010) have also investigated the quality of some surface water sources in Ghana. Water-borne gastrointestinal infections remain one of the major causes of morbidity and mortality worldwide (WHO, 2003). These water-borne gastrointestinal infections are primarily dominant in surface waters. The most important microbes causing infections or epidemics through drinking water include bacteria: *Campylobacter* spp, *Escherichia coli, Salmonella spp., Shigella* spp., *Vibrio cholera, Yersinia enterocolitica,* viruses such as: adeno-, entero-, hepatitis A- and E-, noro-, sapo- and rotaviruses and protozoa: *Cryptosporidium parvum, Dracunculus medinensis ,Cyclospora cayetanensis , Entamoeba histolytica ,Giardia duodenalis* and

Toxoplasma gondii (WHO, 2004). Studies in this direction is therefore of great significance and importance.

However, the various microbiological studies of surface water done in Ghana, have generally been to determine the microbial contamination of the water sources with emphasis on coliforms assesment

Anim, *et al.* (2010) investigated the coliform status in water available to typical rural communities in Ghana. Their study also sought to compare results obtained from the surface water sources with ground waters sources. They observed that, the total coliform values obtained ranged from 60–2,672 cfu, 0–680 cfu and 0–128 cfu for streams, hand-dug wells and boreholes, respectively. Faecal coliform values similarly ranged from 31–1,988 cfu, 0–136 cfu and 0–36 cfu for streams, hand-dug wells and boreholes, respectively. In a related study Obiri-Danso, *et al.* (2005) sought to assess the influence of urban waste, sewage and other human centred activities on the microbiological quality of the river Subin. They found a consistent increase in bacterial loading as the river flows from the source (Racecourse) through Kumasi. Bacterial numbers were significantly ($p \leq 0.05$) higher during the rainy season compared with the dry (harmattan) season.

Previously, Ampofo (1997) had perfomed a microbial assemenst of selected rivers. His study determined the occurrence and levels of microbial pollution in three major rivers in Ghana: the Birim, Kakum and Nakwa rivers. Among conclusions from his study were observations of significant correlation, between total coliform bacteria and faecal coliform bacteria levels. A noteworthy finding from his study was the identification of high socio-anthropological activities in the basins of the three rivers as a contributory factor to the levels of total coliform bacteria and faecal coliform observed form the study.

Remarkably, McGarvey *et al.,* (2008) examined associations between social and demographic characteristics, water sources, sanitation factors and household drinking water quality in a representative sample of residents of the six coastal districts of Ghana's Central Region. The inferences showed that, almost three quarters, 74%, of the households have > 2 *E. coli* cfu per 100 ml. However, the pathways for the contaminations of the water appeared not to have been

investigated yet.

Historically, large water-borne cholera epidemics with numerous casualties in the mid-1800s and the early investigations of cholera epidemics in London by John Snow (1813-1858) and the works of Robert Koch (1843-1910) on *V. cholerae* have remarkably assisted the understanding of the epidemiology and prevention of water-borne diseases (Brock, 1999). Worldwide, *V. cholerae* is still a significant cause of water-borne infections, especially in developing countries where most of the victims are children under five years old (WHO, 2003). However, the relevant published studies reviewed had focused largely on total and faecal coliform counts. Without attention to the other kinds of pathogenic bacteria that may be present in these water sources. Consequently, the season distribution of bacteria flora across seasonal shift in the drinking water sources is unknown. Furthermore, there is no data to form the basis of comparison with other countries, let alone to observe the changing trends of the concentration of these water borne bacteria.

The published studies in Ghana reviewed by this current study, appears largely aimed at determining the microbial contamination of selected water sources. However, it is not known how the various water sources (both ground and surface waters) relate in terms of faecal and total coliform status. Furthermore, most of these studies seldom investigated coliform status across seasons (wet and dry season). This is very much needed in tracking contamination sources as well as anthropogenic influences. Moreover, most of the studies had looked at the presence of microbes in the population, while providing little or no information on the routes by which these bacteria contaminates the water source. Furthermore, the diseases risk assessments of the various water sources were not carried out in most cases.

2.3 Bacteria resistance to antibiotics

Ghana's 24.5 million people face a double disease burden of communicable and non-communicable diseases equally causing morbidity and mortality. In addition to the wide spread cases of water-borne diseases resulting from the contamination of water sources, concerns have been raised when these diseases fail to be cured due to development of resistance to commonly prescribed antibiotics by the contaminating microorganisms originating from livestock feaces

and human sewage (Prescott & Baggot 1993). Now-a-days, antibiotic resistant bacteria species are ubiquitous in the environment and their negative impact has greatly increased (Mathew *et al.,* 2007). Improper antibiotic use and lack of awareness are considered as the most important factors for the emergence, selection, and dissemination of antibiotic resistant bacteria species in the environment (Neu, 1992). Modern food animal production depends on the use of large amounts of antibiotics for disease control and agricultural purposes, particularly for growth enhancement.

These provide favorable conditions for selection, spread and persistence of antimicrobial-resistant bacteria capable of causing infections in animals and humans through food chain (ETAG, 2005; Mathew *et al.,* 2007). As a result, when water bodies are contaminated with faecal matter containing antibiotic resistant bacteria, they could serve as a source and reservoir of antibiotic resistant genetic elements. This could possibly be transferred to other bacteria species and make the quality of water even very hazardous for health (Biyela & Bezuidenhout, 2004) and also create a favorable condition for the spread of antibiotic resistant genetic element from place to place (APUA, 1999).

In Ghana today, a number of studies (Newman & Seidu, 2002; Iruka *et al.,* 2007; Eddoh & Alomatu, 2008*;* Newman *et al.,* 2011; Duredoh, *et al., 2012*; Dayie, *et al*., 2013) have been done to investigate antibiotic resistance. These studies can clearly be identified under two broad categories, retrospective studies and laboratory bases experiments. However, virtually all of these studies have concentrated on clinical isolates. This accounts for the plenty information on clinical isolates we have to day, in contrast to the little information available on environmental isolates.

Newman *et al.,* (2011), undertook a retrospective quantitative study set in various hospitals in Ghana to identifying the agents of bacterial infection in Ghana, their antibiogram, and the possibility of setting up a surveillance program. Their study revealed high percentage of resistance for tetracycline (82%), cotrimoxazole (73%), ampicillin (76%), and chloramphenicol (75%). Multidrug resistance was also observed to a combination of ampicillin, tetracycline, chloramphenicol, and cotrimoxazole. On the other hand a lower percentage of resistance was

observed for ceftriaxone (6.3%), ciprofloxacin (11%) and amikacin (9.9%).

In a similar study by Odonkor *et al.,* (2011) carried out to investigate the pathogen prevalence and antimicrobial susceptibility patterns of the most prevalent pathogen isolated from urine samples of some patients at a large Ghanaian hospital, *E. coli* was found to be susceptible to ampicillin, pipemedic acid and cotrimoxazole. Susceptibility of *E. coli* to nitrofuranton and cefuroxime was 18.5% and 18.3% respectively. Susceptibility to gentamycin, tetracycline, and nalidixic acid, were 15.4%, 11.4%, and 11.1% respectively.

In yet another retrospective study by Eddoh & Alomatu (2008), the authors set out to compare antibiotic resistance patterns obtain between laboratories in Accra. Microbial isolates were found to be resistant to a number of antibiotics. However the results from the different laboratories were found to be similar.

Few studies (Newman & Seidu 2002; Djie-Maletz, *et al.,* 2008; Duredoh, *et al.,* 2012) have investigated antibiotics resistances among *E. coli* isolates in Ghana. A handfull have also looked at antibiotic resistance among enteric bacteria (Iruka, *et al.,* 2007; Djie-Maletz, *et al.,* 2008). According to Murray *et al.,* (1999), the pathogenic enteric bacteria included organisms such as *Salmonella* spp, *Shigella* spp, *Vibrio* spp and *Escherichia coli.* Until the 1940s only *Salmonella* and *Shigella* were considered as gastrointestinal pathogens of medical importance in the family *Enterobacteriaceae.* It is now a well-established fact that *E. coli* is a significant cause of diarrhoeal illnesses both in infants and adults in many parts of the world (Schierack *et al.,* 2009; Kinge *et al.,* 2010). The importance and relevance of theses studies done in Ghana can therefore not be over emphasized.

Duredoh *et al.,* (2012) studied the antibiotic resistance patterns of *Escherichia coli* isolated from Kumasi-South, Tafo and Suntreso Hospitals, Kumasi, Ghana. Though in a clinical setting the isolates were obtained from floors, benches, beds, door handles, and wastewater from drainages, rather than clinical isolates. Their study showed that majority of the *E. coli* isolates (90.7%) exhibited resistance to ampicillin while 6.2% and 3.1% showed intermediate and sensitive respectively. They concluded that (80.4%) exhibited multi-drug resistance. In a similar study

Namboiri *et al.,* (2011) determined the prevalence of resistance to broad-spectrum antimicrobials with particular focus on the quinolones. They found that Quinolone-resistant *E. coli* is commonly present in the faecal flora of Accra residents. They also postulated that the isolates have evolved resistance through multiple mechanisms and belong to very few lineages, suggesting clonal expansion

The focus of another study carried out by Djie-Maletz, *et al.,* (2008) was aimed at the determination of the susceptibility of bacterial enteric pathogens and faecal *E. coli* isolates obtained from children in urban Tamale, Northern Ghana, to antibiotics widely used. Their conclusion was that enteric bacteria from children in urban Northern Ghana are highly resistant to antibiotics used in that area. Similarly, Newman and Seidu (2002) sought to investigate antibiotic resistance patterns of *Escherichia coli* isolated form intestinal flora. Their study revealed a high prevalence of resistant bacteria in faecal flora of healthy adults.

Studies on antibiotic resistances of *E. coli* have been done from different angles in Ghana, but have all primary been in the clinical settings. Little information is available on the prevalence of antibiotic resistance within indicator and pathogenic microorganisms in water sources or in natural wetland systems. The only study in Ghana that appears close to investigating resistance in water was, the one done by Tagoe *et al.,* (2011). In that study, they investigated the antibiotic susceptibility pattern of bacteria isolates in sachet drinking water. They concluded that degree of resistance of isolates showed 100% resistance to ampicillin, flucloxacillin and penicillin, while none of them was resistant to gentamycin.

In many countries today conscious efforts are being made to investigate antibiotic resistance in the environment particularly in water sources. As result, some data is available in some African countries in this regard (Bahiru *et al.,* 2013). However, the same cannot be said of Ghana. Thus, there is no data or adequate information about the extent to which antibiotic resistant bacteria have contaminated different environments in Ghana. It is worth noting that this phenomenon is not peculiar to Ghana alone. As a result, globally, data on clinical isolates is plenty while less attention has been given to environmental isolates (Sharma *et al.,* 2010). Samples from the environment such as water, soil, meat, vegetables, and dry foods are probable reservoirs of these

pathogens. Studying antimicrobial resistance in humans and animals is important in order to detect changes in patterns of resistance, implement control measures on the use of antimicrobial agents, and to prevent the spread of multidrug-resistant strains of bacteria (Duijkeren *et al.,* 2003).

Furthermore, most antibiotics are not fully eliminated during sewage treatment process. Thus, aquatic environment such as rivers or streams could act as an antibiotic resistant genes reservoir and facilitate the dissemination of these genes (Kummerer, 2004; Lupo *et al.,* 2012). The emergence of antimicrobial resistance mechanisms, especially those associated with mobile genetic elements, may enhance the possibility that virulence genes and antibiotic resistance genes are spread simultaneously, inducing the emergence of new pathogens (Chen *et al.,* 2011; Da Silva & Mendoça, 2012; Koczura *et al.,* 2012).

In conclusion, it is evident that a clear cut fissure exist: this is because the progressive increase in antimicrobial resistance among enteric pathogens particularly *Shigella, Vibrio cholerae,* Enteropathogenic *E. coli, Salmonella typhi* and *Salmonella enteriditis* species are becoming a critical concern worldwide, particularly in the developing world where there are high rates of diarrhoeal diseases which are associated with mortality (Fricke *et al.,* 2008; Sang *et al.,* 2011). Yet there is no surveillance data on resistance patterns, sources, and risk factors associated *E. coli* isolates from the water sources. This is needed to define or update guidelines for empirical treatment, as well as a guide for appropriate drug supplies. Furthermore, the study of faecal indicators, such *E. coli* has dominated studies of MAR bacteria in water because of the association of these indicators with disease-causing genera of importance to public health and hygiene. However little work has been done to assess the MAR index value of *E. coli* isolates from different drinking water sources nor to determine virulence genes (such as stx1, stx2, eae gene and IpaH genes) associated with multiple *resistant E. coli* in Ghana.

2.4 Methods of detection and Characterization of antibiotic resistance bacteria.

It should be noted that although speed is of great importance in detecting resistance, accuracy is paramount and must not be sacrificed. For example, falsely labeling an organism as susceptible to a given antibiotic can have disastrous consequences for patients. An error that overstates

resistance is more acceptable than an error that calls an organism falsely susceptible. It is therefore important to compare methods, to determine their specificity and sensitivity.

A review of published data in relationship to detection methods of resistant bacteria in Ghana is trifling. Hackman *et al.,* (2013) studied the phenotypic characters of AmpC beta-lactamase among cefoxitin resistant *E. coli* and *Klebsiella pneumonia*. They concluded that 50 isolates out of 400 were resistant to cefoxitin of which 29 were *Klebsiella pneumonia* and 21 were *E. coli*. Similarly Dayie, *et al.,* (2013), investigated penicillin resistant *Streptococcus pneumonia* in Ghanaian children using stereotyping methods. Their study concluded that, the overall prevalence of pneumococcal carriage among the children was 34% in Accra and 31% in Tamale. The predominant serotypes were 19F, 6B, 23F, and 6A with 23% of the isolates being non-typable in Accra and 12% in Tamale.

In a similar study Donkor *et al.,* (2008) used Kirby-Bauer method to compare the use of two laboratory media for susceptibility testsing. They evaluated the susceptibility of 30 isolates each of *Salmonella typhi* and *Staphylococcus aureus* to various antimicrobial agents on mueller-hinton agar. They concluded that overall, the discrepancy in susceptibility results between nutrient agar and that of mueller-hinton agar was 8.9% while discrepancy between tryptone Soy agar and mueller-hinton agar, and was 17.2%. Their study recommended the discontinuos use of nutrient and tryptone Soy agars for the Kirby Bauer method as practiced by some laboratories in Ghana.

Odonkor & Addo (2011) in a study done on methicillin resistance in *S. aureus* (MRSA) in Accra-Ghana evaluated 3 methods of detection of Methicillin resistance in *S. aureus* and also determine β lactames production profile of MRSA using the MRSA screen assay as gold standard. Out of the 250 isolates, the methicillin disc diffusion test detected 54 - representing 21.6% - as MRSA positive and 196 - representing 78.4% - as negative. Oxacillin disc diffusion test detected 78 (31.26%) to be MRSA and 172 (68.8%) as negative. The MRSA screen assay detected 84 (33.6%) as MRSA and 166 (66.4%) as negative. Their study concluded that all phenotypic methods had significant differences in sensitivity and specificity for detection of

MRSA. However, MRSA screen assay method in comparison to other methods had higher specificity and sensitivity for detection of MRSA in resource constraint settings.

In the past few decades the methods for the identification of bacteria particularly members of the family enterobacteriaceae in both clinical and reference laboratories have undergone major changes. Rapid progress has been made, from conventional tubed biochemical through miniaturized biochemical panels and plates (Washintong *et al.*, 1971; MacCarthy *et al*., 1978; Rhoden *et al.*, 1987) to semi automated systems (Costigan & Hollick, 1984, Pfaller *et al.*, 1986), all of which render identifications in 18 to 21 hours. A few systems exist that give identifications in 5 to 8 hours (Altwegg, 1983; Keville & Doern, 1984). Today's technology allows some fully automated systems to yield answers in 2 to 4 hours with the use of fluorogenic compounds and fluorometric analysis (Nucera *et al.*, 2006).

Review of the work done on detection methods of resistant bacteria has to the large extent been hinged on phenotypic characterization. Phenotypic methods, just as traditional methods of bacterial detection, are effective but can take hours or days to show results and may miss most types of bacteria. There are newer, more rapid methods of bacterial detection being developed to streamline the process and increase rates of detection. However as it stands now, we do not know how various methods compare in the detection of resistant *E. coli*, in terms of specificity and sensitivity.

2.5 Studies on radiation sensitivity (D_{10})

In Ghana today very few published research are available on radiation sensitivity of microorganism. Radiation has been used to improve the safety of food substances as well as in studies involving the use of pure isolates of bacteria. Mahami *et al*., (2012) sought to investigate the relative susceptibilities of planktonic versus biofilm cells of *Listeria monocytogenes* on glass to gamma radiation. Result from their study demonstrated that ionizing radiation effectively reduced the populations of both planktonic and biofilm-associated *L. monocytogenes.* The study further showed that, in contrast to chemical antimicrobial treatments, the antimicrobial efficacy of ionizing radiation is preserved or enhanced when treating biofilm associated bacteria.

In a similar study Adu Gyamfi *et al*., (2012) they determined the D_{10} value (decimal reduction dose) of *Escherichia coli* in refrigerated and frozen retailed chicken. This study was done along side the investigation of the microbiological quality of chicken, at different retail outlets. The study generally confirmed the presence of pathogenic bacteria of public health importance in fresh chicken sold in in Accra. Their study observed low D_{10} values of *E. coli* especially under refrigerated conditions. This suggests susceptibility to low dose irradiation and the possibility of controlling spoilage and pathogenic microflora of fresh poultry.

Radiation sensitivity (D_{10}) is known to be a virulence factor. For example, the radiation sensitivity of three strains of *Escherichia coli* O157:H7 were found to increase after being induced to the antibiotic nalidixic acid (Niemira, 2005). However previous work, studied the radiation sensitivity only in relation to decimal reduction of bacteria but not to antibiotic resistance. Generally the effect of radiation on the antibiotic resistance of bacteria has not been extensively researched worldwide. This is particularly important when antibiotic resistance of inoculated bacteria is used as selective marker. For example, bacteria resistant to the antibiotic nalidixic acid (NalR) have recently been validated for use as a marker in studies of chemical interventions and for use in food (Niemira, 2005). Furthermore, the radiation sensitivity of *E. coli* isolates from water has not been determined. This is critical to further our understanding of radiation doses that will be required to eliminate water borne *E. coli* from water for safe public consumption. In addition, we do not know the relationship between multi antibiotic resistant *E. coli* and radiation sensitivity. This information is needed to provide baseline knowledge for future research in the application of radiation sensitivity to the control of multi antibiotic resistant *E. coli*.

2.6. Theoretical framework of the study

Antimicrobial resistance (AMR) presents a major and growing threat to effective treatment of bacterial infections. For almost a century antimicrobials have been used to control bacterial infections and disease in humans and animals. However, with increasing microbial resistance to these drugs, despite current interventions, we face a return to nineteenth century levels of morbidity (Levy & Marshall, 2004; Hawkey, 2008; Chan, 2011). Hitherto, investigation and

policy development for the control of AMR using surveillance data have focused largely on patterns of resistance to individual antimicrobials (Threlfall *et al.,* 2003; Skjøt-Rasmussen *et al.,* 2009) Quantitative studies have concentrated on theoretical frameworks using simulated and in vitro experimental data (Levin *et al.,* 2000; Smith *et al.,* 2002; Bergstrom *et al.,* 2004), while much of our current understanding of the impacts of individual antimicrobials has been derived from small scale clinical epidemiological studies (Weber *et al.,* 2003; Jiang *et al.,* 2006).

Only recently, has the broader ecological landscape occupied by the bacteria and their hosts been considered (D'Costa *et al.,* 2006; Singer *et al.,* 2006, Martınez, 2008). Novel approaches may bring new perspectives on the origins and spread of AMR, or assist in the development of new or revised targeted interventions. The use of antimicrobials in agriculture as a major driver of AMR in pathogenic bacteria of significance to humans is an issue over which opinions are divided (Davis, *et al*., 1999; Phillips *et al*., 2004; Mølbak, 2004; Angulo *et al.,* 2004; Wassenaar, 2005). The prophylactic and metaphylactic use in animal populations has been a particular concern (Klare, *et al.,* 1999; Aarestrup *et al.,* 2001), especially when the drug classes are the same as or related to the pharmaceuticals used in the control of human infections. Exposure of microbial populations to antimicrobials evidently selects for resistance; however, the critical and unresolved issue is the relative contribution to resistance in these populations from the different host communities.

In conceptualizing an assumption for this study (Figure 2.1), a number of models were reviewed. It is important to note that mathematical modeling has played an integral role in improving our understanding of antibiotic resistance (Opatowski *et al.,* 2011). These models provide a platform for experiments that help us better understand mechanistic sources of uncertainty, generate refined hypotheses, and interpret empirical observations. Models make empirical experimentation more efficient, and experiments can be conducted at reduced cost and avoids ethical dilemmas. Within-host models have focused on:

1) Emergence of antibiotic resistance (D'Agata *et al.,* 2008).

2) Microbial fitness and selection for antibiotic-resistant strains compared with antibiotic-sensitive strains (Levin, *et al.,* 2000; De Gelder *et al.,* 2004).

3) Antibiotic tolerance (Levin *et al.,* 2006).

Population-level models have been used to better understand and predict the effects of treatment protocols such as antibiotic cycling (Bonhoeffer *et al.,* 1997). Published models of the emergence and spread of antibiotic resistance have progressed from resistance models of abstract agents in abstract settings (Massad *et al.,* 1993; Austin *et al.,* 1997) to resistance models of specific agents such as *Mycobacterium tuberculosis* (Blower & Chou, 2004), *Streptococcus pneumoniae* (Temime *et al.,* 2003), *Staphylococcus aureus* (Bergstrom *et al.,* 2004) and *Enterococcus* (Smith *et al.,* 2002) in more specified settings, such as transmission between hospitals (Bootsma *et al.,* 2006), within hospitals and intensive care units (Hotchkiss *et al.,* 2005) and within day-care centers (Andersson *et al.,* 2005).

However, all models of antibiotic resistance include competition between antibiotic-sensitive and antibiotic-resistant strains. To model this competition, a wide array of model structures have been used, each reflecting distinct biological mechanisms of competition, but model structure is rarely justified. In order to establish a sound theoretical framework, this study reviewed the published literature on antibiotic-resistance modeling published from 1993 to 2013.

Having reviewed these models on the influence, emergence, and maintenance of resistance in human pathogens (Mølbak, 2004; Angulo *et al.*, 2004; Wassenaar, 2005) three critical questions arise. First, are the resistance phenotypes in human and environmental microbial populations the same, and do they arise from common or distinguishable microbial communities? Second, in which microbial population are the resistances and resistance profiles common to both animals and humans first identified? Third, can we identify the most probable origins of all resistance phenotypes recovered from human and environmental populations, and thereby infer the directionality of resistance transmission in these microbial communities?

These studies therefore conceptualize a simplified model (figure 2.1). This was done with an assumption that selection for antibiotic resistance is not confined to the human body or even to hospitals, clinics, and farms. Selection takes place anywhere an antibiotic is present, especially in natural environments, most notably sewage and surface water sediments, where antibiotics are likely to be coupled with high densities of various microorganisms.

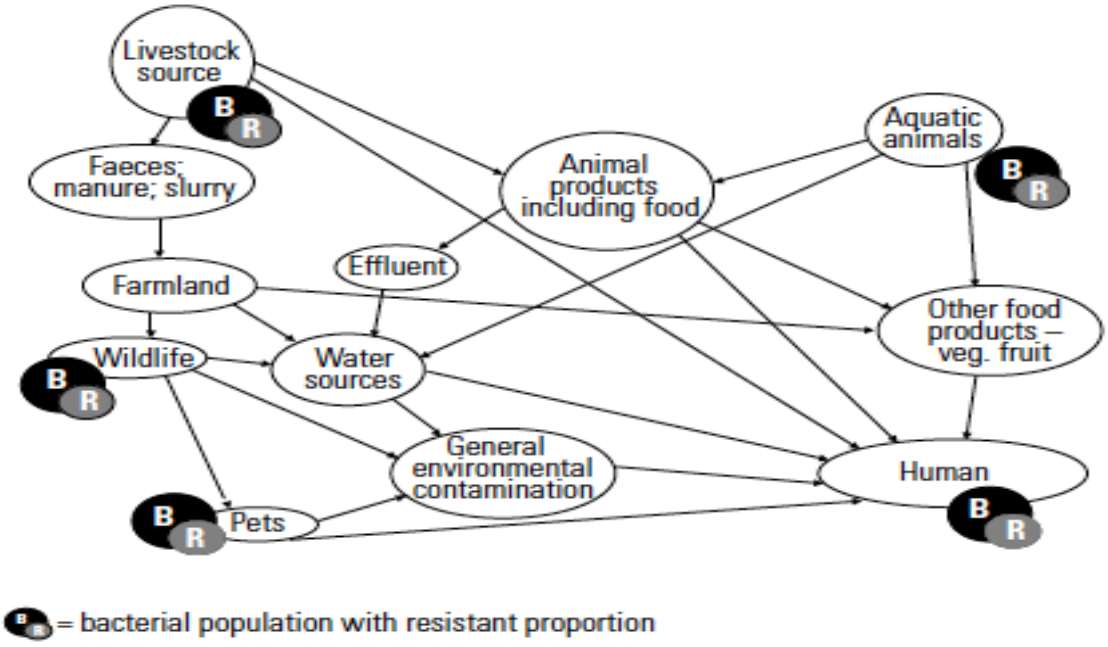

Figure 2.1 Potential route of bacteria transfer within any resistant population

Although a model cannot represent all the complexity of real life or capture all the factors that contribute to the transmission of antibiotic resistance, we can develop valid general predictive models of particular situations like to predict spread from person to person and quantitative prediction of the impact of antibiotics on resistance spread. For example the potential routes of transferring bacteria, with any population ranging from animals to humans, such as the model conceptualize by this study, as presented in figure 2.1

The development of an appropriate model addressing antibiotic resistance from an ecological perspective requires, field data collected from naturally infected hosts occupying their natural ecological niches, and characterized using the same microbiological techniques where we consider the full spectrum of multiple resistant phenotypes. This is important because models that rely on laboratory strains to estimate the transferability of conjugative plasmids might be misleading, because laboratory strains do not possess the virulence factors, restriction enzymes, and other elements that contribute to the spread of resistance.

Large quantities of antibiotics, like tetracycline, florphenicol, and flumequine, are used in aquaculture - an application that considers the continuity between farmed waters, recreational

waters, beaches, and possibly drinking water sources has undoubtedly had an impact on human health. The opportunity for rampant lateral gene transfer in aquatic environments is undeniable. The research on the impacts of antibiotic use on development of resistances in the environment is scant and deserves more attention.

CHAPTER THREE

3.0 RESEARCH DESIGN AND METHODOLOGY

3.1 Introduction

This chapter describes the research design and methodology used to provide data to investigate the research questions. The research was executed in three phases: Phase 1 involved gathering of information of drinking water sources in the study area, sampling sites observations, water sampling, coliforms and *E. coli* counts analysis and phenotypic characterization of bacteria. Phase II involved antimicrobial susceptibility testing, molecular characterization of *E. coli* and the determination of virulence genes associated with multiple antibiotic resistant *E. coli.* Phase III involved determination of the radiation sensitivity (D_{10}) of the multidrug resistant *E. coli* isolates. The chapter then ends with a description of data management and statistical analysis employed; finally, with consent and ethical considerations issues.

3.2 Demarcation of the study area

The Dangme West District is situated in the southeastern part of Ghana, lying between longitude 5° 45’ south and 6° 05’ north and longitude 0° 05’ east and 0° 20’ west. The District has a total land area of 1,442 square kilometers, making it the largest in the Greater Accra Region. The land size represents 41.5% of the regional land area. The District forms part of sixteen (16) metropolitan, municipal and districts in the Greater Accra Region of Ghana.

The administrative capital of the district is Dodowa. The Dangme West District is completely rural. It shares boundaries with North Tongu District to the northeast, Yilo and Manya Krobo Districts to the northwest, Akwapim North District to the west, Tema Metro to the southwest, Dangme East District to the east and the Gulf of Guinea to the south. The District has a 37 Km Coastline and a 17km stretch of the Volta River (TDWD, 2013). Figure 3.1 shows the map of the Dangme West District of Ghana.

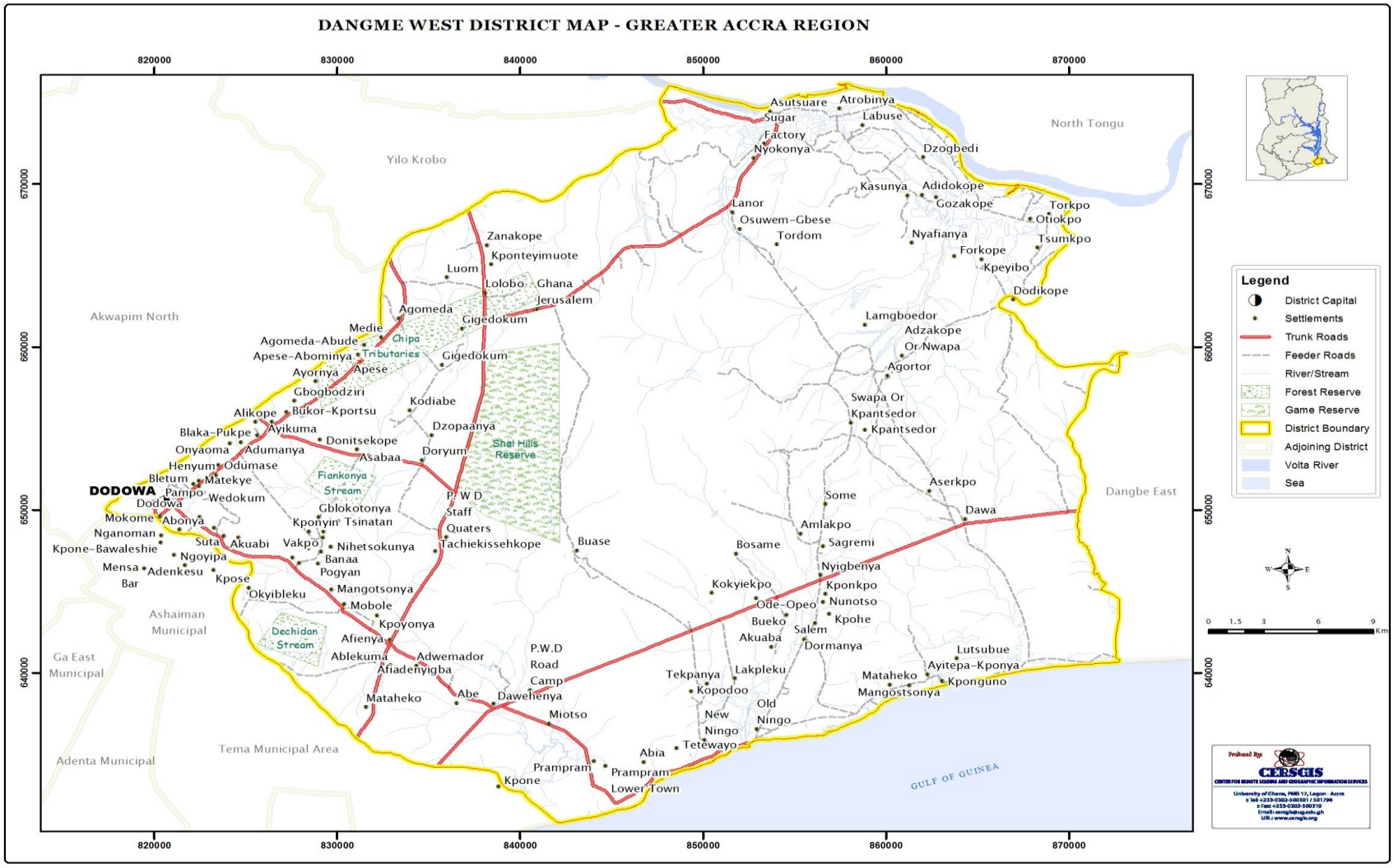

Fig 3. 1. A map of the study area; source: Centre for Remote Sensing and Geographic Information Services, University of Ghana-Legon (2013)

3.3 Geographical description of the study area.

3.3.1 Topography and drainage

The district forms the central portions of the Accra plains. The relief is generally gentle and undulating, a low plain with heights not exceeding 70 metres. The plains are punctuated in isolated areas by a few prominent inselbergs, isolated hills, outliers and knolls scattered erratically over the area. Prominent relief features include the Yongua inselberg (427 metres) which appears conical in the air with a number of outliers close to the north of the district around Asutsuare and Osuwem areas; the Krabote inselberg also to the north and the Shai Hills (289 metres) found towards the western portions of the district (MLGRDE, 2008).

Large rock outcrops and boulders conspicuously stand in the vicinity of the hills in certain places. The rocky hills together with the large boulders provide immense potentials for stone quarrying, which is already a major pre-occupation in the district. The general pattern of drainage in the Dangme West District is dendritic with most of the streams taking their source from the Akwapim range, which also serves as a watershed and then flow in a northwest to southwest direction into lagoons on the coast. Flowing over a fairly low terrain, most of the streams have carved wide valleys for themselves, which are left dry for most parts of the year. Rainfall is generally very low with most of the rains, very erratic in nature and coming mostly between September and November. Mean annual rainfall increases from 762.5 milliliters on the coast to 1220 milliliters to the North and Northeast close to the foothills of Akwapim Range and on the summit. (MLGRDE, 2008).

3.3.2 Climate and Vegetation

The southeastern coastal plain of Ghana, which encompasses the Dangme West District, is one of the hottest and driest parts of the country. Temperatures are however subjected to occasional and minimal moderating influences along the coast and altitudinal influences affected by the Akwapim range in the northwest. Temperatures are appreciably high for most parts of the year with the highest during the main dry season (November - March) and lowest during the short dry season (July - August). They average a few degrees lower on the coast and close to the Akwapim range than they do over most of the plains. The absolute maximum temperature is 40 °C (MLGRDE 2008).

The predominant vegetation type found in the district is of the short grass savannah interspersed with shrubs and short trees, a characteristic of the Sub-Sahel in type. A large portion of vegetation, particularly towards the south, remains dry for most parts of the year except for the short rainy season. The ravaging effects of seasonal bushfires that sweep across most parts of the district especially during the dry season further depreciate the quality of the vegetation.

Along some stream courses, however, higher vegetation type ranging from thickets to light forest is common. Some light forest with tall trees is also found along the foothills of the Akwapim Ridge especially around Dodowa, Ayikuma and Agomeda areas. There is a Forest, Game and Wildlife Reserve around the Shai hills (MLGRDE, 2008).

3.3.3 Geology and soil

Ancient igneous rocks underlie the major part of the district. Strongly metamorphosed ancient sediments occur along the western boundary. There are also important areas of relatively young unconsolidated sediments in the south and southeast. Dahomeyan gneiss and schists occupy most of the plains proper. Basic gneiss forms a number of large inselbergs (isolated rocky hills) in the north and center of the belt. Small rock outcrops are also common in the north close to the inselbergs but are rare in south and southeast. The eastern belt of acidic gneiss consists mainly of the grained metamorphosed rocks rather richer in minerals than the rocks in the western belt (MLGRDE, 2008).

The predominant soil types in the district are the black clays classified as Akuse series and occupy the central to eastern parts of the district. The soils are highly elastic when wet but become hard and compact when dry and then crack vertically from the surface. This renders the soil unsuitable for land cultivation. The soil in the district consists of gray-brown soils loamy for about 15-30 centimeter of the surface.. The topsoil rapidly becomes draughty during the dry seasons. This type of soil fairly supports any level of crop production. Most parts of the area are, however, left for grazing purposes (MLGRDE, 2008).

3.3.4 Water sources and sanitation

One of the major bases for the experimental design of the current study is the water sources in the district. The supply of potable water in the district is woefully inadequate and only few sections of the district have regular supply of pipe-borne water. Analysis of the current water and sanitation in the district shows that more effort is needed to meet the 85% water and sanitation coverage. On the basis of the National Community Water and Sanitation Standards of 600 people per Stand pipe, 350 persons per borehole and 150 persons per hand dug well, the district has achieved about 66% coverage with 34% of the population lacking access to potable water supply. As it stands now, below 37 % of the district population have access to pipe-borne water or tanker services, whilst 6 % use well or borehole. The remaining population in the district depends on untreated water from sources such as ponds, dams, rivers, streams, dugouts and periodic harvesting of rainwater. This means that about 57 % of the settlements do not have access to potable water. Visually the water from the streams and dams are light brownish yellow caused by mostly decayed dead leaves. If turbulently disturbed it turns to deep brownish yellow and some suspended soils can be seen. With the projected population of 118,500 as at 2010, the district would have to increase investment in potable water supply by the provision of 67 Stand pipes or 115 boreholes or 268 hand dug wells to achieve one hundred percent potable water coverage in 2010 and provide 40,290 people with safe water (MLGRDE 2008).

In the area of improved sanitation, the coverage of not more than 10% in the district implies that the District Assembly in collaboration with development partners would have to double its effort to meet the needs of about 90% of the people. About 300 household latrines and hundreds of water closet toilets are needed to meet the sanitation needs of the people. This should also include hygiene and environmental education to address the behavioural needs of the people especially in the area of open defecation (MLGRDE, 2008

3.4 Motivation for the selection of study site

To meet the study objectives, research was conducted at various sites within the Dangme West District of Ghana. The district was selected for a number of reasons. It is the largest district in the Greater Accra Region of Ghana. The district is a typical rural setting, whose

composition and geography is largely representative of most rural settings in Ghana. The water source by which the research design is centered perfectly suits the conditions in the district. Majority of the population in the district depends on untreated water from sources such as dams, rivers, and streams. Increases in water scarcity are expected to grow in the district, due to the current district growth rate of 2.1% per annum. The district has recorded a number of water borne diseases, this allows for investigations of risk associated with bacteriological contaminants of water sources.

3.5 Quality assurance

3.5.1 Sterilization and disinfection

The basic function of an infection control procedure in an environment that is essentially non-sterile is to effectively break the chain of cross-contamination and cross-infection. Therefore, the definitive methods of avoiding microbial cross contamination are to sterilize and disinfect all equipment and materials used for laboratory based microbiological analysis. In pursuance of this, all materials were sterilized and disinfected using standardized procedures. All glassware such as petri dishes and test tubes were washed and rinsed with water and air-dried. They where then placed in a sterilizing oven, face up for two hours at 160 ^{0}C. The working benches were also cleaned and disinfected, swabbing the working surface with 70% alcohol to prevent the introduction of contaminants. The laboratory benches were sterilized before and after use. It was ensured that nothing unsterile came into contact with the laboratory benches. Small pieces of equipment such as glass rods and metal tools were sterilized by dipping them in 70% ethanol (alcohol) and then flaming to burn off the alcohol. Sterilization of inoculating loops and needles were done by holding in a flame until red-hot.

During bacteriological cultures, inoculum transfers were aseptically carried out to prevent contamination of the media used. The inoculation loops were sterilized as described above before and after transfer of samples. The opening ends of all test tubes containing media to be used as well as those containing various samples were also flamed before and after transfers.

3.5.2 Equipment calibrations

All equipment used for the study were properly calibrated. These included pH meters, incubators, water bath, and autoclave. The pH electrodes were calibrated before every set of measurements by using any one (single-point calibration) of the WTW technical buffer solutions (pH values at 25 °C: 2.00 / 4.01 / 7.00 / 10.01). After the calibration, the electrode was thoroughly rinsed with deionized water before the sample measurements were taken.

3.5.3 Media preparation

The various media used for the study were prepared from dehydrated stocks according to the manufacturer's instructions. All media were prepared with distilled water, and pH adjustments were made by using a pH meter (Hanna model). All solid media prepared were sterilized by autoclaving at 121^0C for 15 minutes. It was then well mixed, dispensed aseptically into sterilized petri dishes and allowed to gel before sterility testing. Durham tubes were placed in, test tubes containing prepared broth (e.g. MacConkey) before autoclaving as described above

3.5.4 Media sterility testing

Media sterility test as a measure of quality assurance was carried out to verify freedom from contamination; demonstrate the correct performance of the medium used and ensure against physical or chemical imperfections. All prepared media were aseptically dispensed and incubated at 37^0C for 24 hrs, before inspection. Inspection for physical imperfection included; uneven distribution of media; variable amounts of medium in petri dishes/tubes/bottles; gross deformation of surface of the media. All media that did not meet the required standards were discarded.

3.5.5 Bacteria control strains

Control strains are cultures that exhibit typical microscopic, macroscopic and biochemical characteristics of the species. Control stains were used in this study, with cultures that were verified, validated and whose lineages are documented by American Type Culture Collection (ATTC). All control strains were tested for purity and identification, including particular characteristics utilized for media growth performance.

Positive and negative control cultures of the organism under test were used as checks for the analytical procedures. Numbers of reference organisms (American Type Culture Collection [ATCC]) used with their descriptions are shown in Table 3.1. Investigation and enumeration of Coliforms, faecal Coliforms, members of the family Enterobacteriaecae and all other bacteria were done according to Standard Methods (APHA, 1992).

Table 3.1 ATCC numbers of reference organisms used

Serial Number	Bacterial Isolate	ATCC Number
1	*E. coli*	25922
2	*Enterobacter aerogenes*	13048
3	*Salmonella*	19196
4	*Pseudomonas aeruginosa*	10145
5	*Staphylococcus aureus*	6538
6	*Proteus*	35659

3.5.6 Turbidity testing: McFarland standard.

The McFarland equivalence standards are intended to be part of a quality control program for adjusting densities of bacterial suspensions that are used for identification and susceptibility testing (NCCLS 1990; Chapin & Lauderdale, 2003: ASM 2007). The study used 0.5 McFarland standard to standardize the quantity of bacteria in a liquid suspension for identification and antimicrobial susceptibility testing.

Phase I

3.6 Water sampling

3.6.1 Water sample collection sites

After several preliminary visits to various communities in the districts, 57 sampling sites comprising six different water sources that include dams, boreholes, stream sources, a river, a canal and hand-dug wells in 27 communities were selected.

These 27 communities were selected from the seven (7) area councils in the district namely: Osuwem, Asutsiare, Ayikumah, Dodowa, Prampram, Ningo and Dawa. Samples were taken from locations that were representative of the water sources, based primarily on factors such as population, extent of usage and level of patronage of water from these sources.

In selecting the sampling points, each locality was considered individually; however, the sampling points were selected such that the samples taken included all the different sources from which the community obtains water. This was ensured by the use of appropriate methods and quality-assurance measures to confirm that the field sites selected and the samples collected accurately represent the environment intended for study and can fulfill data-quality objectives in the sample design.

3.6.2 Sample Size and Sampling Frequency

Four hundred and sixty four (464) water samples were collected for the study between June 2011 and May 2012. The sample collection period spanned the two seasons in Ghana: the dry and rainy seasons. Table 3.2 shows the details of water samples collection.

Table 3.2 Water sample collection details

WATER SOURCE	WATER SAMPLE COLLECTION DETAILS		
	RAINING SEASON	DRY SEASON	TOTAL
Dams	60	60	120
Bore holes	32	32	64
Streams	68	68	136
Hand-dug wells	60	60	120
River	6	6	12
Canal	6	6	12
TOTAL			464

Prior to water sampling, important observations were made of sanitary conditions and possible sources of contamination, both anthropogenic and natural events that occur in the proximity of water bodies and are likely to influence water quality from all the sources sampled. For example, it was observed that in some places, refuse dumps, and places of convenience (toilets) were sited close to water bodies. In other cases, organic and inorganic waste as well as wastewater from various human activities had been disposed off near or into water bodies, which also served as sources of water for some communities.

The importance of accurate field records when conducting water sampling cannot be over emphasized. Recording site details and other environmental factors help when interpreting the sample results later on. Field notes including the following were therefore recorded: Date, Time of sampling, Water body type, Site code, etc.

The following environmental factors were also recorded: Water clarity/turbidity (visual clarity in the water i.e. leaves, debris, algae), Weather conditions (temperature, wind, rainfall), presence of animals (birds) and other comments (e.g. faecal accidents).

3.6.3 Water sample collection procedure

All water sampling and preservation procedures were performed according to Standard Methods for the examination of water and wastewater (APHA, 1998; APHA, 1995), and WHO guidelines for drinking water quality (WHO, 1996, 1982). Sampling for bacteriological analysis was done aseptically with care, ensuring no external contamination of samples. In the process, sterilized plastic Polyethylene (PET) bottles were used. The bottles were cleaned and rinsed carefully, given a final rinse with distilled water and then sterilized at 121^{o}C for 15 minutes. Sterilization effectiveness was checked by putting sterilization strips on each sampling bottle and glassware in each run.

During samples collection, enough air space was left in each sampling bottle (at least 3 cm) to aid thorough mixing by the electronic shaker prior to examination. Samples collected were representative of the water being tested.

Borehole water samples were taken from boreholes fitted with hand pumps. Before samples were taken, the pumps were continuously operated for about 5 minutes, after which the mouth of the borehole was cleaned with cotton wool soaked in 70% concentrated alcohol and then flamed for about 5 minutes. Water was again pumped out for a further 3 minutes to allow the metal to cool. Water samples were then collected by direct flow into sterilized bottles and carefully sealed. For hand-dug wells, a sterilized bottle was tied to a rope and lowered into the wells. The lid was first removed and the bottle lowered into the well to a depth of about 1m below the water surface. The bottle was removed and quickly covered. Immediately after collection, samples were placed in an insulated box (an ice chest) filled with ice cubes to keep the temperature below 4 C^{o}. Water samples from streams/river were also collected from depths of about 1m from the active part of the streams/river where people normally collected water for domestic purposes. Steps were taken at all times to avoid contamination using standard procedures. All other equipment used for the exercise was sterilized by autoclaving on the eve of each sampling day. All samples were transported to Noguchi Memorial Institute for Medical Research (NMIMR) of University of Ghana within 2 hours for analysis.

3.7 Coliform populations analysis: MPN Technique

Three tests: presumptive, confirmed and completed tests were used to determine the presence of coliform bacteria in the water samples (APHA, 1998).

3.7.1 Presumptive tests

The presumptive test was used to determine the total coliforms present in the water samples. Double and single strength lactose broth (MacConkey broth) was prepared and dispensed into tubes in 20 ml volumes. Durham tubes were inverted in the broth, which was sterilized at 121 ^{o}C for 15 minutes. The double strength lactose broth was inoculated with 10 ml of the water sample in triplicate. Single strength lactose broth was inoculated with 1 ml and 0.1 ml of the water sample in triplicate. All the tubes were incubated at 37 ^{o}C for 48 hours. Presence of gas in the durham tubes as well as acid production evidenced by colour change to yellow was the presumptive evidence of the presence of coliform bacteria in the water sample

3.7.2 Confirmed test

The confirmed test was used to confirm the presence of coliform bacteria in water samples for which the presumptive test was positive. A sterile inoculating loop was used to transfer a loopful of the inoculum from the positive lactose broth tubes into brilliant green lactose bile broth. The brilliant green lactose broth tubes were then incubated for 48 hours at 37 ^{o}C. The formation of gas any time within 48 hours constituted a positive confirmed test. The distribution of the positive tubes was used to estimate the total coliforms per 100 ml of water using the most probable number (MPN) index (APHA, 1995). The corresponding Most Probable Number (MPN) index was then determined from the McCrady table. Figure 3.2 shows a picture of tubes used for determination of MPN after 24-48 hours of incubation

Figure 3.2 Test tubes used for determination of MPN after 24-48 hours of incubation

3.7.3 Completed test

A sterile inoculating loop was used to streak Eosin Methylene Blue (EMB) agar plates from the positive brilliant green lactose broth tubes. The plates were incubated for 24 hours at 37 ^{o}C. The presence of pink mucoid colonies and green metallic sheen colonies was recorded as a suspected indicative result for coliform bacteria. Well-isolated colonies were further inoculated into brilliant green lactose bile broth and a streak done in a nutrient agar slant. A gram stain on the cultures stored in the agar slants was done. The formation of gas in the lactose broth and the demonstration of gram- negative, nonsporing rods in the agar culture indicated a positive completed test.

3.8 Storage of bacteria isolates

Following incubation, aliquots from the cultured positive tubes (acid and/gas production) were aseptically streaked on MacConkey agar (Oxoid CM7) for total coliform and Eosin Methylene Blue agar [EMB (Oxoid CM69)] for faecal coliform and incubated at 37°C and 44°C, respectively. In situation when there was the need to store the isolates for later identification, discreet colonies from MacConkey agar and EMB agar were sub cultured onto

nutrient agar slants and incubated at 37 ^{0}C for 24 to 48 hours. The cultures on the nutrient agar slants were stored at 4 ^{0}C. The maximum storage time of the isolates at this stage of the study did not exceed 3 weeks. By which time the second step of phenotypic identifications were carried out. The next isolates storage in this study was carried after phenotypic identification of *E. coli*. Confirmed *E. coli* isolates were stored in thioglcollate broth treated with 20% glycerol (to ensure that bacteria cells are preserved) in cryoval tubes and stored at at - 0 ^{0}C. Until, ready for molecular characterization.

3.9 Phenotypic identification and characterization of bacteria isolates

Few colonies of culture from stored nutrient agar slants were suspended in nutrient broth and incubated at 35-37 ^{o}C for 24 hours to reactivate the microorganisms. From these, the following primary isolation media: Eosine Methylene Blue Agar (EMBA), Mac-Conkey agar, Endo agar and Salmonella/Shigella (SS) agar were inoculated by streaking with a metal loop. These were incubated at 35 – 37 ^{o}C. Colonies suspected to be coliform and faecal coliform were identified using their colony morphology, cultural characteristics, gram stain and were differentiated and confirmed through biochemical tests. All organism were confirmed with an API 20E kit (Bio Merieux,SA France).

The selective isolation, cultivation, and differentiation of coliforms and enteric bacteria were based on the ability to ferment lactose. For example in the case of *E. coli*, *a* rapid detection test based on the two properties characteristic of *E. coli*—the ability to produce gas from lactose or mannitol at 44°C and the ability to produce indole at 44°C was carried out.

In addition to inoculating a confirmatory medium with positive presumptive tubes, a tube of tryptone water was inoculated and all tubes were then incubated at 44°C for 24 hours.
Indole formation and gas production confirmed the presence of *E. coli* in the sample. IMViC tests for indole formation, methyl red and Voges Proskauer reactions, citrate utilization and gas production from lactose at 44°C were also performed. This array of tests was used to differentiate *E. coli* from non-faecal enterobacteriaecae as it showed peculiar characteristic pattern of positive and negative results. Figure 3.3 shows a picture of *E. coli* growing on an EMB agar, with metallic shines. Investigation and enumeration of coliforms, faecal

coliforms, members of the family enterobacteriaecae and all other bacteria were done according to Standard Methods (APHA, 1992).

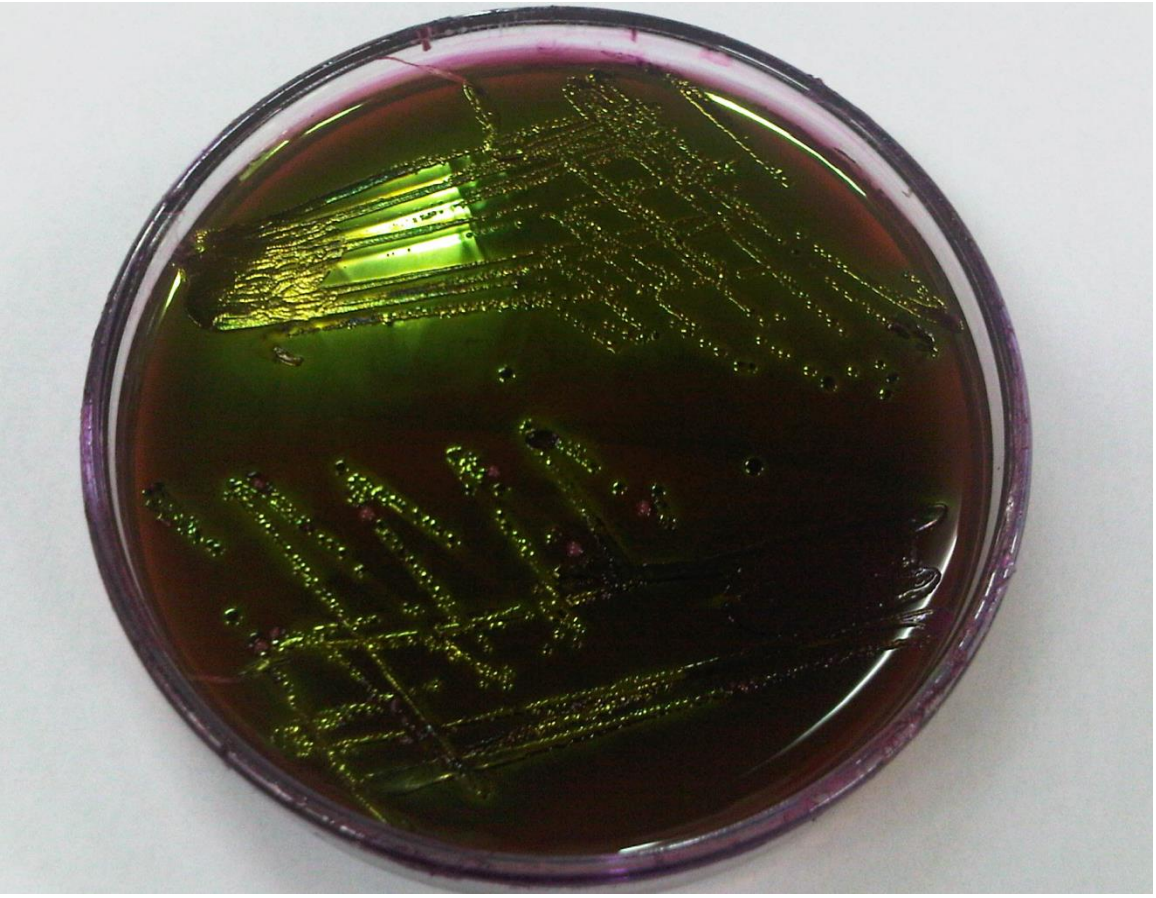

Figure 3.3 A picture of *E. coli* growing on an EMB agar, showing metallic sheen.

3.9.1 Gram staining of bacteria cultures

Gram stain was performed on all, bacteria colonies, to determine their gram reaction. A film smear of each culture under study was prepared by emulsification on a on a clean glass slide. The prepared smear was then gently heat fixed with a bunsen burner flame. The smear was flooded for one minute with crystal violet stain. The crystal violet stain was washed thoroughly with water under slow running tap. After that, the smear was flooded for one minute with gram iodine solution. This was also washed under running tap water and the excess water blotted by shaking. The next step was to delolourize the stained films by flooding the smear on the slide with acetone for few seconds and washed under slow running water. This was followed by the addition of safranin, to counterstain, for a minute. This was

again washed under slow running tap water, and allowed to air dry. The slides were subsequently examined by microscope using the oil immersion objective.

3.10 Biochemical Testing

The various biochemical test used to identify the bacteria isolated are described below:

Indole production

Trypticase soy broth medium containing tryptone 17.0 g, soytone 3.0 g, dextrose 2.5 g sodium chloride 5.0 g, and dipotassium phosphate 2.5 g in 1000 ml of distilled water was dispensed into tubes and autoclaved at 121 °C for 15 min. Using aseptic techniques, each tube was inoculated with a loopful of culture and the tubes were incubated at 37 °C for 24 hrs. A volume of 0.5 ml kovacs reagent was added to each tube and the tubes shaken gently.

Methyl-red test

MR-VP broth medium containing peptone 7.0 g, dextrose 5.0 g and potassium phosphate 5.0 g in 1000 ml of distilled water was dispensed into tubes and autoclaved at 121 °C for 15 min. Using aseptic techniques each tube was inoculated with a loopful of culture and the tubes incubated at 37 °C for 24 to 48 hours for the slow fermenters.

A third of each culture was transferred into a sterile empty tube for the Voges – Proskauer test. To the two thirds of the culture remaining in each tube, 0.2 ml of methyl red indicator was added (Harley and Prescott, 1996).

Voges-Proskauer test

The one third aliquot from the methyl red test was used for this test. A volume of 0.8 ml barrits reagent containing 40 % KOH and a 5 % solution of alpha-naphthol in absolute ethanol was added to each culture and shaken vigorously to aerate (Cheesbrough, 1990).

Citrate utilization test

Simmons citrate agar containing ammonium dihydrogen phosphate 1.0 g, dipotassium phosphate 1.0 g, sodium chloride 5.0 g, sodium citrate 2.0 g, magnesium sulphate 0.2 g, bromothymol blue 0.08 g and agar 15.0 g in 1000 ml of distilled water was prepared as slants

by autoclaving at 121 °C for 15 minutes and allowing to solidify. Using aseptic technique each tube was inoculated with the bacteria culture by means of a stab-and-streak and the tubes were incubated at 37 °C for 24 to 48 hours. The slant cultures were examined for the presence or absence of growth and for any change in colour from green to blue (Cheesbrough, 1990).

Triple sugar iron (TSI) agar test

Triple sugar iron agar containing beef extract 3.0 g, yeast extract 3.0 g, peptone 15.0 g, peptose-peptone 5.0 g, lactose 10 g, saccharose 10.0 g, dextrose 1.0 g, ferrous sulfate 0.2 g, sodium chloride 5.0 g, sodium thiosulfate 0.3 g, phenol red 0.024 g and agar 12.0 g was prepared as slants by dissolving 65 g in distilled water, dispensing into tubes and autoclaving at 121 °C for 15 minutes. Each TSI agar slant was labelled with the bacterial isolate to be inoculated and one tube (uninoculated) was used as a negative control.

An aseptic technique was used to streak the slant and stab the butt with the bacterial culture using a straight inoculating needle. The tubes were incubated for 18 to 24 hours at 37 °C to detect the presence of sugar fermentation, gas production and H_2S production.

The tubes were incubated and checked daily for seven days in order to observe blackening. The cultures were examined for the colour of the slant and butt and for the presence or absence of blackening within the medium (Harley & Prescott, 1996).

Motility test

This test was done to differentiate between *Salmonella* and *Shigella* species. A motility test medium containing tryptose 10.0 g, sodium chloride 5.0 g and agar 5.0 g in 1000 ml of distilled water was dispensed into tubes, autoclaved at 121 °C for 15 minutes and allowed to cool as agar deeps. Each agar deep tube was inoculated with the bacterial culture aseptically by stabbing the medium to the bottom with a straight inoculating needle. The tubes were incubated at 37 °C for 24 to 48 hours. The cultures were examined for the presence or absence of motility. Motility was present when the growth of the culture was not restricted to the stab line of inoculation. Growth of nonmotile bacteria was confined to the line of inoculation (Cheesbrough, 1990).

Oxidase test

Nutrient agar medium was prepared by autoclaving at 121 °C for 15 minutes and dispensed into sterile petri dishes. The agar plates were each divided into two sections and each labelled with the bacterial isolate to be inoculated. A single streak-line inoculation on the agar surface was made aseptically with the cultural isolate. The plates were incubated at 37 °C for 24 hours. Using a wooden applicator stick, an oxidase disk (p-aminodimethylaniline) was placed on an isolated colony and the colony observed for colour change (APHA, 1998).

3.11 API analysis

Specimens (collection and preparation)

The microorganisms to be identified were first isolated on a culture medium adapted to the culture of Enterobacteriaceae and non-fastidious gram-negative rods, according to standard microbiological techniques.

Preparation of the strip

An incubation box (tray and lid) was prepared and 5 ml of distilled water or demineralized water [or any water without additives or chemicals which may release gases (e.g., Cl_2, CO_2, etc.)] was distributed into each of the honeycombed wells of the tray to create a humid atmosphere. Strain references were recorded on the elongated flap of the tray and the strips were removed from their packages.

Preparation of the inoculum

With the aid of a sterile cotton wool, a single colony from the culture plates (young cultures 18-24 hours old) was selected and emulsified. Careful emulsification was done to achieve homogeneous bacterial suspensions and was used immediately after preparation.

Inoculation of the strip

Using the same pipette, both tube and cupule of the tests CIT, VP and GEL were filled respectively with the bacterial suspensions. However, only the tube (and not the cupule) of the

other tests was filled. Anaerobiosis in the tests ADH, LDC, ODC, H_2S and URE were created by overlaying with mineral oil. Incubation was at 36°C ± 2°C for 18-24 hours.

Reading the strip

After the incubation period, the strips were read and in cases where 3 or more tests (GLU test + or –) were positive, the spontaneous reactions were recorded on results sheet and the tests were then revealed with the addition of reagents. A TDA Test was done by the addition of 1 drop of TDA reagent. A reddish brown colour produced indicated a positive reaction, which was also recorded on the result sheet. Likewise, an IND test was performed by adding 1 drop of James reagent and a pink colour developed in the whole cupule indicated a positive reaction, which was recorded on the result sheet. In addition, VP Test was done by adding 1 drop each of VP 1 and VP 2 reagents and waiting for 10 minutes. A reddish brown colour indicated positive reaction for TDA; a pink colour developing in the whole cupule for IND; and a pink colour for VP. Figure 3.4 shows a API result sheet.

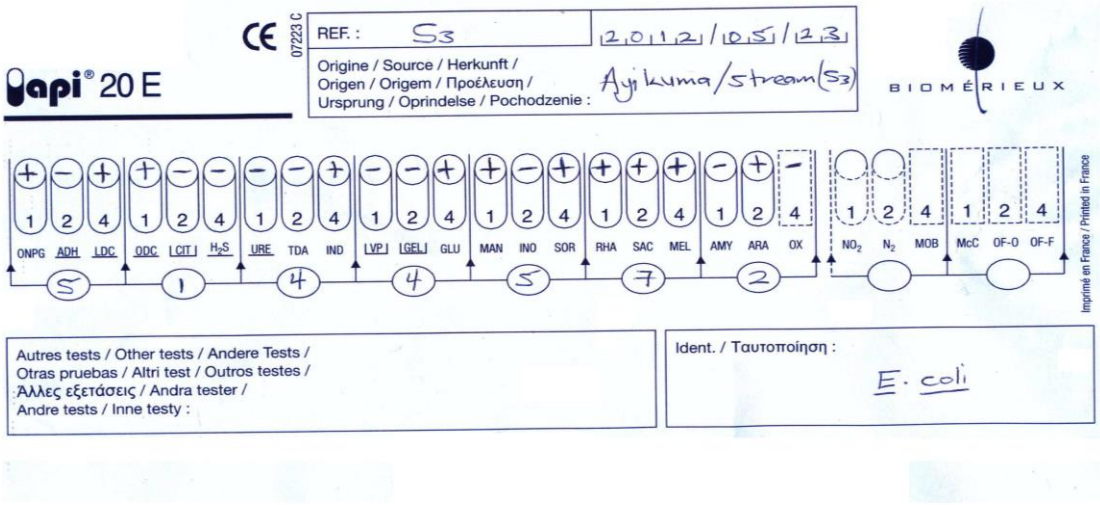

Figure 3.4 API results sheet.

Figure 3.4 shows results sheet of the API 20E identification system for the identification of *E. coli*. Identification of the isolates was determined by the numerical profile using the analytical profile index. On the result sheet, the tests are separated into groups of 3 and a value 1, 2 or 4 is indicated for each. By adding together the values corresponding to positive

reactions within each group, a seven-digit profile number was obtained for the 20 tests of the API 20 E strip. The profile number is then looked up in a codebook having a correlation between numbers and bacteria species

PHASE II

3.12 Anti-bacteria susceptibility testing of *E. coli*

Each isolate of *E. coli* was subjected to antibiotic susceptibility testing. The Kirby–Bauer disc-diffusion method as set by the Clinical and Laboratory Standards Institute (CLSI, 2007) was used. Isolates grown overnight on Nutrient Agar were suspended in sterile normal Saline (0.9% w/v Nacl), using a sterile wire loop until the turbidity was equivalent to 0.5 Mcfarland standards. Sterile non-toxic cotton swabs dipped into the standardized innocula were used to streak the entire surface of Mueller Hinton agar plates.

The antibiotics tested are shown in table 3.3. Antibiotics disks were aseptically placed using a sterile forceps, and all plates incubated (Gallenkamp England model IH-150) at 37^{o}C for 24hrs (Mills –Robertson *et al.*, 2003). After incubation, the isolates were classified/ interpreted as susceptible, intermediate, or resistant by comparing the diameters of inhibition zones with the breakpoints according to CLSI recommendations (CLSI, 2007).

Table 3.3 Description of antibiotics used

Antibiotic description		Disc concentration
Class of antibiotics	Name of antibiotic	
Aminoglycosides	Amikacin (AMK)	30 µg
	Gentamicin (GEN)	10µg
Cephalosporins	Cefuroxime (CXM)	30 µg
	Cefotaxime (CTX)	30 µg
Chloramphenicol	Chloramphenicol (CHL)	30 µg
Co-Trimoxazol	Co-trimoxazole (COT)	25 µg
Macrolides	Erythromycin (ERY)	15µg
Nitrofurantoin	Nitrofurantoin (NIT)	300 µg
Penicillins	Ampicillin (AMP)	10 µg
	Penicillin (PEN)	10 units
Pyridopyrimidine	Pipemidic acid (PA)	20 µg
Quinolones	Ciprofloxacin (CIP)	5 µg
	Nalidixic acid (NAL)	10 µg
Tetracyclines	Tetracycline (TET)	30 µg

3.13 Identification of multiple antibiotic/drug resistance (MDR)

Multi drug/antibiotic resistance is defined as resistance to all the tested antibiotics in at least two of the following three classes: -lactams, aminoglycosides, and quinolones (Bauer *et al.*, 1996). The MDR of the isolates were identified by observing the resistance pattern of the isolates to the antibiotics.

3.14 MAR (multiple antibiotic resistance) index study:

The MAR Index as described by Webster *et al*. (2004) aims to demonstrate the presence of either simple or multiple resistances to antibiotics which results are directly related to the human impact on aquatic environment system.

The MAR index of an isolate is defined as a/b, where a represents the number of antibiotics to which the isolate was resistant and b represents the number of antibiotics to which the isolate was subjected (Krumperman, 1983). The aggregate MAR index for a sampling sources (MARq) is defined as the ratio between the number of resistant test at the sampling sources and the total number of test performed at that point.

3.15 Genotypic characterization of *E. coli*

Molecular characterization of *E. coli* isolates was carried out using a DNA•STRIP technology (GenoType EHEC). This technology permits the combined identification of the Shiga toxin genes *stx1* and *stx2*, the intimin gene *eae*, and the *ipaH* (invasion plasmid antigene H) gene. The whole procedure was divided into three steps: DNA extraction from cultured material, a multiplex amplification with biotinylated primers and a reverse hybridization.

3.15.1 Cultivation and DNA extraction

The *E. coli* strains were isolated from bacterial culture plates and were identified by standard bacteriological methods (Koneman *et al.,* 1997). The isolates were then confirmed using API 20E. Overnight cultures of *E. coli* were prepared and incubated for 24 hours at 37°C. The colonies were washed from the well-grown culture plate ("lawn of growth") with 1.5 ml of sterile 0.9% saline solution. The supernatant was then discarded. The resultant pellets were re-suspended in 500ul of molecular biology grade water. The resultant suspension was incubated for 10 minutes at 95°C in a heating block. It was span down for 5 min at maximum speed in a standard tabletop centrifuge and supernatant transferred to a new tube. A volume of 5 µl of the lysate was taken for amplification.

Amplification

Two separate PCR reactions were performed with each sample: one with PNM EHEC 1 and the other one with PNM EHEC 2. The DNA amplification mix (45 µl) was prepared in a DNA free room. The DNA sample was added in a separated area. The following per tube mix was prepared: thirty five microliters (35 µl) PNM EHEC 1 and PNM EHEC 2, five microliters (5µl) polymerase incubation buffer, two microliters (2 µl) $MgCl_2$ two (2) unit(s) thermostable DNA polymerase and two microliters (2 µl) water to obtain a volume of 45 µl.

Five microliters (5 µl) of freshly extracted DNA solution was added to the tube mix leading to a final volume of 50 µl. The performance evaluation of the GenoType EHEC assay was carried out using the HotStarTaq DNA Polymerase from Qiagen. Four different PCR amplification cycles (1, 10, 10 and 1) profile were carried out according to the manufacturers instruction. The amplification cycles together with their respective times used are as follow: 1 cycle (15 min 95°C, 10 cycles 30 sec 95°C, 2 minutes 58°C), 10 cycles (25 seconds 95°C, 40 seconds 53°C, 40 seconds 70°C and 40 seconds 70°C) and 8 minutes 70°C 1 cycle.

Taq polymerase was used for validation. Amplification products were stored at +4 to –20°C. In order to confirm amplification reaction, exactly 5 µl of each sample was directly applied to a gel without the addition of loading buffer. Agarose (sigma) 1.0 g was dissolved in 100 ml of tris boric acid buffer (TBA). The mixture was heated in a microwave for 2 minutes while shaking at intervals of 30 seconds to release any gas. A comb with well-spaced teeth was placed on an agarose electrophoresis plate to create wells. The agarose solution was allowed to cool slightly and stained with 5 µl of ethidium bromide before pouring on the plate. The stained agarose gel was allowed to polymerise for 1 h in the plate. The comb was carefully removed and the plate was placed in the electrophoresis chamber. The TBA buffer was added until the gel was completely submerged. About 20 µl of plasmid DNA mixed with 10 µl of loading buffer was pippeted into the wells. The chamber was connected to a power supply and the gels were run at 100 volts for one hr (Umolu *et al.*, 2006).

The amplicons generated with PNM EHEC 1 had a length of 42 bp *(E. coli)*, 65 bp *(ipaH)*, and 50 bp *(eae)*, respectively; the amplicons generated with PNM EHEC 2 had a length of 63 bp (Universal Control), 93 bp *(stx1)* and 115 bp *(stx2)*, respectively.

Hybridization preparation

The hybridization included the following steps: chemical denaturation of the amplification products, hybridization of the single-stranded, biotin-labeled amplicons to membrane-bound probes, stringent washing, addition of a alkaline phosphatase (AP) conjugate, and an AP mediated staining reaction. A template ensures the easy and fast interpretation of the banding pattern obtained.

The two amplification reactions for each sample (generated with PNM EHEC 1 and PNM EHEC 2, respectively) were hybridized together with a single strip. Twenty microliters (20µl) of Denaturation Solution (DEN, blue) was dispensed in a corner of each of the wells. Ten microliters (10 µl) of each of the two amplification reactions was added and, pipetted up and down to mix well and incubated at room temperature for 5 minutes. One millilitre (1ml) of pre-warmed Hybridization Buffer was then carefully added to each well. The tray was then gently shaken until the solution had a homogenous color. Care was taken not to spill solution into the neighboring wells. A strip was placed in each well.

The strips were completely covered by the solution and the coated side (identifiable by the colored marker near the lower end) placed face upward. Tray was placed in shaking water bath and incubated for 30 minutes at 45°C. Hybridization Buffer was completely aspirated. One milliliter (1 ml) of stringent wash solution was then added to each strip and incubated for 15 minutes at 45°C in an incubator.

Work was done at room temperature from this step forward. Stringent wash solution was completely removed. Each strip was washed once with 1 ml of rinse solution for 1 minute.

Exactly one milliliter (1 ml) of diluted conjugate was added to each strip and incubated for 30 minutes. Solution was removed and each strip washed twice for 1 minute with 1 ml of rinse solution. One (1 ml) of diluted substrate was added to each strip and incubated protected from light without shaking. Reaction was stopped by briefly rinsing twice with distilled water. Using tweezers, strips were removed from the tray and dried between two layers of absorbent paper

Evaluation and interpretation of results

Paste strips were stored protected from light. An evaluation sheet was provided with the kit. The supplied template served as an aid for evaluation and was aligned with the conjugate control band of the strip. Each strip had a total of 7 reaction zones (Figure 3.5).

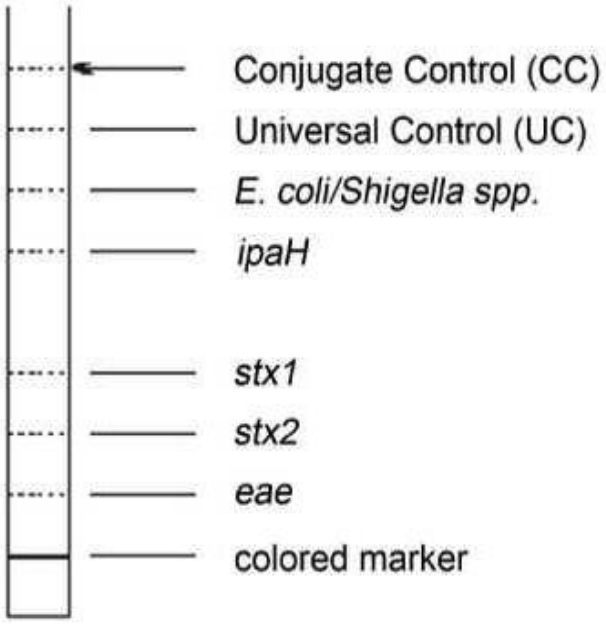

Figure 3.5 A diagram explaining interpretation of results

In order to validate the correct performance of the test and the proper functioning of reagents, each strip included 3 control zones: A conjugate control zone to check the binding of the conjugate on the strip and a correct chromogenic reaction, a universal control zone which detects, as known, all bacterial species and an *E. coli* control zone.

Phase III

3.16 Determination of radiation sensitivity (D_{10}) of *E. coli*

The Radiation sensitivity (D_{10}) of *E. coli* was carried out on 29 (60%) of the multidrug resistant *E. coli* isolates. Stored isolates of *E. coli* were revived by culturing them on EMB and nutrient agar media overnight at 37^{o}C in an incubator. Colonies from the overnight cultures were taken into a 100ml of peptone water. The peptone water was allowed to stand for a while for the organism to reach the stationary phase. The broth was dispensed 10ml into 7 McCarty bottles and labeled 0, 500, 600, 700, 800, 900, 1000 Gray for irradiation using the (^{60}Co source).

The exposure to the various doses of radiation was controlled from a radiation-controlled system. After irradiation with the doses mentioned above, the samples were aseptically

dispensed into conical flacks containing 90 ml of peptone water. The samples were placed on a mechanical shaker for 5 minutes. The 100 ml peptone water was serially diluted into 8 other McCarty bottles containing 9ml of peptone water.

The dilutions were immediately plated in triplicates on EMB and incubated at 37^{o}C to estimate survival rates. The samples were incubated at 38^{o}C. The average of the surviving population of each dose was determined after 24 hours of incubation. This selection protocol was repeated independently three more times. Each replicate was initiated using an isolated colony derived from frozen stock of the founder.

The formula -log [N/ N_o] was used to calculate the survival curve and the dose resistance of the *E. coli* organism where N is the number of surviving *E. coli* on a plate after each dose administered and N_o is the inoculums concentration of the samples sent for irradiation. The data was analyzed and graphs were drawn. The inverse of the slope of each graph was taken as the dose to reduce the population of the *E. coli* organism by 1log cycle (D_{10}).

3.17 Data management and analysis

Field data was recorded in a tabular form in a notebook at the sampling sites while laboratory data was collected and recorded stepwise in laboratory notebooks. Quantitative and qualitative analysis of the laboratory results was done using a computer package, Genstat and SPSS version 11.5. The data was presented using tables, graphs, and charts. Measures of variability were reported with the means. T test, and analysis of variance (ANOVA) were used to determine the variations. Simple regression was used to establish the relationships investigated. A probability value, $p < 0.05$ was considered significant.

3.18 Consent and ethical considerations

An application for ethical clearance in respect of this study was submitted, reviewed, and approved by the Research Ethics Review Committee of the College of Agriculture and Environmental Sciences, University of South Africa.

CHAPTER FOUR

RESULTS

4.1 Introduction

In chapter three the experimental design employed to answer the research question. The current chapter presents the results and their relevance to the stated research questions. The main empirical findings are presented in tabular, pictorial, and graphical formats, so that the reader can appreciate the underlying facts in the data. The organization of the results is presented in a logical and chronological form in line with how the research questions that were advanced in the introduction, literature review, and methodology.

4.2 Bacteriological contamination of water and associated risk.

Testing drinking water for biological quality is essential in providing information about disease risk and is needed to protect public health. This section (4.2) provides results on the experimental analysis done, on bacteriological contamination of water and associated risk. The results presented include: total and faecal coliform populations assessments; *E. coli* population analysis; analysis of bacteria flora in the drinking water sources; assessments of disease risk associated with the drinking water sources and, analysis of *E. coli* isolates from the water sources.

4.2.1 Total and faecal coliform populations analysis

Table 4.1 shows the range and geometric means of total and faecal coliforms observed in the water sources during the rainy season. Majority of the water sources showed high faecal and total coliform levels. The highest range of total coliform contamination (5.4×10^2 - 2.4×10^3 MPN/100ml) in the rainy season was recorded in dam water sources with a geometric mean of 1.089×10^3. This was followed by stream water sources with a range and geometric mean of 3.5×10^2 - 2.4×10^3 MPN/100ml and 7.96×10^3 MPN/100ml respectively. The least range of total coliform counts was observed in boreholes and hand-dug wells. The counts range recorded was 0 - 1.1×10^1 MPN/100ml and 0 - 2.6×10^1 MPN/100ml for borehole and hand-

dug wells respectively. The faecal coliform counts ranged between 0 - 2.4 x 10^3 MPN/100ml cross all the water sources.

Table 4.1 Range and geometric mean of total coliform and faecal coliform bacteria counts in water sources during the rainy season

Water sources	Total coliform		Faecal coliform	
	Counts (MPN/100ml)	Geometric mean	Counts	Geometric mean
Borehole	0 - 1.1x10^1	1.4 x10^1	0 - 0.2x10^1	5 x10^0
Canal	9.2x10^2 - 1.6x10^3	1.331x10^3	3.5x10^2 - 9.2x10^2	5.58x10^2
Dam	5.4x10^2 - 2.4x10^3	1.089 x10^3	1.4 x10^2 - 2.4x10^3	5.91x10^2
Hand-dug well	0 - 2.6x10^1	1.8 x10^1	0 - 0.5x10^1	3 x10^0
River	9.2x10^2 - 1.6x10^3	1.160 x10^3	1.4x10^2 - 2.4x10^2	1.92 x10^2
Stream	3.5x10^2 - 2.4x10^3	7.96 x10^3	9.4x10^1 - 9.2x10^2	2.46 x10^2

The highest range of faecal coliform contaminations of 1.4 x 10^2 - 2.4 x 10^3 MPN/100ml with a geometric mean of 5.91 x 10^2MPN/100ml were observed in dam water sources. However, the lowest range (0 - 0.2 x 10^1MPN/100ml; geometric mean: 5 x 10^0 MPN/100ml) was recorded in borehole water sources in the rainy season. With the exception of some few samples from hand-dug wells and borehole water sources, all the other water sources had faecal coliform counts exceeding the WHO recommended guideline and the Ghana Standard Authority drinking water standard of 0 MPN/100ml.

The range and geometric means of dry season total and faecal coliform counts recorded within the various water sources in the dry season are presented in table 4.2. Generally, the patterns of ranges of total coliforms and faecal coliforms counts observed in the rainy season followed similar trends in the dry season. However, the ranges observed were higher in the dry season than in the rainy season. The total coliform counts ranged between 0 - 0.2 x 10^1 MPN/100ml to 9.2 x 10^2 - 2.4 x 10^3 MPN/100ml from hand-dug wells and river water sources respectively.

Table 4.2 Range and geometric mean of total coliform and faecal coliform bacteria in water sources during the dry season

Water sources	Total coliform		Faecal coliform	
	Counts (MPN/100ml)	Geometric mean	Counts	Geometric mean
Borehole	$0 - 0.2x10^1$	$1.3x10^1$	$0 - 0.2x10^1$	$4.6 x10^0$
Canal	$9.2x10^2 - 2.4x10^3$	$1.523x10^3$	$5.4x10^2 - 1.6x10^3$	$9.26 x10^2$
Dam	$5.4x10^2 - 2.4x10^3$	$1.476 x10^3$	$1.4 x10^2 - 2.4x10^3$	$7.96 x10^2$
Hand-dug well	$0 - 7.9x10^1$	$3.2 x10^1$	$0 - 1.2x10^1$	$4.13x10^0$
River	$9.2x10^2 - 2.4x10^3$	$1.523 x10^3$	$1.4x10^2 - 5.4x10^2$	$2.63 x10^2$
Stream	$5.4x10^2 - 2.4x10^3$	$1.238 x10^3$	$1.4x10^2 - 9.2x10^2$	$4.33 x10^2$

The highest range of total coliform counts in a single water source was between $5.4 \times 10^2 - 2.4 \times 10^3$ with a geometric mean of 1.476×10^3 MPN/100ml. This was observed in dam water sources. The corresponding dry season range and geometric means of faecal coliform counts were $1.4 \times 10^2 - 2.4 \times 10^3$ and 1.476×10^3 MPN/100ml respectively. The least observed range and counts for both faecal and total coliform were observed in borehole water sources. These were between $0 - 0.2 \times 10^1$ MPN/100ml and geometric mean of 1.3×10^1MPN/100ml for total coliforms and $0 - 0.2 \times 10^1$ MPN/100ml and geometric mean of 4.6×10^0 MPN/100ml for faecal coliform counts.

Table 4.3 provides a comparison of total coliform count (MPN/100ml) in water sources across seasons. The highest total coliform counts (1373.33 ± 392.60) were observed in canal water sources during the dry season. Mean total coliform count increased in the dry season (1640 MPN/100ml ± 740.81 MPN/100ml), two-sample t-test, and d.f. = 2, $P<0.05$. This indicates a significant difference between the total counts of the two seasons. The second highest total counts after canal water sources were observed in dam water sources. Mean total coliform count was higher in the dry season (1660 MPN/100ml ±6350.65 MPN/100ml) two-sample t-test, and d.f. = 14, $P<0.05$. Thus, there was a significant difference of total coliforms recorded in the dry season, as compared to the rainy season.

Table 4.3 Comparison of total coliform count (MPN/100ml) in water sources across seasons

Water sources	Rainy season				Dry season				
	Mean±SD	Min	Max	d.f	Mean±SD	Min	Max	d.f	P value
Borehole	8.63±7.48	0	17	7	15.63±12.22	0	33	7	0.09
Canal	1373.33±392.60	920	1600	2	1640±740.81	920	2400	2	0.31
Dam	1185.33±609.54	540	2400	14	1660±635.66	540	2400	14	0.03
Hand-dug well	16.13±8.14	0	26	14	32.93±20.42	0	79	14	0.00
River	1146.67±392.60	920	1600	2	1640±740.81	920	2400	2	0.18
Stream	937.10±553.87	540	2400	16	1347.10±581.63	540	2400	16	0.02

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

The least total coliform counts were observed in borehole water sources, where mean total coliform counts were higher in the dry season (15.63 MPN/100ml ±12.22 MPN/100ml), two-sample t-test, and d.f. = 7, $P<0.05$. General observation from the results show that the there was significant differences ($P<0.05$) between total coliform counts observed in the rainy season compared to the dry season. This was observed in all the five water sources under study. With the exception of dam, hand-dug well and stream waters sources all the other water sources did not show significant difference (P> 0.05) of total coliforms counts recorded between the dry and wet season.

Table 4.4 shows a comparison of faecal coliform count (MPN/100ml) in water sources across the dry and wet seasons. Dam water sources presented with the maximum faecal coliform counts (804.67 ± 639.72 MPN/100ml). There were more faecal coliforms (1058.67 MPN/100ml ± 612.93 MPN/100ml) observed in the dry season than in the rainy season (804.67 MPN/100ml ± 639.72 MPN/100ml), two-sample t-test, and d.f. = 14, $P<0.05$. Canal water sources recorded with the next highest observations in faecal coliform counts. The canal water sources also recoded higher faecal coliforms (1020 MPN/100ml ± 537.03 MPN/100ml) in the dry season than in the rainy season (603.33 MPN/100ml ± 290.22 MPN/100ml), two-sample t-test, and d.f. = 7, $P<0.05$.

Table 4.4 Comparison of faecal coliform count (MPN/100ml) in water sources across seasons

Water sources	Rainy season				Dry season				
	Mean±SD	Min	Max	d.f	Mean±SD	Min	Max	d.f	P value
Borehole	3.13±2.95	0	7	7	4.25±4.10	0	12	7	0.27
Canal	603.33±392.60	920	1600	2	1020±537.03	540	1600	2	0.15
Dam	804.67±639.72	140	2400	14	1058.67±612.93	140	2400	14	0.13
Hand-dug well	2.13±1.73	0	5	14	4.13±3.38	0	12	14	0.03
River	186.67±50.33	140	240	2	306.67±208.17	140	540	2	0.19
Stream	309.30±249.31	16	94	16	497.10±308.32	140	920	16	0.03

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

The least counts in faecal coliforms were observed in hand-dug wells. There were also statistically more faecal coliforms (4.133 MPN/100ml ± 3.37 MPN/100ml) found in the dry season than in the rainy season (2.13 MPN/100ml ± 1.72 MPN/100ml), two-sample t-test, and d.f. = 16, $P<0.05$. The general observation from the results shows that the there was significant differences ($P<0.05$) between faecal coliform counts observed in the rainy season compared to the dry season in all the water sources. There were significant differences ($p<0.05$) in the faecal coliform counts in dry season compared to rainy season in hand-dug well and stream water sources

Table 4.5 shows a T test comparing the total coliform counts observed in all the water sources in the dry and rainy seasons. The mean total coliform count observed was higher in the dry season.

Table 4.5 T test for the seasonal distribution of total coliforms

Season	Mean±SD	Min	Max	d.f	P value
Dry	955.07 ± 875.29	0	2400	60	
Rainy	681.66 ± 697.65	0	2400	60	P<.001

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

The mean total coliform count increased in the dry season (955.07 MPN/100ml ± 875.29 MPN/100ml), two-sample t-test, and d.f. = 2, $P<0.05$. There was also a significant difference between the total counts of the two seasons.

Table 4.6 presents a test comparing the faecal coliform counts observed in all the water sources in the dry and rainy seasons. The mean total coliform count observed was also higher in the dry season

Table 4.6 T test for the seasonal distribution of faecal coliforms

Season	Mean±SD	Min	Max	d.f	P value
Dry	465.67± 557.25	0	2400	60	
Rainy	323.85± 466.10	0	2400	60	0.000102

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

The mean total coliform count increased in the dry season (465.67 MPN/100ml ± 557.25 MPN/100ml), as against (323.85 ± 466.10) in the rainy season, two-sample t-test, and d.f. = 2, $P<0.05$

4.2.2 *E. coli* population analysis

Table 4.7 shows the range of *E. coli* counts observed in the rainy season. The widest range of *E. coli* counts ($1.2x10^1$ - 7.9 x 10^1MPN/100ml) were obtained from in dams during the dry season. Hand-dug wells, borehole, and stream water sources recorded counts of 0 MPN/100ml of *E. coli* as minimum counts. No *E. coil* was counted in river water sources during the rainy season.

Table 4.7 Range of *E. coli* counts (MPN/100ml) in water during the rainy and dry seasons

Water sources	Drinking water sources (MPN/100ml)	
	Rainy season	Dry season
Borehole	$0 - 0.2x10^1$	$0 - 0.2x10^1$
Canal	$3.1x10^1 - 6.3x10^1$	$2.3x10^1 - 1.1x10^2$
Dam	$0.7x10^1 - 1.1x10^2$	$1.2x10^1 - 7.9x10^1$
Hand-dug well	$0 - 0.2x10^1$	$0 - 0.4x10^1$
River	0	$0.2x10^1 - 0.7x10^1$
Streams	$0 - 3.3x10^1$	$0 - 3.3x10^1$

Table 4.8, shows a statistical summary of comparison of *E. coli* count (MPN/100ml) in the various water sources. The highest *E. coli* counts (59.67 ± 45.08) was recoded from canal water sources during the dry season. However, the least *E. coli* count of 0MNP/100ml was observed in river water sources in the rainy season.

Table 4.8 Comparative summary of *E. coli* counts (MPN/100ml) in the water sources

Water sources	Rainy season				Dry season				
	Mean±SD	Min	Max	d.f	Mean±SD	Min	Max	d.f	P value
Bore hole	0.63±0.74	0	2	7	1± 0.76	0	2	7	0.09
Canal	47.67±16.04	31	63	2	59.67±45.08	23	110	2	0.28
Dam	38.07± 30.71	7	110	14	39.87±19.76	12	79	14	0.35
Hand-dugwell	0.93±0.80	0	2	14	1.47±1.06	0	4	14	0.00
River	0± 0	0	0	2	4.33±2.52	2	7	2	0.05
Stream	15.06±13.59	0	33	16	20.77± 11.05	0	31	16	0.02

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

Table 4.9 and Table 4.10 show Analysis of mean differences and confidence intervals for *E. coli* levels during rainy season and analysis of mean differences and confidence internals for

E. coli levels in the dry season. The results shows that mean difference were significant for boreholes and canal sources in the rainy season and borehole, river and canal in the dry season

Table 4.9 Analysis of mean differences and confidence intervals for *E. coli* levels in the rainy season

Water sources	T	df	Sis (2-talied)	Mean difference	95% confidence interval	
					Upper limit	**Lower limit**
Borehole	2.376	7	.049	.625	.00	1.25
Canal	5.147	2	.036	47.667	7.82	87.52
Dam	4.800	14	.000	38.067	21.06	55.08
Hand-dug well	4.525	14	.000	.933	.49	1.38
River	-	2	-	-	-	-
Stream	4.569	16	.000	15.059	8.07	22.05

Test Value = 6 The mean difference is significant at P ≤ 0.05.

Table 4.10 Analysis of mean differences and confidence intervals for *E. coli* levels in the dry season

Water sources	T	df	Sis (2-talied)	Mean difference	95% confidence interval	
					Upper limit	**Lower limit**
Bore hole	3.742	7	.007	1.000	.37	1.63
Canal	2.292	2	.149	59.667	-52.32	171.66
Dam	7.814	14	.000	39.867	28.92	50.81
Hand-dug well	5.358	14	.000	1.467	.88	2.05
River	2.982	2	.096	4.333	-1.92	10.58
Stream	7.745	16	.000	20.765	15.08	26.45

Test Value = 6. The mean difference is significant at P ≤ 0.05.

4.2.3. Analysis of disease risk associated with drinking water sources

Figure 4.1 and figure 4.2 presents results of analysis of the disease risk level of the various water sources during the rainy season and the dry season respectively. The evaluation is based

on the WHO drinking water risk assessment. An *E. coli* count of 0MPN/100ml is considered to be in conformity with WHO guidelines for safe drinking water. A count between 1-10 MPN/100ml is considered low risk; 11-100MPN/100ml is intermediate risk and finally, *E. coli* counts >100 MPN/100ml is considered high risk.

From figure 4.1 only a single source (7%) presented with high diseases risk during the rainy season. This was observed in dam water sources. Water (100%) from canal water sources had intermediated risk level. This was followed by dam water sources also with an 87 % recorded intermediate risk levels. Stream water sources presented with 59% recorded level of intermediate risk.

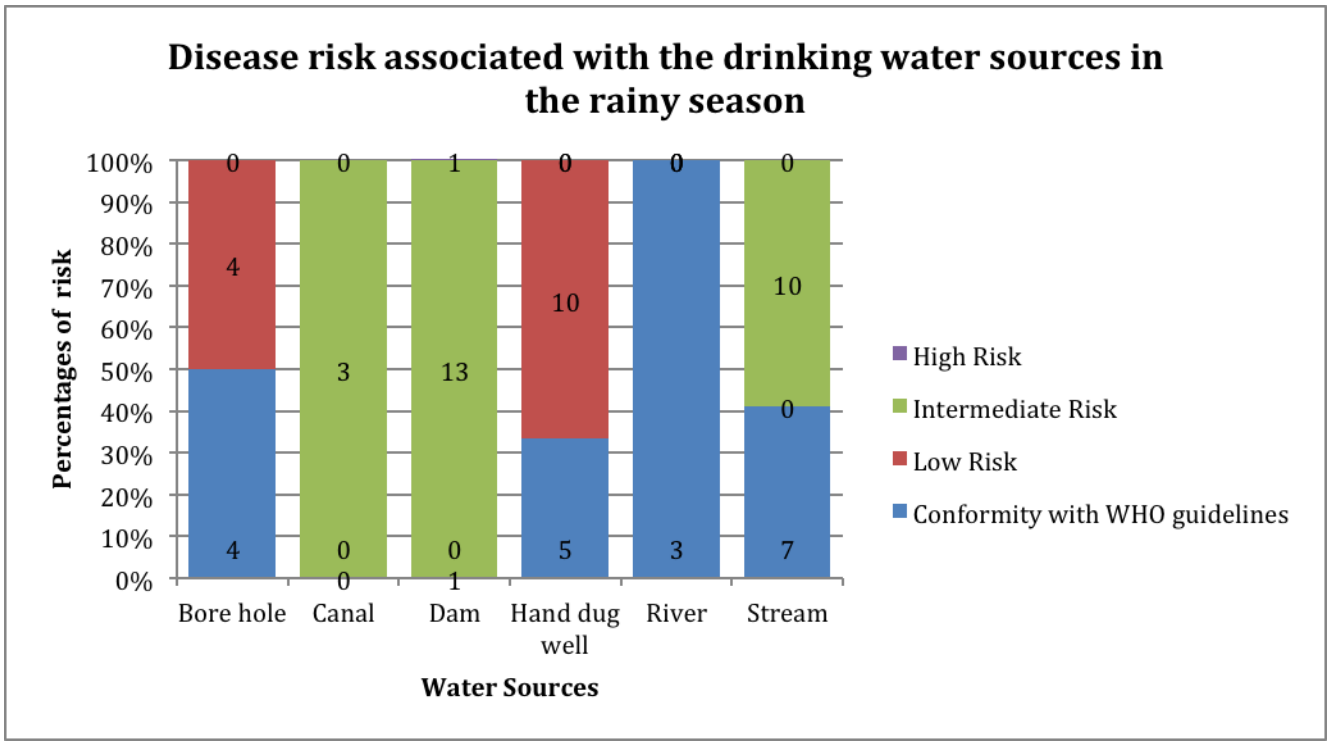

Figure 4.1 Shows the various water sources at each WHO disease-risk level in the Dangme West District during the rainy season. The number in each column section represents the number of sources at each WHO disease-risk level.

Hand-dug wells recoded a 67% low risk levels within its sources. A half (50%) of all the water sources in boreholes were of low risk. Interestingly, all the water sources from rivers were all in conformity with the WHO guidelines. Fifty percent (50%) of borehole water sources were also in conformity with the WHO guidelines. Stream water sources had 41% of

its sources in conformity with the WHO guidelines. None of the water from canal and dams was in conformity with the WHO guidelines. Water from these sources therefore poses great public health threat.

Figure 4.2 shows the disease risk levels of the water sources in the dry season. Interesting, observational patterns occurred. First, none of the water sources posed a high risk during the dry season, compared to what was observed in the rainy season. Second, three of the water sources: canal, dam, and river had none of their sources in conformity with the WHO guidelines during the dry season.

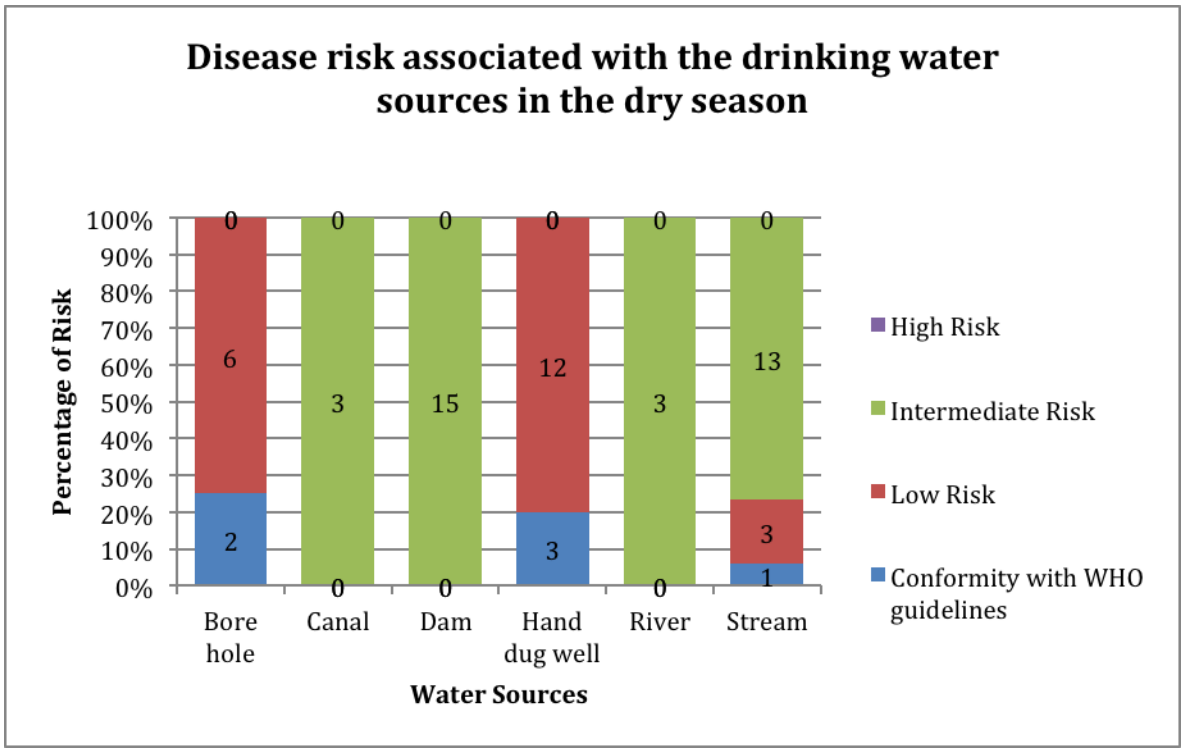

Figure 4.2 shows the various water sources at each WHO disease-risk level in the Dangme District during the dry season. The number in each column section represents the number of sources at each WHO disease-risk level.

River hand-dug wells and canals sources all had their water sources recording intermediate risk (100%). Boreholes and hand-dug wells recorded low risk levels of 75% and 80% respectively among their various water sources. Furthermore, 25% of borehole water sources and 20% of hand-dug well water sources were all in conformity with the WHO guidelines. In the nutshell dam water sources have high disease risk whiles borehole water sources posed low risk.

4.2.4. Analysis of bacteria flora in the drinking water samples.

This section presents the results of the various bacteria species that were isolated from the water sources across the dry and rainy season.

4.2.4.1 Gram-positive and gram-negative bacteria populations

Figure 4.3 shows the distribution of gram-negative bacteria isolated during the rainy season. Stream sources recorded the highest number of gram-negative bacteria followed by dam water sources and hand-dug wells.

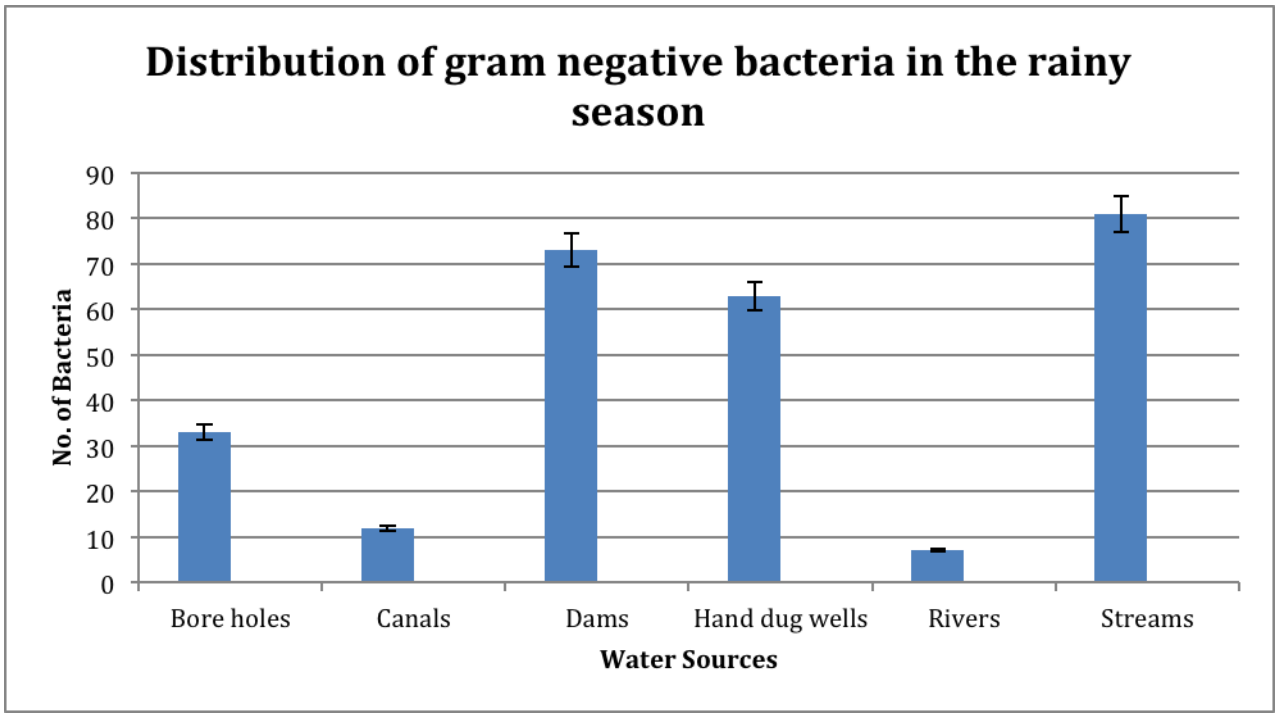

Figure 4.3. The distribution of gram-negative bacteria isolated in the rainy season.

The least number of gram-negative bacteria isolates in the dry season was obtained from river water sources.

Figure 4.4 shows the distribution of gram-negative bacteria isolates during the dry season. Stream water sources recoded the highest number of gram-negative bacteria isolates in the dry season. This was followed by dams and wells. The least number of isolates (7) was obtained form river water sources

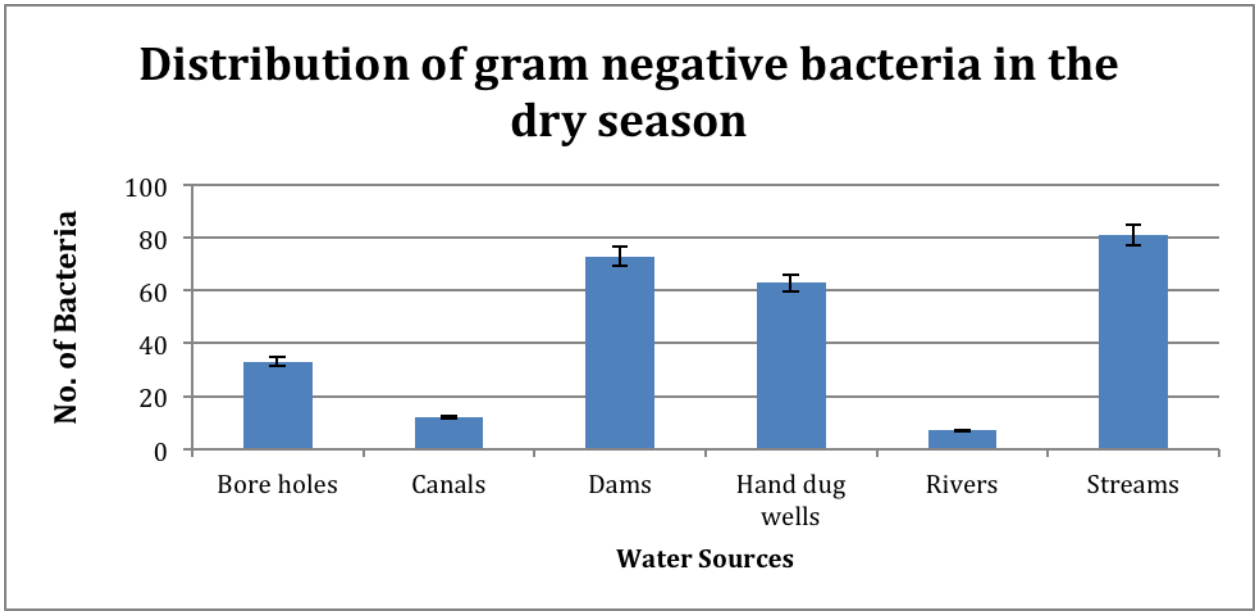

Figure 4.4 The distribution of gram-negative bacteria isolated in the dry season.

Figure 4.5 shows the distribution of gram-positive bacteria isolated during the dry season. Stream water sources had the highest number (10) of gram-positive bacteria in the rainy season.

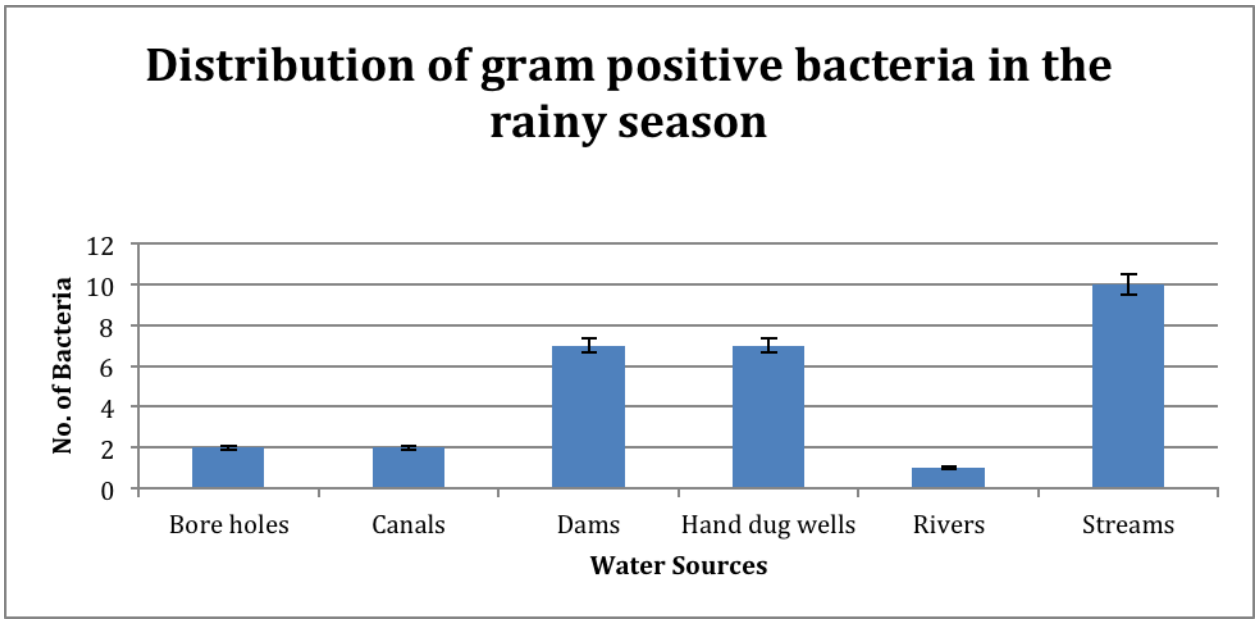

Figure 4.5 The distribution of gram-positive bacteria isolated in the rainy season.

This was followed by hand-dug wells and dam water sources with (7) bacteria isolates each. The least number (1) of gram-positive bacteria were obtained from river water sources.

Figure 4.6 shows the patterns of gram-positive bacteria isolated in the dry season. Hand-dug wells presented with the highest number (11) of bacteria. The second highest number (10) of gram-positive bacteria isolates in the dry season was observed in dam water sources. The least number (1) was recoded in river water sources.

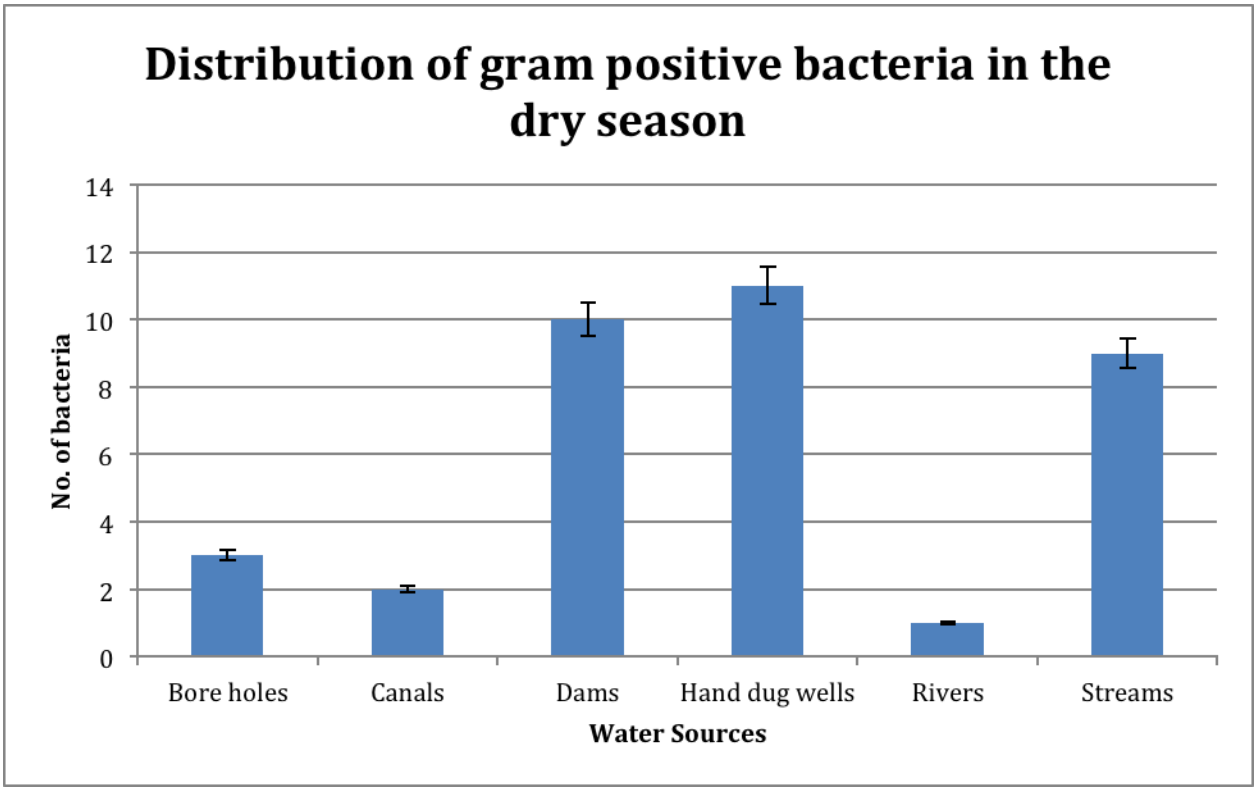

Figure 4.6 The distribution of gram-positive bacteria isolated in the dry season.

Generally, there were more gram-positive bacteria isolated in the dry season as compared to the rainy season.

Figure 4.7 shows the over all percentage distribution of bacteria isolated from the various water sources in terms of gram stain reactions**.** Five hundred and twenty (520) bacteria were isolated. Four hundred and fifty two (452) representing 87.5% were found to be gram negative; whiles sixty eight (68) representing 12.5 % were gram positive.

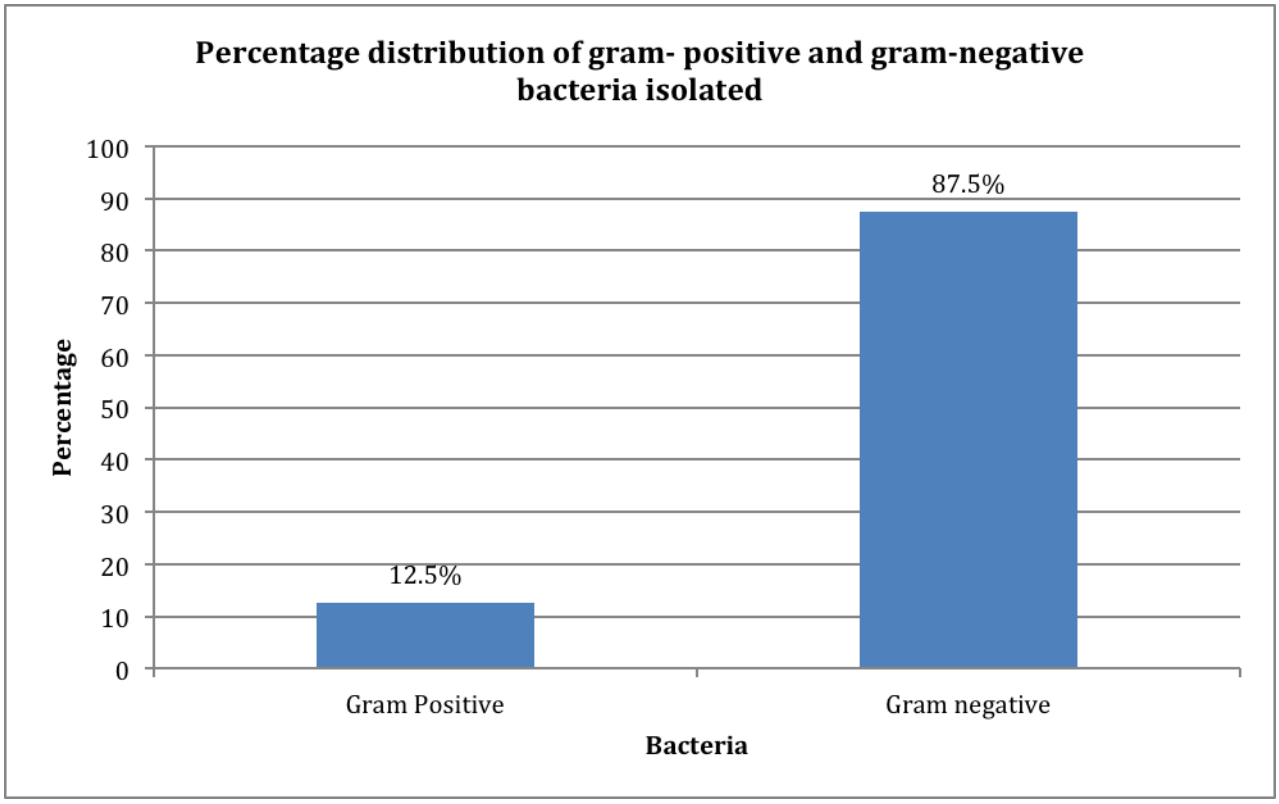

Figure 4.7. Percentage distribution of gram-positive and gram-negative bacteria isolates

4.2.4.2. Profile of different species of bacteria isolated from water sources

Microbiological pathogens that are transmitted by the faecal-oral route, especially those originating from human faeces are of particular concern for public health. Result from the bacteria isolation analysis (tables 4.11 and 4.12), indicates the presence of these oral-faecal pathogens in the various drinking water sources. Bacteria that cause faecal-oral infections such as *Escherichia coli* (diarrhoeal infection or dysentery), *Shigella* spp. (dysentery), *Salmonella typhi* (typhoid fever and acute diarrhoeal infection), *Salmonella typhi* (typhoid fever), and *Vibrio cholerae* (cholera), were isolated.

Table 4.11 shows the numbers and distribution of bacteria isolated during the rainy season. The results show that 215 bacteria were isolated from the different water sources during the rainy season. *Klebsiella* spp. was the highest isolated bacteria (45) representing 20.9% of the total bacteria isolated. *E. coli* followed with 39 isolates representing 18.1% of the total isolates in the rainy season. This was followed by: *Pseudomonas auriginosa* (15.8%);

Enterobacter spp. (14.0%); *Proteus vulgaris* (12.6%); *Enterococcus faecali* (910.7%); *Streptococcus* spp. (2.8%); *Salmonella typhi* (21.4%).

Table 4.11 Distribution of bacteria species isolated from different water sources in the rainy season

Type of Bacteria	Number of bacteria isolates from each water source						Total (%)
	Bore holes	Canals	Dams	Hand-dugwells	Rivers	Streams	
E. coli	4	3	12	10	0	10	39 (18.1)
Enterobacter spp.	2	1	11	8	0	8	30 (14.0)
Klebsiella spp.	4	3	12	10	1	15	45 (20.9)
Salmonella typhi	2	0	0	1	0	1	4 (1.9)
Streptococcus spp.	0	1	2	2	0	1	6 (2.8)
Proteus vulgaris	2	0	10	5	0	10	27 (12.6)
Vibrio cholerae	0	0	1	1	0	2	4 (1.9)
Shigella spp.	0	0	1	0	0	2	3 (1.4)
Pseudomonas aeruginosa	2	1	10	8	1	12	34 (15.8)
Enterococcus faecalis	2	1	5	5	1	9	23 (10.7)
					Total		215 (100)

The least isolated organisms were *Vibrio cholerae* (1.9%) and *Shigella* spp. (1.4%). The three most significant bacteria isolates in terms of public health importance isolated during the rainy season were *E. coli, Vibrio cholerae* and *Shigella s*pp. *E. coli* was isolated in all the water sources except river. *Vibrio cholerae* was isolated in two (2) water sources namely: streams, and dams, whiles *Shigella s*pp. was isolated in streams, and dams water sources only. The highest occurring gram positive organism isolated was *Enterococcus faecalis* (23) representing 10.7% whiles that of gram negative organisms *Klebsiella* spp. (45) *20.9 %*. Generally, the patterns of bacteria isolated in the dry season (table 4.12) did not differ much from that observed in the rainy season. However, the total bacteria isolated in the dry season were 305. The highest occurring bacteria isolated during the dry season were *Klebsiella* spp.

(59), accounting for 19% of the total bacteria isolates of 305. *E. coli* followed with (58) 19.3%, thus almost the same percentage as that of *Klebsiella* spp. The occurrences of the other bacteria were: *Pseudomonas aeuruginosa* (15.4%); *Enterobacter* spp. (8.2.0%); *Proteus vulgaris* (13.4%); *Enterococcus faecalis* (910.7%); *Streptococcus* spp (2.8%); *Salmonella typhi* (21.4%). The least isolated organisms were *Vibrio cholerae* (0.7%) and *Shigella* spp. (1.0%). *Vibrio cholerae* was isolated from borehole and stream sources, whiles Shigella spp. was isolated from stream sources only.

Table 4.12 Distribution of bacteria species isolated from different water sources in the dry season

Type of Bacteria	Number of bacteria isolates from each water source						Total (%)
	Bore holes	Canals	Dams	Hand-dug wells	Rivers	Streams	
E. coli	7	3	16	14	2	16	58 (19.0)
Enterobacter spp.	6	2	15	13	1	13	50 (16.4)
Klebsiella spp.	6	3	16	14	2	18	59 (19.3)
Salmonella typhi	2	1	2	2	0	2	9 (3.0)
Streptococcus spp.	0	0	7	3	0	1	11 (3.6)
Proteus vulgaris	5	1	12	9	0	14	41 (13.4)
Vibrio cholerae	1	0	0	0	0	1	2 (0.7)
Shigella spp.	0	0	0	0	0	3	3 (1.0)
Pseudomonas aeruginosa	6	2	12	11	2	14	47 (15.4)
Enterococcus faecalis	3	2	3	8	1	8	25 (8.2)
					Total		305 (100)

More of the Isolates (305) representing 58.65% of the total were obtained during the dry season, as against (205) representing 41.35% in the rainy season. The most commonly occurring organism in the water samples was *Klebsiella* spp. (20%). The next most occurring bacterial isolate after *Klebsiella* spp. was *E. coli* (18.7%) of the total bacterial Isolates. This

was followed by: *Pseudomonas aeruginosa* (15.61%); *Enterobacter* spp. (15.4%); *Proteus vulgaris* (13.1%); *Enterococcus faecalis* (9.2%); *Streptococcus* spp. (3.1%); *Salmonella typhi* (2.4%). The least isolated organism was *Vibrio cholerae* (1.2%) and *Shigella* spp. (1.2%). *Vibrio cholerae* was isolated in four (4) water sources namely: stream, borehole, hand-dug wells and dam water sources, whiles *Shigella* spp. was isolated in stream, borehole and dam water sources only.

Table 4.13 presents the statistical summary of the bacteria species isolated from different water sources. Generally there was a statistical differences (P> 0.05) of the microbes isolated seasonally. The highest occurring was *Klebsiella* spp. (9.83 ± 6.99, P> 0.05) in the dry season and the least *Shigella* spp. P> 0.05.

Table 4. 13 Statistical summary of bacteria species isolated from different water sources

Bacteria	Rainy season				Dry season				
	Mean±SD	Min	Max	d.f	Mean±SD	Min	Max	d.f	P value
E. coli	6.5±4.81	0	10	5	9.67± 6.47	2	16	5	0.01
Enterobacter spp.	5±4.56	0	11	5	8.33±6.12	1	15	5	0.00
Klebsiella spp.	7.5±5.61	1	15	5	9.83±6.99	2	18	5	0.01
Salmonella typhi	0.67±0.82	0	2	5	1.5±0.84	0	2	5	0.02
Streptococcus spp.	1±0.89	0	2	5	1.83±2.79	0	3	5	0.20
Proteus vulgaris	4.5±4.64	0	10	5	6.83±5.78	0	14	5	0.01
Vibrio cholerae	0.67±0.82	0	2	5	0.33±0.52	0	1	5	0.20
Shigella spp.	0.5±0.84	0	2	5	0.5±1.23	0	3	5	0.50
Pseudomonas aeruginosa	5.67±4.93	1	12	5	7.83±5.23	2	14	5	0.00
Enterococcus faecali	3.83±3.13	1	9	5	4.16±3.06	1	8	5	0.33

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

Figure 4.8 shows the number of bacteria that was isolated from each water source across seasons. The highest number (70) of bacteria isolated in the rainy season was obtained form stream water sources. This was followed by dam water sources with 64 bacteria isolates.

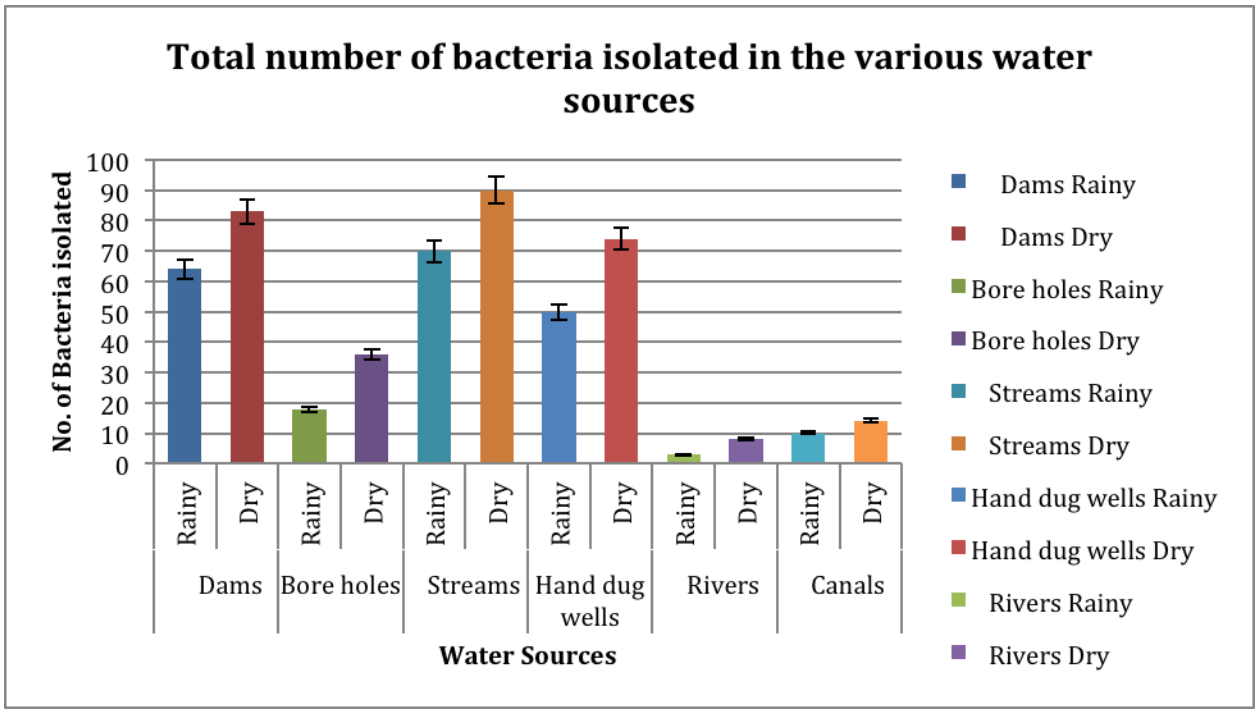

Figure 4.8 Total number of bacteria isolated across seasons, per water source.

The least number (3) of bacteria isolated was obtained from river sources. The highest number (90) of bacteria isolated in dry season was obtained from stream water sources. This was followed by dam water sources with 83 isolates. The least (3) number of bacteria isolated in the dry season was from river water sources. The highest number of bacteria isolated per water source across both the dry and rainy season was 160 representing (21%) this was obtained form stream water sources. The least was 12 (2.1%) obtained from river water sources.

4.2.4.3. Coliform bacteria and non-coliform bacteria

Figure 4.9 shows the percentage distribution of coliform bacteria and non-coliform isolated from the various water sources. Coliform bacteria are generally lactose fermenters and belong to the family enterobacteriaciae. Out of the five hundred and twenty (520) bacteria isolated, three hundred (300) representing 57.7 % were found to be coliform bacteria; whiles two hundred and twenty (220) representing 42.3 % were non-coliform bacteria.

Percentages of coliform and non-coliform bacteria Isolated

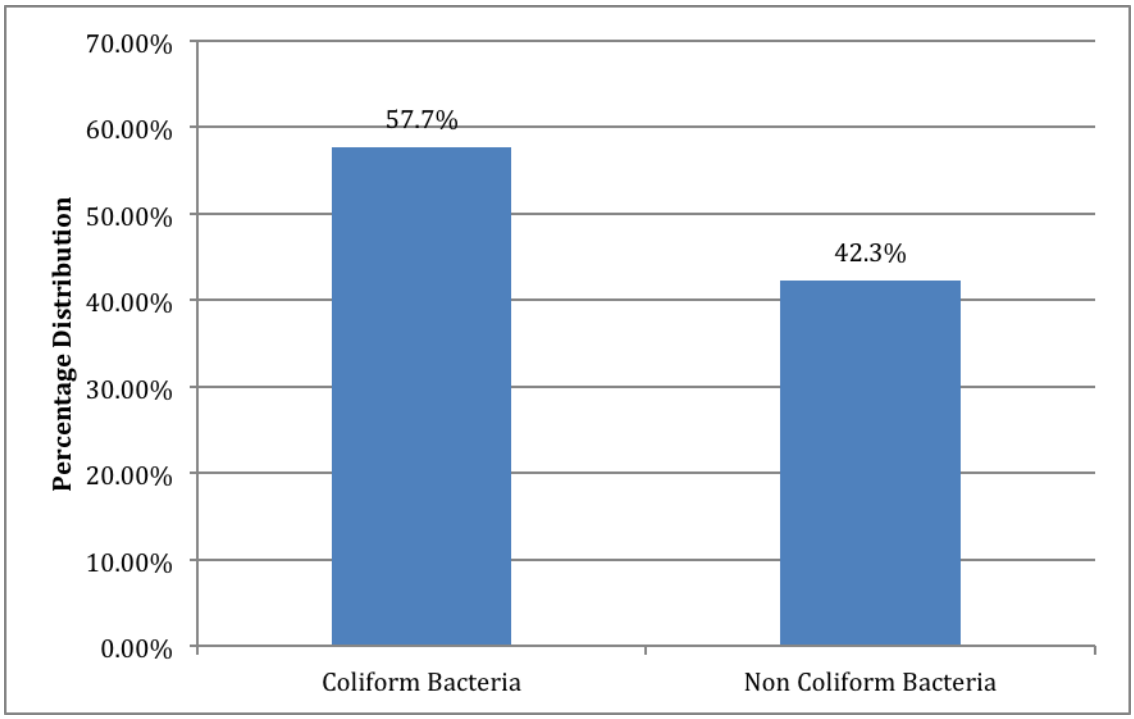

Figure 4.9 Percentage distribution of coliform and non-coliform bacteria obtained from water.

4.2.5. Seasonal distribution of *E. coli* isolates from the water sources

Table 4.14 shows the isolates of *E. coli* and other bacteria from the different water sources. A total number of seventy nine (79) *E. coli* isolates were obtained. This was part of the total number of five hundred and twenty (520) bacteria that were isolated. Dam water sources produced the highest number (28) of *E. coli* isolates representing 28.78% of total *E. coli* Isolated. This was followed by stream water sources with twenty six (26) representing 26.80%. The rest were as follows: hand-dug wells water sources (24) 24.74%, boreholes water sources (11) 11.43%, canal water source (6) 6.19 % and finally river water source two (2) isolates representing 2.06%.

Table 4.14 Isolates of *E. coli* and other bacteria from different water sources

Water sources	Total No. of bacteria n= 520	No of *E. coli* n= 97	*% E. coli*
Borehole	54	11	11.43
Canal	24	6	6.19
Dam	147	28	28.78
Hand-dug well	124	24	24.74
River	11	2	2.06
Streams	160	26	26.80
Total	520	97	100

Table 4.15 presents the paired t test analysis for occurrences of other bacterial and *E. coli* in the various water sources. The occurrences of other bacteria (70.50 ± 53.94) were higher than as compared to that of *E. coli* (16.17 ± 11.22), two-sample t-test, and d.f. = 5, $P<0.05$.

Table 4.15 T- test for the occurrence of other bacteria and *E. coli* in the water sources

Parameter	Mean±SD	Min	Max	d.f	P value
Other Bacteria	70.50±53.94	2	28	5	0.0269
E. coli	16.17±11.22	9	134	5	

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

Table 4.16 shows the seasonal distribution of *E. coli* isolates. Results indicate that out of the ninety-seven (97) *E. coli* isolates, fifty-eight (58) were obtained during the dry season as against thirty-nine (39) in the rainy season. The highest number of isolates from a single water source was from dams (28) representing 29%. This was followed by stream water sources (26) representing 27%, hand-dug wells (24) representing 25%, borehole water sources (11) representing 11%.

Table 4:16 Seasonal distribution *E. coli* isolates

Water sources	No of *E. coli* isolates		
	Rainy	Dry	Total (%)
Borehole	4	7	11(11)
Canal	3	3	6(6)
Dam	12	6	28(29)
Hand-dug well	10	14	24(25)
River	0	2	2(2)
Streams	10	16	26(27)
Total	39	58	97(100)

River water sources produced the least number of isolates (2) representing 2% and then canal water sources (6) representing 6%. The highest isolates during the rainy season were obtained from dam water sources (12) followed by stream water sources (10) and hand-dug wells (10). The highest isolates during the dry season were obtained from stream water sources (17). However, least isolates during the rainy season were obtained from canals (3) followed by borehole water sources (4). No *E. coli* was isolated from river water sources in the rainy season. The least isolates during the dry season were obtained from rivers (2) followed by canal water sources (3)

4.3 Antibiotic resistant profile of *E. coli* isolates

This section presents results and analysis of the antibiotic resistance patterns of the *E. coli* isolated from the water sources. The general susceptibility profiles is presented first in table 4.17. This is followed by susceptibility analyst (sensitive, intermediate and resistant) per the water sources (tables 4.18 - 4.20). The results for the general antibiotic resistance patterns of *E. coli* isolates from the various water sources, are presented in table 4.17. The result (table 4.17) shows that the *E. coli* isolates were most resistant to penicillin (32) representing 32.99%, this was followed by cefuroxime (28) representing 28.87%, erythromycin (23) representing 23.71%, Tetracycline (21) representing 21.45% chloramphenicol (18) representing 18.65%, pipemidic acid (13) representing 13.40%, ampicillin (11) representing 11.32%.

Table 4.17 Antibiotic susceptibility patterns of *E. coli* isolated from the various water sources

Antibiotic description			Susceptibility profile		
Class of antibiotics	Name of antibiotic	Disc con.	Resistant Number (%)	Intermediate Number (%)	Sensitive Number (%)
Aminoglycosides	Amikacin (AMK)	30 µg	7 (7.22)	1 (1.03)	89(91.75)
	Gentamicin (GEN)	10µg	5(5.15)	4(4.12)	88(90.72)
Cephalosporins	Cefuroxime (CXM)	30 µg	28(28.87)	18(18.65)	51(52.58)
	Cefotaxime (CTX)	30 µg	4(4.12)	4(4.12)	89(91.75)
Chloramphenicol	Chloramphenicol (CHL)	30 µg	18(18.56)	12(12.37)	67(69.07)
Co-Trimoxazol	Co-trimoxazole (COT)	25 µg	10(10.31)	6(6.19)	81(83.50)
Macrolides	Erythromycin (ERY)	15µg	23(23.71)	24(24.74)	50(51.55)
Nitrofurantoin	Nitrofurantoin (NIT)	300 µg	4(4.12)	2(2.060	91(93.81)
Penicillins	Ampicillin (AMP)	10 µg	11(11.32)	41(42.27)	45(46.39)
	Penicillin (PEN)	10 units	32(32.99)	51(52.58)	14(14.43)
Pyridopyrimidine	Pipemidic acid (PA)	20 µg	13(13.40	20(20.62)	64(65.98)
Quinolones	Ciprofloxacin (CIP)	5 µg	8(8.25)	17(17.53)	72(74.22)
	Nalidixic acid (NAL)	10 µg	4(4.12)	6(6.19)	87(89.69)
Tetracyclines	Tetracycline (TET)	30 µg	21(21.45)	47(48.45)	29(29.90)

Seven (7) out of the fourteen (14) antibiotics had ten (10) or less number of isolates showing resistance. Four (4) isolates representing 4.12% were resistant to each of the following antibiotic: cefotaxime, nalidixic acid and nitrofurantoin. This was followed by gentamicin (5) representing 5.15%, amikacin (7) representing 7.2%. Ciprofloxacin (8) representing 8.5% and finally, co-trimoxazole (8) representing 8.5%. The results also shows that *E. coli* isolates were most sensitive to nitrofurantoin (91) representing 93.8 %, this was followed by cefotaxime and amikacin (89) representing 91.75 %, gentamicin (88) representing 90.7%, nalidixic acid (87) representing 89.65. ciprofloxacin, (72) representing 74.2%, chloramphenicol (67) representing 69.07.%, pipemidic acid (64) representing 65.97%, and cefuroxime (51) representing 52.58 %. Six (6) out of the fourteen (14) antibiotics had fifty (50) or less number of isolates showing resistance. These were Penicillin (14), tetracycline (29) ampicillin (45) and erythromycin (50). The result (table 4.17) shows that the *E. coli* isolates were most resistant to penicillin (32) representing 32.99%.

Table 4.18 shows the sensitive suceptibility patterns of the *E. coli* Isolates across water sources. Generally, nitrofurantoin was the most sensitive antibiotics across the water sources. The least sensitive was penicillin.

Table 4.18 *E. coli* sensitive antibiotic susceptibility patterns in the water sources

E. coli **sensitive** antibiotic susceptibility patterns in the water source							
Antibiotics description		Boreholes	Canals	Dams	Hand-dug well	River	Streams
Antibiotic type	Disc con.	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)
Amikacin (AMK)	30 µg	10	6	25	22	2	24
Gentamicin (GEN)	10µg	10 (11.36)	5(5.68)	25(28.41)	22(25.00)	2(2.27)	24(27.27)
Cefuroxime (CXM)	30 µg	6 (11.76)	3 (5.88)	14 (27.45)	13 (25.49)	1 (1.96)	14 (27.45)
Cefotaxime (CTX)	30 µg	10 (11.24)	6 (6.74)	25 (28.09)	22 (24.72)	2 (2.25)	24 (26.97)
Chloramphenicol (CHL)	30 µg	8 (11.94)	4 (5.97)	19 (28.36)	17 (25.37)	1(1.49)	18 (26.87)
Co-trimoxazole (COT)	25 µg	9 (11.11)	5 (6.17)	23 (28.40)	20 (24.69)	2 (2.47)	22 (27.16)
Erythromycin (ERY)	15µg	7 (14.00)	3 (6.00)	14 (28.00)	12 (24.00)	1 (2.00)	13 (26.00)
Nitrofurantoin (NIT)	300 µg	10 (10.99)	6 (6.59)	26 (28.57)	23 (25.27)	2 (2.20)	24 (26.37)
Ampicillin (AMP)	10 µg	5 (11.11)	3 (6.67)	13 (28.89)	11 (24.44)	1 (2.22)	12 (26.67)
Penicillin (PEN)	10 units	2 (14.29)	1 (7.14)	4 (28.57)	3 (21.43)	0 (0.00)	4 (28.57)
Pipemidic acid (PA)	20 µg	7 (10.94)	4 (6.25)	18 (28.13)	16 (25.00)	1 (1.56)	18 (28.13)
Ciprofloxacin (CIP)	5 µg	9 (12.50)	4 (5.56)	21 (29.17)	18 (25.00)	1 (1.39)	19 (26.39)
Nalidixic acid (NAL)	10 µg	10 (11.49)	5 (5.75)	25 (28.74)	22 (25.29)	2 (2.30)	23 (26.44)
Tetracycline	30 µg	3 (10.34)	2 (6.90)	8 (27.59)	7 (24.14)	1 (3.45)	8 (27.59)

Table 4.19 shows the intermediate antibiotic patterns of the *E. coli* isolates across water sources. Generally. *E. coli* showed the least intermediate susceptibility patterns to Amikacin. The highest *E. coli* intermediate susceptibility observed was penicillin.

Table 4.19 *E. coli* intermediate antibiotic susceptibility patterns in the water sources

E. coli antibiotic **intermediate** patterns across water sources							
Antibiotics description		Bore holes	Canals	Dams	Hand-dug well	River	Streams
Antibiotic type	Disc con.	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)
Amikacin (AMK)	30 µg	0 (0.00)	0 (0.00)	1(100)	0 (0.00)	0 (0.00)	0 (0.00)
Gentamicin (GEN)	10µg	1 (25.00)	0 (0.00)	1 (25.00)	1(25.00)	0 (0.00)	1 (25.00)
Cefuroxime (CXM)	30 µg	2 (11.11)	2 (11.11)	5 (27.78)	4 (22.22)	0 (0.00)	5 (27.78)
Cefotaxime (CTX)	30 µg	0 (0.00)	1 (25.00)	1 (25.00)	0 (0.00)	1 (25.00)	1 (25.00)
Chloramphenicol (CHL)	30 µg	1 (8.33)	1 (8.33)	4 (33.33)	3 (25.00)	0 (0.00)	3 (25.00)
Co-trimoxazole (COT)	25 µg	1(16.67)	0 (0.00)	3 (50.00)	1 (16.67)	0 (0.00)	1 (16.67)
Erythromycin (ERY)	15µg	3 (12.50)	1 (4.17)	7 (29.17)	6 (25.00)	1 (4.17)	6 (25.00)
Nitrofurantoin (NIT)	300 µg	1 (50.00)	0 (0.00)	1 (50.00)	0 (0.00)	0 (0.00)	0 (0.00)
Ampicillin (AMP)	10 µg	5 (12.20)	3 (7.32)	11 (26.83)	10 (24.39)	1 (2.44)	11 (26.83)
Penicillin (PEN)	10 units	6 (11.76)	3 (5.88)	14 (27.45)	13 (25.49)	1 (1.96)	14 (27.45)
Pipemidic acid (PA)	20 µg	2 (10.00)	1 (5.00)	6 (30.00)	5 (25.00)	0 (0.00)	6 (30.00)
Ciprofloxacin (CIP)	5 µg	2 (11.76)	1 (5.88)	5 (29.41)	4 (23.53)	0 (0.00)	5 (29.41)
Nalidixic acid (NAL)	10 µg	1 (16.67)	0 (0.00)	2 (33.33)	1 (16.67)	0 (0.00)	2 (33.33)
Tetracycline (TET)	30 µg	5 (10.64)	3 (6.38)	14 (29.79)	12 (25.53)	0 (0.00)	13 (27.66)

Table 4.20 shows the resistant antibiotic susceptibility patterns of the *E. coli* Isolates across water sources. The subsequent section gives a detail analysis of the resistant patterns.

Table 4.20 *E. coli* resistant antibiotic susceptibility patterns in the water sources

E. coli antibiotic **resistant** patterns across water sources							
Antibiotics description		Bore holes	Canals	Dams	Hand-dug well	River	Streams
Antibiotic type	Disc con.	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)
Amikacin (AMK)	30 µg	1 (14.29)	0 (0.00)	2 (28.57)	2 (28.57)	0 (0.00)	2 (28.57)
Gentamicin (GEN)	10µg	1 (20.00)	0 (0.00)	2 (40.00)	1 (20.00)	0 (0.00)	1 (20.00)
Cefuroxime (CXM)	30 µg	3 (10.71)	1 (3.57)	8 (28.57)	7 (25.00)	1 (3.57)	8 (28.57)
Cefotaxime (CTX)	30 µg	0 (0.00)	1 (25.00)	1 (25.00)	0 (0.00)	1 (25.00)	1 (25.00)
Chloramphenicol (CHL)	30 µg	2 (11.11)	1 (5.56)	5 (27.78)	5 (27.78)	0 (0.00)	5 (27.78)
Co-trimoxazole (COT)	25 µg	2 (20.00)	1 (10.00)	3 (30.00)	3 (30.00)	0 (0.00)	1 (10.00)
81Erythromycin (ERY)	15µg	3 (13.04)	1 (4.35)	7 (30.43)	4 (17.39)	1 (4.35)	7 (30.43)
Nitrofurantoin (NIT)	300 µg	0 (0.00)	1 (25.00)	1 (25.00)	0 (0.00)	1 (25.00)	1 (25.00)
Ampicillin (AMP)	10 µg	2 (18.18)	1 (9.09)	2 (18.18)	3 (27.27)	0 (0.00)	3 (27.27)
Penicillin (PEN)	10 units	3 (9.38)	1 (3.13)	10 (31.25)	8 (25.00)	1 (3.13)	9 (28.13)
Pipemidic acid (PA)	20 µg	2 (15.38)	1 (7.69)	2 (15.38)	4 (30.77)	1 (7.69)	3 (23.08)
Ciprofloxacin (CIP)	5 µg	1 (12.5)	0 (0.00)	2 (25.00)	3 (37.50)	0 (0.00)	2 (25.00)
Nalidixic acid (NAL)	10 µg	1 (25.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (25.00)	2 (50.00)
87Tetracycline (TET)	30 µg	0 (0.00)	1 (4.76)	7 (33.33)	2 (9.52)	1 (4.76)	10 (47.62)

Table 4.21 shows the seasonal distribution of Multiple resistant *E. coli* isolates in the water. The number of multiple resistant *E. coli* isolated ranged between 1 to 8 in the dry season. The highest number (8) of multidrug resistant isolates in the dry reason was obtained from stream water sources, whilst the least was from river water sources (1).

Table 4.21 A seasonal distribution of multiple resistant *E. coli* isolates

Water source	No. of Multiple resistance isolates	
	Dry Season	Rainy Season
Borehole	4	6
Canal	3	1
Dam	8	6
Hand-dug well	5	4
River	1	0
Streams	7	3
N	6	6
Mean	4.67	3.33
Std Dev	2.58	2.50
S. E	1.05	1.02

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

Table 4.22 presents the paired t test analysis for season distribution of multiple resistances in *E. coli*. The total number of multi resistance isolates (n=6) averaged 4.67 ± 2.58 and (n=6) averaged 3.33 ±2.50 in the dry and rainy seasons respectively. There was a significance difference (t-test, and d.f. = 5, $P<0.05$) between the number of multiple antibiotic resistance *E. coli* isolated in the dry and rainy seasons.

Table 4.22 T test for the seasonal distribution of multiple resistant *E. coli* isolates

Season	Mean±SD	Min	Max	d.f	P value
Dry	4.66 ±2.58	1	8	5	0.1576
Rainy	3.33 ±2.50	1	6	5	

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

Table 4.23 shows the distribution of the antibiotic resistant *E. coli* isolates and the number of antibiotic to which they were observed to be resistant. The highest number (16) of antibiotics to which *E. coli* was resistance in a single water source was observed in stream water sources

and the least (1) was from borehole and river water sources. Two *E. coli* isolates were observed to be resistance to 10 different antibiotics. This was recorded in dams and stream waters sources. A summary of the resistant profiles and the kind of antibiotics to which the *E. coli* showed resistant are presented in tables 4.24 and 4.25

Table 4.23 Summary of the distribution of antibiotic resistance *E. coli* in water sources

Water source	Number of isolates	Number of isolates that showed resistance									
		1	2	3	4	5	6	7	8	9	10
Borehole	11	1	7	2	1	0	0	0	0	0	0
Canal	6	2	1	1		1	1	0	0	0	0
Dam	28	14	4	1	3	1	0	2	1	1	1
Hand-dug well	24	15	2	1	4	1	1	0	0	0	0
River	2	1	0	0	0	1	0	0	0	0	0
Streams	26	16	0	0	5	1	0	1	1	1	1

Table 4.25 shows a summary of the number of antibiotic to which *E. coli* isolated showed resistance in water sources. Out of the 97 *E. coli* isolates, the table reveals that forty-nine isolates (49) representing 50.52% exhibited resistance to one antibiotic or the other. However, forty eight (48) isolates showed resistances two (2) or more antibiotics, thus classified as multi antibiotic resistance (Hill *et al.*, 2005).

Table 4.24 Summary of antibiotic resistance profile of *E. coli* isolated from various water sources

Antibiotic tested	Number of resistant isolates (%)
One antibiotic	49(50.52)
Two antibiotic	14(14.43)
Three antibiotic	5(5.15)
Four antibiotic	13(13.40)
Five antibiotic	5(5.15)
Six antibiotic	2(2.06)
Seven antibiotic	3(3.09)
Eight antibiotic	2(2.06)
Nine antibiotic	2(2.06)
Ten antibiotic	2(2.06)

Two (2) isolates showed resistances to six (6), eight (8), nine (9), and ten (10) different antibiotics. Two other isolates showed resistance to two (2) antibiotics and four isolates to thirteen (13) antibiotics. Fives isolates showed resistance to three(3) different antibiotics, whiles another five isolates also showed resistance five antibiotics.

Table 4.25 shows the various antibiotics to which the *E. coli* isolates were resistance. PEN-CM had the highest (14) combination of multi resistances. This was followed by CXM-ERY-TET

Table 4.25 Antibiotic resistance profile (antibiogram) of *E. coli* from various water sources

Antibiotic resistant profile										Number of resistant isolates
PEN	CXM	ERY	TET	CHL	PA	AMP	COT	CIP	NIT	2
PEN	CXM	ERY	TET	CHL	PA	AMP	NAL	GEN		2
PEN	CXM	ERY	TET	CHL	PA	AMK	COT			2
PEN	CXM	ERY	TET	CHL	PA	CTX				3
PEN	CXM	ERY	TET	CHL	PA					2
PEN	CXM	ERY	TET	CHL						5
PEN	CXM	ERY	TET							13
PEN	CXM	ERY								5
PEN	CXM									14
PEN										49

CHL= Chloramphenicol; COT= Co-trimoxazole; ERY= Erythromycin; NIT= Nitrofurantoin; AMP= Ampicillin; PEN= Penicillin; PA= Pipemidic acid; CIP= Ciprofloxacin; NAL= Nalidixic acid; TET = Tetracycline; CXM = Cefuroxime

4.4 Multiple antimicrobial resistance (MAR) index profiles of *E. coli* isolates

Figure 4.10 Shows the seasonal occurrence of *E. coli* isolates with MAR index>2. The MAR Index of an isolate is defined as a/b, where a represents the number of antibiotics to which the isolate was resistant and b represents the number of antibiotics to which the isolate was subjected. It can be observed from the graph that sixteen (16) *E. coli* isolates with MAR index>2 representing 53% were obtained in the dry reason. Fourteen (14) isolates *E. coli* isolates with MAR index>2 representing 47 % were obtained in the rainy season.

Seasonal occurrence of *E. coli* isolates with MAR index > 2

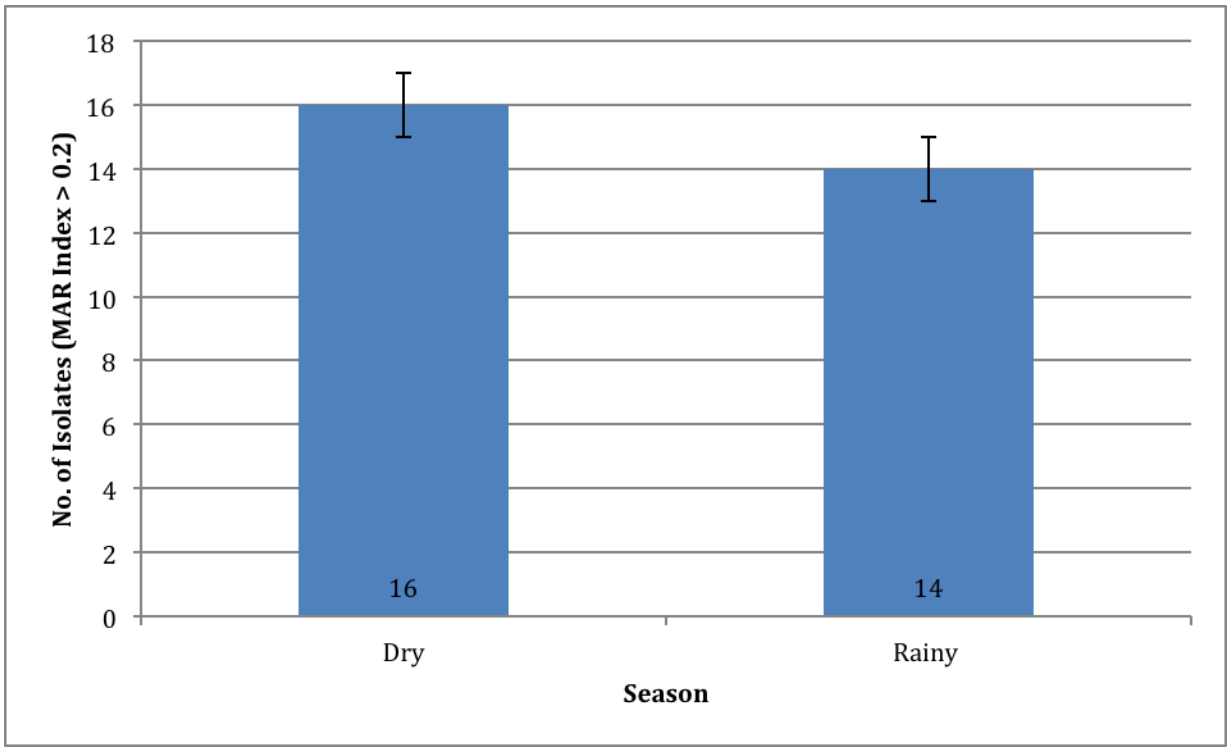

Figure. 4.10. The seasonal occurrence of *E. coli* isolates with MAR index>2

Table 4.27 shows the multiple antibiotic resistant indexes of *E. coli* isolates at various water sources. Borehole water sources recorded the highest MARp values of 0.9. This was followed by canal water source with a value of 0.7. The least was obtained from stream water sources and hand-dug well water sources, both recorded values of 0.4

Table 4.26 Multiple antibiotic resistant indexes of *E coli* isolates at various water sources

Sampling site	Total numbers of test (isolates)	No of resistant test (resistant isolates)	MAR p
Borehole	11	10	0.9
Canal	6	4	0.7
Dam	28	14	0.5
Hand-dug well	24	9	0.4
River	2	1	0.5
Streams	26	10	0.4

MAR p = MAR index per sampling source.

4.5 Distribution and diversity of *E. coli* virulence factors

The results for the molecular characterization of multi drug resistant *E. coli* is presented in this section.

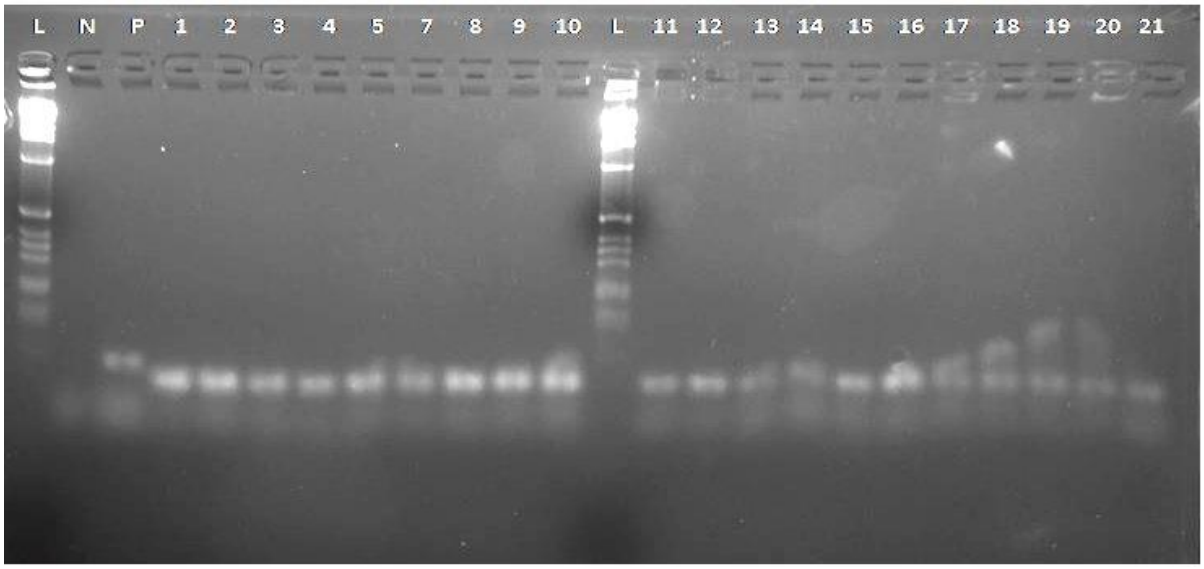

Figure 4.11 picture showing amplicons on a gel

Figure 4.11 shows the amplification of bands on the gel, for the first few multidrug resistant isolates. This is critical before the next step of hybridization can be carried out. The presence of bands indicates that DNA has been successfully extracted and amplified. The amplicons generated are for 42bp (*E. coli*), 5bp (*IpaH*) and 50bp (*eae*), 63bp (universal control). If *stx1* and *stx2* were present, they would have been shown on length 93bp and 115bp respectively.

Table 4.27 below presents a summary of the molecular characterisation of the multi drug resistant isolates. First, the results from the table show that all 48 isolates were confirmed as *E. coli* by the PCR. Second, none of the isolate had gene producing *Stx1* and *stx2*. However, three (3) of the multidrug resistant *E. coli* isolates representing 6% were found to be *eae* producers. The strip band hybridization pictures are presented in appendix 13.

Table 4.27 Summary of molecular characterization of virulence genes

Molecular parameters	Positive		Negative	
	Number	Percentage (%)	Number	Percentage (%)
Cc	48	100	0	0
Uc	48	100	0	0
E.coli	48	100	0	0
ipaH	0	0	48	100
stx1	0	0	48	100
stx2	0	0	48	100
Eae	3	6	45	94

4.6 Comparison of methods for detection of *E. coli*

This section presents the analysis for the detection methods of *E. coli*. Table 4.29 shows the comparison for the specificity and sensitivity of three (API, Culture/BCT and PCR) test methods used for detection of *E. coli*. The analytical profile index (API) detected ninety six (96) out of the total number of ninety seven (97) *E. coli* isolates.

The specificity evaluations came up to 99.7% and sensitivity was 98.50 %. The culture/ biochemical based methods for detection of *E coli* showed a specificity of 81.82% and a sensitivity of 96.91%. The PCR was the gold standard for the detection.

Table 4.28 Evaluation specificity and sensitivity of test methods for detection of *E. coli*

Test method	Number of E. *coli* detect	Specificity (%)	Sensitivity (%)
API 20E	96	99.77	98.50
Culture/BCT	94	81.82	96.91
PCR	97	100	100

Key: API = analytical profile index; BCT= biochemical testing; PCR= polymerase chain reaction

4.7 Association Between Antibiotic Resistance and Radiation Sensitivity (D_{10})

This section presents results and analysis of the radiation sensitivity (D_{10}) of the *E. coli* isolated from the water sources. The general statistical summary is presented first in table.4.29. A scatter plot of radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) of *E. coli* are also provided. Finally, a simple regression analysis of the association between D_{10} and multiple antibiotic resistances index of *E. coli* is presented.

Table 4.30 shows the descriptive statistical summary of the measures of radiation sensitivity (D_{10}) obtained from the multi-resistant *E. coli* isolates.

The radiation sensitivity (D_{10}) for 29 multidrug resistant isolates was measured. The mean radiation sensitivity (D_{10}) is 0.33±0.11 kGy. This means that average radiation doses of 0.33±0.11 kGy will be required to reduce the number of *E. coli* by 10-fold (one log cycle) or required to kill 90% of the total number (Whitby & Gelda, 1979).

Table 4. 29 Statistical summary of the radiation sensitivity (D_{10}) of the multi-resistant *E. coli* isolates

Parameter	Mean±SD	Min	Max	d.f	95.0% Confidence Interval	
					Lower Boundary	Upper Boundary
D_{10}	0.33±0.11	0.05	0.5	28	-0.133	0.79

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

Table 4.30 presents a simple regression analysis of the test of association between the radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) index of *E. coli*. The correlation coefficient (r) value of 0.27 indicates that there is no linear relationship between the radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) index of *E. coli*. The corresponding significance level of 0.156 also implies that there is no relationship or association between the radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) of *E. coli.* The larger insignificant P value (0.156) also suggests that changes in the predicator (multiple resistance indexes of multiple resistant *E. coli*) are not associated with the changes in the response) radiation sensitivity (D_{10})

Table 4.30 A simple regression analysis correlation between D_{10} and multiple antibiotic resistances of *E. coli* index.

	Coefficient	Standard error	Standardized coefficient	*t*	*P*
Intercept	0.270	0.043	0	6.237	0.000
Slope	0.223	0.153	0.270	1.459	.0.156
Correlation coefficient (r)=0.270, r^2=0.039					
Source	df	MS	*F*	*P*	
Regression	1	0.025	2.12	0.156	
Residual	27	0.012			

Significant at 0.05

CHAPTER FIVE
5.0 DISCUSSIONS

5.1 Introduction

This chapter focuses on interesting and important findings, which have been adduced from the empirical evidence emanating from the analysis of the study. The chapter has been structured into several sections to correspond with the different aspects of the analyses carried out. Thus the discussion centers around five themes reflecting the results namely: (a) bacteriological contaminants of water: total coliforms, faecal coliforms and *E. coli* assessment and bacteriological profile of microbes in the water sources. (b) Prevalence and susceptibility profiles of antibiotic resistant water-borne *E. coli.* (c) Virulence genes associated with multiple antibiotic resistant *E. coli* isolates. (d) Comparison of three laboratory based techniques (PCR, API 20E, and culture/biochemical test) used for detection of *E. coli,* and, (e) the association between antibiotic resistance and radiation sensitivity (D_{10}).

5.2 Bacteria Contamination of water: coliforms, and bacteriological profiles.

What is the faecal and total coliform status of the drinking water sources in the Dangme West District? In addition, what is the seasonal distribution of bacterial flora in the drinking water sources? The findings from this research have answered these research questions, eliciting some very interesting phenomenon and observation. In developing countries such as Ghana, where the majority of populace live in rural settings, raw water sources such as streams, dams, rivers, hand dug wells and lately boreholes appear to be the main sources of water for drinking and domestic uses. However, public and environmental health protection requires safe drinking water, which means that it must be free of pathogenic bacteria. Among pathogens disseminated in water sources, enteric pathogens are the ones most frequently encountered.

The coliform test is therefore the starting point for determining the domestic pollution level in water samples. This test is of great importance because of the acute risk that disease-causing organism poses to users of water (Pinfold, 1990; Nala *et al.,* 2003; Ampofo & Karikari,

2006). Microbial hazards are know to represent an overall greater threat than chemical hazards, and in developing countries account for 5.7% of the total global burden of disease (Larmie & Paintsil, 1996). Ideally, drinking water is expected to be pristine and indicator organisms must not be detected in any 100 ml water sample (WHO, 2006). Furthermore, groundwater sources are considered and known to be of better microbial quality than surface water (Omari & Yeboah-Manu, 2012) however, the results obtained from this study showed a high total and faecal coliform contaminations. Groundwater sources were observed to be as polluted as surface water sources.

The current study also evaluated the *E. coli* counts in the water as a confirmation of the observations made on the coliform populations analysis and as basis for the risk assessments of the water sources. *E. coli* counts are sometimes used as a surrogate of faecal coliform counts due to the reduced chance of having false positives, for example *Klebsiella* spp counted as faecal coliforms (Shireman & Cichra, 1994). *E. coli* counts are also better indicators of faecal pollution than faecal coliform counts because some faecal coliform bacteria may replicate in the environment and falsely elevate indicator organism levels (McLellan *et al.,* 2001). The use of *E. coli* as an indicator of faecal contamination is recommended by the United States Environmental Protection Agency (U.S. EPA) based on studies demonstrating a direct relationship between the density of *E. coli* organisms in the water and the occurrence of swimming-associated gastroenteritis (Dufour 1984; U.S. EPA 1986, Pruss 1998). Furthermore, *E. coli* counts is know to be an indicator of human, domestic, and natural sources of faecal contamination (McLellan & Jensen, 2003; Odonkor & Ampofo, 2013).

The results from the current study also observed the presence of other bacteria of public health importance in the water sources. These included *Shigella* spp. (dysentery), *Salmonella typhi* (typhoid fever and acute diarrhoeal infection), *Salmonella typhi* (typhoid fever), and *Vibrio cholerae* (cholera). Obviously, the detection of these bacterial species further demonstrates the level of faecal contamination of the water sources in the study area. High incidence of diarrhoea is associated with drinking of contaminated water, and people who are at high risk include the very young and the very old, as well as immune-compromised

individuals, such as those suffering from HIV/AIDS (Howard *et al.,* 2006; Mahvi & Karyab, 2007).

Recent studies have shown that rural waters sources have high concentration of faecal coliforms (Obiri-Danso, *et al.,* 2005; Nkansah, *et al.,* 2010*;* Omari & Yeboah-Manu, 2012). Similarly, Nogueira *et al.* (2003) reported that untreated water sources were more heavily contaminated with both total and faecal coliforms than treated water sources. The empirical findings from this current study support and augment these findings. Thus bacteriological quality of both surface and underground water of the rural district is usually poor. At first it was difficult to make definite pronouncements on the entirety of pollution levels of the entire water sources available in the rural communities as various investigators researched into one or few ground water or surfaces water sources.

There was yet another interesting observation from this current study. Higher coliform counts $P \leq 0.05$ were recorded in the dry season compared to the rainy season. This was in contrast to the observations made in other studies (Obiri-Danso, *et al*., 2005*;* Anim, *et al.,* 2010). However enumeration of *E coli* MPN/100ml generally recorded higher counts in the rainy season than in the dry season. This was in agreement with previous studies in which higher counts were observed in rainy season compared to dry season. The bacteria isolated in this current study, agrees with various studies that reported high levels of microbial contamination in many rural water sources. For example the isolation and identification of several bacteria species in the water sources in the current study, corroborates findings by Obi *et al.*, (1998), which revealed that the majority of the water sources in rural communities in Nigeria harboured various enteric pathogens and were also reported to be of poor microbiological quality and unsafe for consumption.

Normally, overland wastes move into water sources such as rivers and streams during periods of heavy or extended precipitation. This is expected to cause a higher indicator bacteria numbers during the rainy season compared with the dry season. The opposite of higher bacteria counts in the dry season was observed in this current study as compared to the rainy season. A number of reasons could be adduced for this observation. First, this observation

could be as a result of scarcity of water and rapid multiplication of the bacteria, due to favourable weather condition in the dry season. Another reason could be that rains lead to dilution due to run-offs thus less pollution. Additionally, bacterial contaminants from incinerators, refuse dumps, and human effluents wash down into the various water sources daily multiplying in the dry season and subsequently washed down during the rainy season. This also makes them potential sources of conveying microbial pathogens creating greater health complications in rural communities.

Another interesting and important observation of this study was the apparent predominance of *Klebsiella* spp. (Table 4.13). *Klebsiella pneumoniae* is a rod shaped non-motile, gram negative, lactose fermenting and facultative anaerobic bacterium, which are usually found in the normal flora of skin, mouth, and intestines. *Klebsiella* spp. is responsible for pneumonia (the destructive lung inflammation disease). Besides *Klebsiella* is found to cause infections in the urinary and lower biliary tract (Ryan & Ray, 2004; Lopes *et al*., 2005). *Klebsiella* is an opportunistic pathogen that primarily attacks immune-compromised individuals and hospitalized patients (Podschun & Ullmann, 1998). The predominance of *Klebsiella* spp. as opposed to *E. coli* is because *Klebsiella* spp. can survive and remain physiologically active under diverse environmental conditions under which they are exposed (Lopez-Torees *et al.,* 1987). Second, they multiply to high numbers in waters rich in nutrients, such as pulp mill wastes, etc. The environmental condition of the water sources in the area under study therefore made it conducive for their growth and survival than *E. coli*. Earlier works done though inconclusive appears to support the observation in the current study (Podschun *et al.,* 2001).

What could account for the high coliform and *E. col*i counts observed in the water sources? Human activities as well as faecal discharges from animals may be major contributing factors. However the relative importance of specific animals as contributors to the high faecal coliform numbers observed here is difficult to assess with confidence and was not formally examined in this study. However, it is probably related to factors such as animal population density and utilization of the territory adjacent to the sample sites.

While human input was not the major cause of elevated faecal coliform levels for many of the samples analyzed for this study, these findings suggest that human faecal pollution may be masked by that of other sources such as wild and domestic animals, particularly when indicator organism counts are high. This is an important consideration when assessing risk of environmental waters contaminated with feaces. While the faecal input from wild animals may be a natural and unstoppable occurrence that results in alarming indicator levels, low levels of human faecal material containing human pathogens may go undetected by standard monitoring practices.

However, some observations in the district suggest the reasons for human faecal contaminations of the waters sources. For example, lack of proper and permanent disposal sites for both solid and liquid wastes in the district may result in the use of streams as receptacles for these untreated wastes. In addition, some residents resort to insanitary practices such as defecating or urinating into open space, gutters which ultimately find their way into bodies of water.

Furthermore, the groundwater (wells) did not have proper physical barriers. For example the wells were observed to have missing covers, lockable sanitary lids and well linings, which could prevent overland runoff containing human, animals and domestic wastes from contaminating the water sources. This could account for the detection of bacteria of faecal origin in groundwater in the study area. The WHO (2006) reported that groundwater is less vulnerable to contamination due to the barrier effect, and that once the protective barrier is breached direct contamination may occur. In the cases of boreholes, Chapman (1996) noted that due to the relatively slow movement of water through the ground, once polluted, a groundwater body could remain so for decades, or even centuries.

The implication and importance of this finding is momentous and cannot be overemphasized. Findings from this study indicate that rural folks residing in the Dangme West District are at high risk and are highly vulnerable to waterborne diseases resulting from the presence of pathogenic bacteria in the water. This results from several activities, which include increased pollution from various human activities.

Cyclic assessment of the quality of water available to the rural communities may not only be deemed expedient but also fitting. Since many rural people usually rely chiefly on untreated water sources, the presence of coliform bacteria in all the water bodies then calls for concern from the government, corporate bodies as well as the council of elders of the respective communities involved in rural water provision. Taking into account the socio-economic significance of access to safe and potable water, it may be deemed necessary to consider all the water sources for rural communities rather than concentrating on only a single source such as boreholes which may not only serve a handful of the residents but also be accompanied by high drilling costs.

5.3. Prevalence and susceptibility profiles of antibiotic resistant water-borne *E. coli*

What are the prevalence and susceptibility profiles of antibiotic resistant water-borne *E. coli*? Findings from the current study have provided answers to this question and have offered a great deal of insights to antibiotic resistances in aquatic environments in Ghana. Antimicrobial resistance in bacteria associated with different ecological niches has been a global concern. This is because emergence of antimicrobial resistant strains of pathogenic bacteria has become a great threat to public health. The study of resistances in environmental bacteria helps predict future emergence and guide the development of strategies to counteract this resistance. Drinking waters sources are expected to be free from microbes, let alone antibiotic resistance bacteria. However data from this current study revealed a high level of multiple resistance patterns between *E. coli* isolated from the water sources. Indeed the high prevalence (49.48%) of multiple antibiotic resistant *E. coli* observed in this current study coupled with high resistances to penicillin (32.99%), Cefuroxime (28.87%), Erythromycin (23.71%), and tetracycline (21.45%), is of serious concern. More seriously the large number (49.5%) *E. coli* isolates that exhibited resistance against two (2) or more antibiotics (Table 4.25), thus classified as multi drug/antibiotic resistance (Hill *et al.,* 2005) is also of serious concern.

Although antibiotic resistances in aquatic environments remains a grey area in Ghana and as such not much work has been done to evaluate the prevalence of antibiotic resistant bacteria

from drinking water sources in Ghana, several other studies have been conducted on the prevalence of antibiotic resistant *E. coli* isolates from water bodies elsewhere in many other countries. The prevalence of resistance among *E. coli* isolates in the aquatic environment to penicillins and tetracyclines class of antibiotics has previously been reported in parts of Asia, Turkey, and South Africa. These range from 76 - 89% (Abdul, 2006; Osman *et al.*, 2007; Atif, 2010), 26 - 54% (Boon & Cattanach, 1999; Abdul Malik, 2006), 41 - 80.6% (Raida *et al.*, 2005; Watkinson *et al.*, 2007) and 51 - 76% (Abdul, 2006; Osman *et al.*, 2007; Watkinson, 2007). Comparatively this indicates a lower level of resistances to these two classes of antibiotics in the present study.

In another research, TNAS (2004) and Biyela & Bezuidenhout (2004) isolated *E. coli* from river water and demonstrated that none of the isolates were resistant to ciprofloxacin, however in contrast, to this current study, ciprofloxacin showed 8.82% resistance. Similarly, a study done on antibiotic resistant *E. coli* isolated from tap and spring waters in a coastal region in Turkey (Osman *et al.*, 2007) and surface water of subtropical areas in Argentina (Lilliana *et al.*, 2008), indicated that resistance to cotrimoxazole was approximately 19.6% for the Turkish study and 11.1% for the Argentine study. This is also in variance with the observations in the current study.

In this current study, all of the water isolates showed high level of multiple antibiotic resistances. In terms of prevalence, 14 (14.43%), 5 (5.5%) 13 (13.40%) and 5 (5.15%) of the water isolates showed multiple antibiotic resistance for two, three, four and five respectively (Table 5.25). This is in accord with a similar study carried out by Olowe *et al.*, (2008). Their study showed that more than 90% of *E. coli* isolates were multiple drug resistant for three or more commonly used antibiotics. The study of Wolde-Tenssay (2002), around Jimma, Ethiopia, also showed that almost all *E. coli* isolates from environmental sources were found to be multiple drug resistant to the commonly used antimicrobials including amoxicillin, tetracycline, and cotrimoxazole. A study done on water samples from southwest Nigeria also showed that most of the *E. coli* isolates were resistant to different antibiotics ranging from two to seven kinds including cotrimoxazole, tetracycline and amoxicillin (Lateef *et al.*, 2003). Atif *et al.*, (2010) also showed that 96.7% of *E. coli* in drinking water samples from

Hyderabad, India was resistant for two to six antibiotics.

After studying the nature of the Mhlathuze River in South Africa for harboring antibiotic resistant bacteria and genes, Biyela and Bezuidenhout (2004) concluded that the river could act as reservoir as well as a medium for the spread of bacterial antibiotic resistance genes. Resistance genes may be horizontally or vertically transferred between bacterial communities in the environment (Biyela & Bezuidenhout, 2004). The same might be concluded for the water sources investigated in this current study. This is because of the high prevalence of multiple antibiotic resistant *E. coli* observed in the study, as well as the known mechanism to which the *E. coli* organism exhibits to the tested antibiotics. The multiple antibiotic resistances of *E. coli* established in this study also agree with other findings (Rowe *et al*., 1997; Yurdakok *et al*., 1997; Saenz *et al*., 2001).

The frequency of penicillin resistance in the current study was high among the *E. coli* isolates as compared with chloramphenicol and ampicillin resistance observed. This may be due to the blanket use of inexpensive antibiotics in the Ghanaian community or may be due to production of beta-lactamase enzymes by the *E. coli* cell. *E. coli* resistance against ampicillin was observed by Celebi *et al.* (2007); Olowe *et al*, (2008) and Uma *et al*. (2009). The emerging co-trimoxazole and ciprofloxacin resistance from the water sources as observed in this study are of serious concern, as these are the preferred drugs for many gram-negative bacteria (Rowe *et al.*, 1997). The most common mechanism of resistance to co-trimoxazole is the acquisition of plasmid mediated, variant diaminopyrimidine folate reductase enzymes (Sharma & Bimala, 2012). Low resistance to amikacin and gentamycin might be due to the less use of these antibiotics in clinical practice and veterinary medicine.

The rising trend of resistance in all the *E. coli* isolates from particularly streams, dams and canal water sources, affirms the fact that disposed antibiotics may have been washed down the water sources and accumulated downstream especially during the rainy season accounting for the high resistance. The differences in resistance profiles in this environmental study clearly reflect the differences in selection procedure pressure in the investigated water sources. The data do not only correlate with elevated faecal and total coliform counts at sampling sites but

also with higher MAR indices. The higher level of resistance to antibiotics in the sampling Ghanaian communities is worrisome since most inhabitants take thire bath, wash clothes and even dispose human sewage into water sources at upstream and midstream sites whiles some occupants and non-occupants use these water sources for drinking and domestic purposes downstream. In Mangalore, it was reported that untreated or partially treated domestic sewage released into open estuaries, accounted for the high presence of antibiotic resistance (Hill *et al*., 2005).

Inexpensive drugs are widely available without prescription from authorized health institutions and pharmacies, as well as from unauthorized patent medicine shops and other distributors (Okeke *et al*., 1999). Ingestion of antibiotics is known to provide selective pressure ultimately leading to a higher prevalence of resistant bacteria (Levin *et al*., 1997). This current study shows the need to monitor commensal organisms as well as pathogens by susceptibility testing to guide treatment. The data observed suggest that the antibiotics against which all the isolates were sensitive may be useful in treating infections caused by pathogenic *E. coli* and other related enteric gram negative pathogenic bacteria in the locality where the sources of water supply largely are similar to those investigated in this study. In addition, different pathologies may alter antibiotic sensitivity patterns; consequently, periodic evaluation of antibiotic susceptibility is recommended to guide management of patients requiring antibiotic treatment (Obi *et al*., 2006).

Though this present study did not investigate mechanisms of resistances, it is worth noting that the widespread use of antibiotics has been shown to create pressure that encourages the selection of multi-drug resistance among bacteria (Hoge *et. al.*, 1998; Pratts *et al.*, 2000). Such rising resistance is due to mechanisms of mutation. Since a plasmid or transposon can carry several resistance indices, resistance to several antimicrobial agents may be acquired simultaneously and results in multiple drug resistant organisms (Hughes & Datta, 1983; Davies, 1994; Hall & Collis, 1995; Labee-lund & Sorum, 2001). It is known that the majority of antibiotic resistance genes in *E. coli* are plasmid borne and such plasmids are often transferable to same species or other enteric pathogenic species.

Observation of the incidence of multidrug resistant *E. coli* isolates in this study may serve as database for formulation of immediate and future sanitation and health related programmes in this area. The study compared the results obtained with various studies done on clinical isolates of *E. coli.* The results revealed a high level of agreement in sensitivity patterns with the clinical isolates. (Djie-Maletz, *et al.,* 2008; Odonkor *et al.,* 2011; Newman *et al.,* 2011)

The current study also evaluated multiple antibiotic resistance (MAR) index studies of the multiple resistant isolates as well as the various water sources. Multiple antibiotic resistance (MAR) among others is used to identify the origin of the faecal pollution, and offers information about the source of water pollution. This is a very useful tool for water management. High multiple antibiotic resistance index of isolates as well as sampling sources observed, suggests high-risk sources such as human and non-human faeces as the origin of contamination.

The study revealed from the multiple antibiotics index analysis that thirty (30) of the multidrug resistance *E. coli* strains had a very high MAR index value (>0.2). Seasonal occurrences of MARs of different *E. coli* strains isolated in the current study are presented in figure 4.10. Sixteen isolates (16) representing 53% were obtained in the rainy reason. Bacteria isolate with a value of MAR > 0.2 is an indicator of an area with a high risk of contamination (e.g. animal farms, increased human population), where the antibiotics are frequently used. The MAR indexes of isolates from different water sources were comparable with those of previous studies (Parveen *et al*., 1997; Chandran *et al.,* 2008; Akturk *et al.,* 2012). Nine isolates recoded MAR index <0.30. Six out of the 9 were obtained in the dry season and 3 in the rainy season. This could be an indication that the isolates had human faecal origin, suggesting those samples were contaminated with *E. coli* arising from high-risk sources.

Similar observation was made by Tambekar *et al*., (2006) who reported high MAR index due to human and non human faecal contamination, of surface, ground and public supply water sites in Akola and Buldhana of Vidarbha district. In a similar study Chatterjee *et al*., (2012) noted that drinking water sources of Uttarakhand region were contaminated with high MAR

index *E. coli* originating from potential risk sources. On the other hand, the high MAR index recorded in this current study can also be attributed to the fact that the water sources may have been highly contaminated with antibiotics. This contamination may be due to the usages of chemicals for farming as oberved in the surrounding areas of the various water sources. This is in accordance with Tambekar *et al.*, (2006) report which states that, bacteria originating from an environment where several antibiotics are used usually produce MAR Index greater than 0.2. The MAR indexes of those few samples that were below 0.2 in this study are below the value of risk contamination (Krumperman, 1983). However, the high number (30) of multidrug resistant isolates that yielded MAR indexing value above 0.2 indicated high risk of contamination. It may be argued that the difference in MAR indexing in the different water sources indicated the impact of urbanization on antibiotic resistance levels.

The findings from this current study have highlighted the need for controlled use of antibiotics and strict pollution monitoring programmes in the Dangme West District of Ghana. The problem is more dangerous if the water bodies from where the local inhabitants get their water for drinking purpose are severely contaminated with harmful microbes such as *E. coli*, which in turn is resistant to many antibiotics i.e. multi-drug resistant. Thus, this study is highly informative in terms of the evaluation of faecal contamination of water bodies as well as to determine the resistance of *E. coli* against the commonly available clinically significant antibiotics.

There are severe consequences for the high multiple resistances observed in *E. coli* in the current study. This is because, the presence of *E. coli* in the various water sources may spell health hazards such as diarrhoeal diseases, which accounts for a substantial degree of morbidity and mortality in adults and children (Black, 1993; Du Pont, 1995; El-Sheikh, & EL-Assouli, 2001; Van-Vuuren *et al.*, 2009). However, control of diarrhoea may require the administration of antibiotics. Nonetheless, several strains of *E. coli* are known to be resistant to a wide array of antibiotics (Black, 1993, Yurdakok, *et al*., 1997; Aseffa, 1997; Obi, *et. al*, 1997; Van-Vuuren *et al.*, 2009). This disease therefore may not be cured due to development of resistance to commonly prescribed antibiotics by the contaminating microorganism originating from livestock feaces and human sewage (Bahiru *et al.*, 2013). It could be recalled

that strains of *E. coli* and *Salmonella* accounted for several outbreaks in the United States and worldwide, partly due to resistance to chloramphenicol, ampicillin and trimethoprim (Mermin *et al*., 1998).

Now it is obvious that, antibiotic resistant bacteria species are ubiquitous in the environment and their negative impact has greatly increased (Mathew *et al.*, 2007); improper antibiotic use and lack of awareness are considered as the most important factors for the emergence, selection, and dissemination of antibiotic resistant bacteria species in the environment (Neu, 1992).

5.4 Virulence genes associated with multiple antibiotic resistant *E. coli*

What are the virulence genes associated with multiple *resistant E. coli*? The current study has revealed the virulence genes associated with the multiple antibiotic resistant *E. coli.* The widespread species *E coli* includes a broad variety of different types, ranging from highly pathogenic strains causing worldwide outbreaks of severe disease to avirulent strains, which are part of the normal intestinal flora. The pathogenicity of a given *E. coli* strain is mainly determined by specific virulence factors, which include adhesins, invasins, toxins, and capsule. They are often organized in large genetic blocks either on the chromosome (pathogenicity islands), on large plasmids or on phages and can be transmitted horizontally between strains. The findings from this research revealed that 6% of the multi drug resistant *E. coli* were found to posses the *eae* virulence gene.

Current technology has made the identification of potentially new virulence genes a relatively simple endeavor. Infection models that study isogenic strains differing by a specific virulence gene provide strong evidence for pathogenicity when infectivity is determined by a single characteristic. To establish an organism's pathogenic potential requires some information on the relative prevalence of virulence genes among pathogenic and nonpathogenic isolates. By the *eae, stx1* and *stx2* virulent genes and their associations with multidrug resistant *E. coli*, we learned of the profiles of these virulent genes in aquatic environments.

Despite a low percentage of *eae* virulence genes that were identified, the potential to transfer

these genes to other *E. coli* isolates do exis. Another observation from this research indicates that all the multidrug resistant *E. coli* isolates were non *stx1* and *stx2* genes producing. *E. coli* belonging to the group of entero-bacteria and are usually apathogenic. However, there also exist *E. coli* strains with pathogenic properties in humans and animals such as Shiga toxin–producing *E. coli* (STEC), also known as verotoxin-producing *E. coli,* and enterohemorrhagic *E. coli* (EHEC). They are the cause of a significant emerging infectious disease (Jaeger & Acheson, 2000).

The foremost diagnostic criterion in detecting an EHEC infection therefore must be the shiga toxin produced by the bacteria. This identification however requires the use of molecular biological diagnostic procedures. The presence of *eae* gene in the water sources is a major public health concern, because transmission of these virulence factors is not only through food, drinking and bathing water but from person to person (Karch *et al.*, 2000). Secondly, the implications are that the *eae* gene do not live in isolation, there is therefore high chance that there may be other bacteria carrying related genes such as: shiga toxin gene *stx1* and *stx2*, and the IpaH in the water sources. Third, *E. coli* has also been shown to be a significant reservoir of genes coding for antimicrobial drug resistance (Bucknel *et al*., 1997). Thus main risk for public health is that resistance genes can transfer from environmental bacteria to human pathogens. The ability of the resistant bacteria and resistance genes to move from one ecosystem to another is documented by the various cases in which transmission of resistant bacteria has been demonstrated between animals and humans (Flor *et al.*, 2013).

The *eae* genes have been found to be responsible for disease outbreaks. *E. coli,* outbreak strain caused the hemolytic–uremic syndrome in 101 children even though the strain lacked the intestinal adherence factor intimin (encoded by the gene *eae*). Similarly, *eae*-negative strains have previously been isolated from adults with the hemolytic–uremic syndrome (Bielaszewska *et al.,* 2006) but rarely from children. For example, 97% of shiga-toxin–producing *E. coli* isolated from children with the hemolytic–uremic syndrome in Germany and Austria carried the *eae* gene (Gerber *et al.,* 2002). Another unique feature of this outbreak, probably attributable to the pathogen, was the estimated median incubation period of 8 days, which was longer than the 3-day to 4-day incubation period reported for shiga-

toxin–producing *E. coli* O157: H7.13 (Mead & Griffin, 1998).

Anthropogenic-driven selective pressures is known to contribute to the persistence and dissemination of virulence genes and antimicrobial resistant bacteria usually relevant in clinical environments (Tacão *et al.,* 2012). Moreover, the highest number of multi-resistant isolates was found in samples from surface water suggesting discharges from human sewage, and farms. This indicates the importance of wastewater discharges in the dissemination of antimicrobial resistance strains.

The permanent influx of pollutants such as antimicrobial agents, detergents, disinfectants, heavy metals, and livestock waste may contribute to the emergence of antibiotic resistant bacteria in water as well as the spread of antimicrobial resistance genes and virulence bacteria in water sources. The results show that it is urgent to evaluate the management of wastewater and the water quality in the Dangme West District and if necessary, implement a local wastewater treatment to prevent the emergence of infectious outbreaks.

5.5 Comparison of three laboratory based techniques for detection of *E. coli*

How does PCR, API 20E, and culture based laboratory methods compare in the detection of *E. coli?* The current study has successfully evaluated these methods and has duly determined their specificity and sensitivity. In the past few decades, the methods for the identification of members of the enterobacteriaceae family of bacteria in both clinical and reference laboratories have undergone major changes. Rapid progress has been made, from conventional tubed biochemicals through miniaturized biochemical panels and plates (Washintong *et al.,* 1971; MacCarthy *et al*., 1978; Rhoden *et al.*, 1987) to semi-automated systems (Costigan & hollick, 1984; Pfaller *et al.,* 1986), all of which render identifications in 18 to 21 hours. A few systems exist that give identifications in 5 to 8 hours (Altwegg, 1983, Keville & Doern, 1984). Today's technology allows some fully automated systems to yield answers in 2 to 4 hours with the use of fluorogenic compounds and fluorometric analysis (Nucera *et al.,* 2006).

Currently, public and environmental health protection requires safe drinking water, which

means that it must be free of pathogenic bacteria. Among the pathogens disseminated in water sources, enteric pathogens are the ones most frequently encountered. Therefore, sources of faecal pollution in water devoted to human activity must be strictly controlled. Entero-pathogens, such as *E. coli* are generally present at very low concentrations in environmental waters within a diversified microflora. Effective and efficient methods are required to detect them thus; the need for more rapid, sensitive, and specific tests in the water industry. The study assessed routine and widely accepted techniques from recent research developments. The data obtained from the study revealed a high specificity and sensitivity for API 20E very close to that of the PCR, which is in general regarded as gold standard (Rompre *et al.*, 2002).

The API 20E diagnostic, which detects 20 biochemical reactions, is a traditional method for the identification of *E. coli* and other enterobacteriaceae (Koneman, 1997). Previous studies of API 20E have reported good (O' Hare *et al*., 1992; Peele *et al.,* 1997) and inaccurate (Aldridge *et al.,* 1978; Aldrige & Hodges 1981; Jones *et al*., 2000), sample classifications. Genetic identification systems can improve *E. coli* identification (Vaneechoutte & Eldere 1997; Hoorfar *et al.,* 2000). An alternative is the highly sensitive and specific PCR (Chiu & Ou 1996; Malony *et al*., 2003). PCR accuracy for *E. coli* detection needs to be confirmed for clinical and environmental samples. With the release of each new product over the years, there has been a flurry of publications reporting on the product's ability to accurately identify members of enterobacteriaceae. However, the current data available on PCR identification of *E. coli* has primarily been on clinical isolates.

The near-perfect sensitivity and specificity of API 20E compared to PCR observed in this current study, indicates that both tests can provide accurate diagnoses for isolates obtained through culture on selective media for *E. coli*. Previous studies have accepted only high API 20E likelihood levels as accurate (90% correct) in the classification of suspected *Salmonella* and *E. coli* isolates (Kelly & Latimer, 1980; Brucker *et al.,* 1982). The present study, using PCR DNA strip technology as a diagnostic standard, indicates that caution should be exercised in classifying as *E. coli* in any result from API 20E for which the likelihood of *E. coli* is less than 99.9%. The reliability of the results presented above was enhanced by a complete agreement between the DNA strip technology results and the band widths obtained

on the gel (figure 4.11). These findings from the current study affirm previous reports demonstrating the PCR to be a reliable, accurate tool in the detection of *E. coli* (Chiu & Ou 1996; Olivera *et al.*, 2002; Malony *et al*., 2003).

Validation of both PCR and API 20E (at the < 99.0% likelihood level) as accurate diagnostic tests suggests that either can be used with similar results. The selection of PCR versus API 20E depends in part upon cost. API 20E strips are relatively expensive, currently about GHC 60.00 per sample, compared to less than GHC 15 current cost in laboratory materials per PCR test. There are also reduced labour costs using PCR. However, PCR requires an initial investment in equipment (e.g., thermocyclers and electrophoretic gel apparatus). Another cost saving for API 20E is that each sample can be tested individually; with PCR, cost efficiency is realized only when samples are tested in quantities sufficient to populate a gel for electrophoresis. Thus, in small laboratories with limited equipment and low sample numbers, API 20E may still be economical. However, in larger well-equipped laboratories with higher sample numbers, PCR becomes more economical. In any case, PCR has the additional advantage of obtaining results within hours of isolation of *E. coli* suspects on selective media. The interpretation of results is straightforward, avoiding problems of subjective evaluation of API 20E biochemical reactions. However, PCR is able to identify large numbers of a single species eg. *E. coli* and not different organism eg. enterobacteriaceae as does API 20E, thus limiting its identification to one specific pathogen at a time. The biochemical testing/ culture based methods on the other hand did not perform badly in comparison to the API and the PCR. However, one will certainly not settle on a sensitivity and specificity of 96.91% and 81.82% respectively, when there are other methods that give higher levels of accuracy such as the API and PCR.

In summary, both PCR and API 20E have demonstrated to be accurate methods for *E.coli* identification. The results of the study of *E. coli* isolates from water sources, are in general agreement with those of previous studies of samples collected for diagnosis in both veterinary and human medicine, thus indicating wide applicability of both diagnostic tools for *E. coli* identification and characterization.

5.6 Association between antibiotic resistance and radiation sensitivity (D_{10})

Is three any association between antibiotic resistance and radiation sensitivity (D_{10})? The results obtained from this research have answered this question, providing new data on radiation sensitivity values for multidrug resistant *E. coli.* Ionizing radiation is a suitable method to control pathogenic bacteria in food and water, a large number of D_{10} values have been published (e.g. Buchanan & Doyle, 1997; Olson, 1998). Antibiotic resistance of bacteria is a commonly used selective marker. Bacteria resistance to antibiotics is believed to have an increased sensitivity to irradiation. The data obtain from the current study indicates that the multidrug resistant *E. coli* had low D_{10} values with a mean of 0.33 ± 0.11KGy. Second, test of association between D_{10} and antibiotic resistance, was $P>0.05$, an indication of no association between the two parameters.

The range of *E. coli* D_{10} values obtained from the current study is consistent with previously published D_{10} values for *E. coli* (Thayer *et al.,* 1999; Niemira, 2003). The primary mode of action of ionizing radiation is via hydrogen and hydroxyl radical molecules resulting from the ionization of water molecules within the target organism. These radicals can disrupt membranes and interfere with the functioning of proteins, but the most significant target within the cell is DNA, where radicals are responsible for strand breakage (Niemira, 2003). NalR strains of *E. coli* O157:H7 and *Salmonella* have been recently shown to be more sensitive to irradiation than the NalS parent strains from which they were derived (Niemira, 2005). A comprehensive understanding of why a given isolate may be more or less sensitive to irradiation than related isolates of the same pathogen is yet to be formulated (Thayer *et al.,* 1999; Niemira, 2003).

This current study did not find any association between the radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) of *E coli*. Furthermore, The results showed that antibiotic-resistant bacteria were preferentially associated with low D_{10} values. Thus, the study has demonstrated that ionizing radiation effectively reduces the populations of both antibiotic resistant *E. coli.* However it is worth noting the likelihood that a survey of a larger number of isolates would result in a more linear progression of D_{10} values, that can bridge the

gap seen among the statistical clusters observed among the isolates evaluated in this current study.

The implications and uses of radiation sensitivity (D_{10}) are of enough benefit to the society. First, in countries such as Austria, Czech Republic irradiation is used for drinking water disinfection (Swinwood, *et al.*, 1994). Safe drinking water should not present any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages (WHO, 2006). However *E. coli* in waters is a major cause of water borne diseases particularly in developing countries, where the chunk of WHO's estimated 30 000 deaths daily from water related diseases occurs (Dauda, 2010). Irradiation is one of the best means of water disinfection. However, radiation sensitivity of bacteria depends on several factors (Mayer-Miebach 1993; Mayer-Miebach & Spiess, 1999). This requires that the radiation sensitivities be evaluated for each and every organism. This study has proven that a radiation doses of 0.33±0.11 kGy could be used to disinfect *E. coli* including resistant isolates of the same in drinking water. Sachet water and bottle water producers in Ghana may have their water products disinfected for public consumption by the uses of the established recommended doses from this current study.

CHAPTER SIX

6.0 CONCLUSIONS, LIMITATIONS OF THE STUDY AND RECOMMENDATIONS

6.1 Conclusions

6.1.1 Bacteriological condition of drinking water sources and associated risk

There is increasing recognition that continual surveillance has a legitimate place in the consideration of options for water quality management. This is because they are sensitive indicators of changes or deterioration in overall water quality, providing a useful addition to physical, chemical and biological information. The effects of the high bacteriological contaminants in the drinking water sources are cause for concern. They could trigger outbreaks of epidemics and isolated water borne diseases in the very near future if measures are not taken to get the water decontaminated before consumption.

In the bacteriological analysis of the water sources, the study found that there are significantly high counts in total coliforms, faecal coliforms, and *E. coli* across seasons but specifically higher in the dry season as against the rainy season. This was observed despite, run-off, and heavy rain during the rainy season. Second, was the observation of a correlation between faecal coliform and total coliform counts in the dry season and the rainy season.

Furthermore dam water sources was observed to poses a high disease risk among the five water sources investigated, whiles borehole water sources possess a lower diseases low risk. Even much more alarming was the observation of the presences of bacteria of public health importance in the water sources. This included *Shigella* spp. (dysentery), *Salmonella typhi* (typhoid fever and acute diarrhoeal infection), *Salmonella typhi* (typhoid fever), and *Vibrio cholerea* (cholera).

The various observation made above led to the conclusion that majority of the water sources used for drinking and domestic purposes in the study area are usually highly contaminated with faecal coliforms above the recommended standards (WHO, GSA) for drinking water. Both animals and humans are the possible sources of faecal bacteria contamination of the

drinking water sources. Most of the faecal coliform isolates identified are opportunistic pathogens capable of causing infection and disease.

In a nutshell, to reduce the level of bacterial contamination of drinking water sources there should be an incessant education on issues such as: environmental awareness, (cultivation sanitation habits and ensure that their surroundings and water sources are not indiscriminately polluted), causes, modes of transmission and prevention of water and sanitation related diseases. Furthermore, education on modes of storing water in proper storing facilities, proper handling of stored water, the treatment of collected water and hand-washing, etc. to help reduce the consumption of contaminated water should be done.

6.1.2 Prevalence and susceptibility profiles of antibiotic resistant water-borne *E. coli*

New resistance mechanisms emerge and spread globally threatening our ability to treat common infectious diseases, resulting in death and disability of individuals who until recently could continue a normal course of life. When treatment options are limited, healthcare providers might need to use antibiotics that are more expensive or more toxic to the patient. When no antibiotic is effective, healthcare providers may be limited to providing supportive care rather than directly treating an infection. This underlines the importance of an examination of prevalence and susceptibility profiles of antibiotic resistant water-borne *E. coli*

Analysis of the prevalence and susceptibility profile of *E. coli* has provided the basis to conclude that: E. *coli* has very high level of multiple antibiotic resistances patterns in the water sources. The prevalence of multiple antibiotic resistances E. *coli* in the water sources is 49.48 %. This makes the water unsafe for consumption. However good hygienic practices including sanitary water handling, safe use of antibiotics, adequate water source protection mechanisms when implemented in the study area will protect the surrounding community and livestock in general from waterborne disease. Furthermore the widespread distribution of antibiotic-resistant *E. coli* isolates documented in this study leads to the conclusion of a clear management implications related to water quality and public health risk in the study area.

Two observations have led to the conclusions stated above. First, the results, demonstrated that majority of the *E. coli* strains had a very high MAR index value (>0.2), with a higher seasonal occurrences of MARs of in the rainy season than in the dry season. Second, the study that E.coli isolates were sensitive to nitrofurantoin, cefotaxime, amikacin, gentamicin ,nalidixic acid and ciprofloxacin. Furthermore The study the high percentage of *E. coli* isolates that were multiple antibiotic resistant (MAR) to penicillin, cefuroxime, erythromycin and tetracycline. This suggest, misuses of these drugs, which is potential threat to humans in the area.

The finding from this research suggests the need for urgent action to alleviate the risk of antibiotic resistance. Messages about antibiotic resistance to the various stakeholders need to be intensified and tailored to achieve need results. The public needs to be educated about risks of antibiotic resistances to individuals without denying the benefits of antibiotic treatment in the right contexts. Second, people should be made aware, for example, what the risks are from taking antibiotics or staying in a hospital. Third, comparable figures about the risks from contracting a resistant infection should be calculated. Fourth, The fact that antibiotic resistance is a global issue must be communicated. Two or three illustrative examples relayed to the public and to politicians would help convey the scope of the problem. The public also needs to know that these conditions are not limited to any single country or segment of the population

Moreover, the toll that antibiotic resistances take on human life and productivity ought to be bought to the fore; it creates longer hospital stays and often requires expensive treatments. Additionally, gains made through massive global spending on malaria, tuberculosis, and HIV prevention are in jeopardy because of resistance. Generally, speaking, education for physicians on infectious diseases and antibiotics is needed. Specific messages for physicians should include: the importance of molecular diagnostics and the need for the physicians close cooperation with the clinical microbiology laboratory. Finally, Surveillance is key and should be a national policy. Investment in rapid diagnostics is also vital for maintaining the utility of antibiotics.

6.1.3 Virulence genes associated with multiple antibiotic resistant *E. coli*

Synthesis of the findings on the virulence genes associated with multiple antibiotic resistant *E. coli* provides evidence for the following conclusions. First carriage of the *eae* virulence genes on the multiple antibiotic resistant isolates is 6%. However none of the *E. coli* isolates were *stx1* and *stx2 virulent* producing.

Second, there is a high risk for public health via the transfer of these virulence genes from environmental bacteria to human pathogens. Finally, the *eae* gene found on the *E. coli* in the water sources is a major public health concern with a potential of these virulence genes transmitting from person to person through the water to cause infections.

6.1.4 Comparison of *E. coli* detection methods

The comparison of methods for detection *of E. coli* aspect of the study showed that API 20E has a high specificity and sensitivity close to that of the PCR which is regards as gold standard, indicating that it can provide accurate diagnoses for isolates obtained through culture on selective media for *E. coli*. Furthermore, though culture methods showed slightly lower specificity and sensitivity as compared to the API 20E, it can be used in combination with API or PCR to provide an accurate result.

The conclusion drawn from the observations was that both PCR and API are accurate diagnostic tests tools that either can be used with similar results. Therefore the selection of PCR versus API 20E will depend in part upon cost. A penultimate point is that PCR is the best detection tool with a 100% specificity and sensitivity in detection of *E. coli*, however it can only detect one specific organism at a time as compared to the API 20E and the culture based method.

6.1.5 Association between antibiotic resistance and radiation sensitivity (D_{10})

Finally, analysis of association between antibiotic resistance and radiation sensitivity (D_{10}) showed that that antibiotic-resistant bacteria were preferentially associated with low D_{10} values. Also a radiation dose of (0.33±0.11KGY) can be used to disinfect water contaminated with multidrug resistant *E. coli*, for safe human consumption. Furthermore, ionizing radiation effectively reduces the populations of antibiotic resistant *E. coli.*

We can therefore conclude that there is no association between multiple antibiotic resistant indexes and radiation sensitivity (D_{10}) of antibiotic resistant *E. coli.* The analysis of the findings further suggest that inactivation kinetics for controlling pathogen inactivation in food and water systems have to be estimated on the basis of specific microorganisms in food or water matrices of concern and should include further extrinsic factors. Lastly, this study has provided a model for which further research on the association between bacteria antibiotics resistances and their corresponding radiation sensitivities could be investigated.

6.2 Limitations of the study

- The scope of sample collection for this research work is limited to the Dangme West District of the Greater Accra region in Ghana. Ideally, collection of samples from the entire Greater Accra region of Ghana would provide a more representative sample range.
- The medium used for the quantification of faecal coliforms during the contamination pathway study is a bit selective and could not possibly promote all the types of faecal coliforms likely to be in the water.
- The water samples were collected over a one-year period only. A better picture would have been obtained if the duration were longer than a year, as well as a much larger samples size.
- Biochemical identification can be subjective. This can result in some minimal discrepancies in the result obtained in identification of bacteria.
- Determination of the antimicrobial zones of inhibition was not without problems. Defining the edge of the zone of inhibition in the antimicrobial susceptibility testing procedures was liable to variations and illuminations. This may affect the

measurements of zone sizes, making it difficult to see the zones.

- PCR has inherent limitations particularly those that result in biases in the template to product ratios of target sequences amplified from environmental DNA. Such amplification biases increases with increasing numbers of PCR cycles. These limitations presented a significant challenge in determining the abundance of individual genes present in environmental samples.
- Coloured media may not provide the proper contrast with McFarland equivalence Standards. This might have an effect on the results. Though negligible, the effect is worth nothing.
- Bacterial suspensions of older cultures may not compare precisely to bacterial counts.

6.3 Recommendations

The following are recommended for further studies:

- It is recommended that a further research on the assessment of contamination pathways should be carried out using other members of the enterobacteriaes (e.g., *Enterobacter* spp., *Klebsiella* spp., *Proteus vulgaris,* etc.) as a test organism instead of *E. coli.* This could further determine how much theses other bacteria contributes to the microbiological contamination of the water sources.

- It is suggest that further studies consider the quantification of other faecal coliforms to ascertain the real danger of consumption of contaminated water. This will help in the determination of the various levels of each organisms in the faecal coliform level so obtained.

- The direction for future research in antimicrobial resistance may include, quantification of antimicrobial residues in the water sources. This is critical because knowing the concentration of the antimicrobial agents in the exposed environments could be useful for risk assessment.

- There is a need to carry out further research to identify the other virulence gene that may be present in the aquatic environment as well as their association with the various

water sources in different seasons; this will further improve our understanding of the roles of antibiotic resistant genes in the aquatic environment.

- Recommendation towards future research in radiation sensitivity of waterborne bacteria may be to determine the radiation D_{10} values of all other coliform bacteria. This will enable the determination of the correlation between antibiotic resistances with sensitivity to radiation of the coliform bacteria.

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APPENDIX

APPENDIX 1

ZONE DIAMETER INTERPRETATIVE CHART

Antimicrobial Agent	Code	Disc Potency	ZONE DIAMETER		
			Resistant	Intermediate	Sensitivity
Amikacin	AMK /AK	10 µg	≤ 14	15-16	≥ 17
Ampicillin	AMP	10 µg	≤ 13	14-16	≥ 17
Cefotaxime	CTX	30 µg	≤ 14	15- 17	≥ 23
Cefuroxime	CXM	30 µg	≤ 14	15-17	≥ 18
Chloramphenicol	CHL	30 µg	≤ 12	13-17	≥ 18
Ciprofloxacin	CIP	5 µg	≤ 15	16-20	≥ 21
Co- trimoxazole	COT	25 µg	≤ 10	11- 13	≥ 16
Erythromycin	ERY	Mg15	≤ 13	14- 22	≥23
Gentamicin	GEN	10 µg	≤	13- 14	≥15

Nalidixic acid	NAL	30 µg	12 ≤	14-18	≥ 19
Nitrofurantoin	NIT	300 µg	13 ≤	13 – 16	≥17
Penicillin	PEN	10Units	14 ≤	------	≥ 29
Pipemidic acid	PA	20 µg	28 ≥	23 – 25	≤ 22
Tetracycline	TET	30 µg	26 ≤	12 – 14	≥ 15
			11		

APPENDIX 2

ANTIBIOTIC SUSCEPTIBILITY CHARACTERIZATION OF *E. COLI* ISOLATES

Sample NO.	AMK 30 µg	AMP 10 µg	CTX 30 µg	CXM 30 µg	CHL 30 µg	CIP 5 µg	COT 25 µg	ERY 15 µg	GEN 10 µg	NAL 10 µg	NIT 300 µg	PEN 10 U	PA 20 µg	TET 30 µg
01	S	I	S	S	I	S	S	S	S	S	S	R	I	R
02	S	R	S	I	S	I	S	R	S	S	S	R	S	I
03	S	I	S	S	S	S	S	S	S	S	S	I	R	I
04	S	I	S	R	S	I	S	I	S	R	S	I	S	I
05	R	S	S	R	I	S	S	R	S	S	S	R	S	S
06	S	S	S	R	R	S	R	R	S	S	S	R	S	S
07	S	I	S	I	S	S	I	I	S	S	S	I	S	I
08	S	I	S	I	S	I	S	S	S	S	S	R	S	I
09	S	S	I	S	S	I	I	S	S	S	S	I	S	S
10	R	I	S	S	R	S	S	I	S	S	S	I	S	I
11	S	R	S	I	I	S	S	R	S	S	S	I	R	I
12	S	S	S	I	S	R	S	R	R	S	S	R	S	S
13	S	I	S	I	S	S	S	R	S	S	S	I	I	S
14	S	S	S	I	S	I	S	I	S	S	S	I	S	I
15	S	I	I	S	R	S	S	I	S	S	S	R	S	I
16	S	I	S	S	I	S	I	I	S	S	I	I	I	I
17	S	R	S	R	S	S	R	I	S	S	I	I	S	I
18	S	I	S	S	R	S	S	S	S	I	S	I	S	I
19	S	S	S	I	S	I	I	S	S	I	S	I	S	I
20	S	S	S	I	S	S	S	I	S	S	S	R	S	R
21	R	I	S	S	I	I	S	S	I	S	S	I	S	I
22	I	I	S	I	S	R	I	S	S	S	S	R	S	I
23	S	I	S	S	I	S	S	I	S	S	S	I	I	S
24	S	I	S	I	S	S	S	I	S	S	S	I	S	I
25	S	I	S	S	S	I	I	S	R	S	S	I	S	I
26	S	I	S	I	S	S	S	S	S	S	S	I	S	I
27	S	I	S	R	S	I	S	R	S	S	S	R	S	I
28	S	S	S	S	I	S	S	I	S	I	S	I	S	I
29	S	R	S	S	R	S	R	R	S	S	S	R	S	R
30	S	I	S	R	S	I	S	R	S	S	S	I	S	I
31	S	R	S	S	R	I	S	S	I	S	S	R	S	R
32	S	I	S	S	S	S	S	I	I	S	S	I	S	I
33	S	R	S	R	S	I	S	I	I	S	S	R	I	I
34	S	I	S	S	S	S	S	S	S	S	S	I	S	S
35	S	R	S	S	S	S	S	I	S	S	S	R	S	S
36	S	S	S	S	S	R	S	R	S	S	S	R	R	R
37	S	I	S	S	S	S	S	I	S	S	S	I	I	I

38	S	S	S	S	R	S	S	I	S	S	S	I	I	S
39	S	I	S	S	I	S	S	S	S	S	S	I	I	I
40	S	S	S	S	S	S	S	I	S	S	S	I	S	I
41	S	S	S	S	R	S	S	S	S	S	S	R	I	S
42	S	S	S	S	S	S	S	I	S	S	S	I	S	I
43	R	S	S	R	S	S	S	S	S	S	S	R	I	I
44	S	S	S	S	S	R	S	S	S	S	S	I	I	R
45	S	I	R	S	I	S	R	S	S	S	S	R	R	I
46	S	R	I	S	I	S	S	S	S	S	S	R	S	R
47	S	S	S	S	S	I	R	S	S	S	S	I	S	I
48	S	I	S	S	S	S	S	S	S	S	S	I	S	I
49	S	R	I	I	S	S	S	R	S	S	S	S	S	S
50	S	I	S	S	I	I	S	S	S	S	S	R	S	R
51	S	I	S	R	S	S	R	S	S	S	S	R	S	R
52	S	S	S	S	S	S	S	S	S	S	S	S	S	R
53	S	S	S	S	S	S	S	S	S	S	S	I	S	S
54	R	R	R	R	R	S	R	R	S	S	S	R	R	R
55	S	S	S	S	S	S	S	S	S	S	S	I	I	I
56	S	S	S	R	S	I	S	R	S	S	S	I	I	I
57	S	S	S	S	S	S	S	S	S	I	S	I	I	S
58	S	I	S	R	R	S	S	I	S	S	S	S	R	S
59	S	S	S	S	S	I	S	S	S	S	S	I	S	I
60	S	R	S	I	R	S	R	R	S	I	R	R	S	R
61	S	S	S	S	S	S	S	S	S	S	S	S	S	S
62	R	I	S	S	R	R	S	R	R	S	S	R	R	R
63	S	R	S	R	S	S	S	R	S	S	S	S	S	I
64	S	S	S	S	S	S	S	I	S	S	S	I	S	S
65	S	I	S	R	R	I	S	S	S	S	S	I	R	R
66	S	S	S	S	S	S	S	S	S	S	S	I	R	S
67	S	S	S	S	S	R	S	R	S	S	S	R	R	R
68	S	I	S	S	S	S	S	S	S	S	S	I	S	I
69	S	S	S	S	S	S	S	S	S	S	S	I	S	S
70	S	I	S	S	S	S	S	S	S	S	S	R	R	R
71	S	S	S	S	S	S	S	S	S	S	S	S	I	S
72	S	I	S	S	S	S	S	R	R	R	S	I	S	R
73	S	S	S	S	S	S	S	S	S	S	S	S	S	S
74	S	I	R	I	S	S	S	S	R	S	S	I	R	I
75	S	S	S	S	S	S	S	S	S	S	S	I	S	S
76	S	S	S	I	S	R	S	S	S	S	S	S	S	R
77	S	I	S	S	S	S	S	S	S	S	S	I	S	S
78	S	S	S	I	S	S	S	S	S	S	S	I	S	I
79	S	S	S	R	I	S	S	S	S	S	S	R	S	S
80	S	S	S	S	S	S	S	R	S	S	S	S	S	I
81	S	I	S	S	S	S	S	S	S	S	S	I	I	S
82	R	R	R	R	R	S	S	R	S	S	R	R	S	R
83	S	S	S	R	S	S	S	R	S	S	S	I	S	S

84	S	S	S	R	S	S	S	I	S	S	S	I	I	I
85	S	S	S	R	S	S	S	S	S	R	R	R	R	I
86	S	S	S	R	S	S	S	S	S	S	S	S	S	S
87	S	I	S	R	S	S	S	I	S	S	S	R	S	R
88	S	S	S	S	S	S	S	I	S	S	S	I	S	I
89	S	I	S	R	R	R	R	I	S	S	S	S	S	I
90	S	S	S	I	S	S	S	S	S	S	S	S	S	I
91	S	S	S	R	S	S	S	S	S	S	S	I	I	S
92	S	S	S	R	S	S	S	S	S	S	S	S	I	I
93	S	I	S	S	R	S	R	R	S	R	R	R	S	R
94	S	I	S	R	S	S	S	S	S	S	S	I	S	I
95	S	S	S	R	R	S	S	S	S	S	S	S	S	S
96	S	S	S	R	S	S	S	S	S	I	S	I	I	I
97	S	S	S	R	R	S	S	R	S	S	S	R	S	S

KEY: R- RESISTANT S- SENSITIVE I-INTERMEDIATE

APPENDIX 3

MPN REFERENCE TABLE (MPN/100mL)

10mL	1mL	0.1 mL	MPN	10mL	1 Ml	0.1 mL	MPN
0	0	0	0	4	2	0	22
0	0	1	2	4	2	1	26
0	1	0	2	4	3	0	27
0	2	0	4	4	3	1	33
1	0	0	2	4	4	0	34
1	0	1	4	5	0	0	23
1	1	0	4	5	0	1	31
1	1	1	6	5	0	2	43
1	2	0	6	5	1	0	33
				5	1	1	46
2	0	0	5	5	1	2	63
2	0	1	7	5	2	0	49
2	1	0	7	5	2	1	70
2	1	0	9	5	2	2	94
2	2		9	5	3	0	79
2	3	0	12	5	3	1	110
				5	3	2	140
3	0	0	8	5	3	3	180
3	0	1	11	5	4	0	130
3	1	0	11	5	4	1	170
3	1	1	14	5	4	2	220
3	2	0	14	5	4	3	280
3	2	1	17	5	4	4	350
				5	5	0	240
4	0	0	13	5	5	1	350
4	0	1	17	5	5	2	540
4	1	0	17	5	5	3	920
4	1	1	21	5	5	4	1,600
4	1	2	26	5	5	5	>2,400

APPENDIX 4

LOCATION OF DAM WATER SOURCES

Serial Number	Sample Number	Location
1	D1	Osuwem
2	D2	Asutsiare
3	D3	Ayikumah
4	D4	Dodowa
5	D5	Prapram,
6	D6	Ningo
7	D7	Dawa
8	D8	Osuwem
9	D9	Asutsiare
10	D10	Ayikumah
11	D11	Dodowa
12	D12	Prapram,
13	D13	Ningo
14	D14	Dawa
15	D15	Osuwem

APPENDIX 5
Location of borehole water sources

Serial Number	Sample Number	Location
1	B1	Osuwem
2	B2	Asutsiare
3	B3	Ayikumah
4	B4	Dodowa
5	B5	Prapram,
6	B6	Ningo
7	B7	Dawa
8	B8	Dodowa

APPENDIX 6

Location of stream water sources

Serial Number	Sample Number	Location
1	S1	Osuwem
2	S2	Asutsiare
3	S3	Ayikumah
4	S4	Dodowa
5	S5	Prapram,
6	S6	Ningo
7	S7	Dawa
8	S8	Osuwem
9	S9	Asutsiare
10	S10	Ayikumah
11	S11	Dodowa
12	S12	Prapram,
13	S13	Ningo
14	S14	Dawa
15	S15	Osuwem
16	S16	Dodowa
17	S17	Ningo

APPENDIX 7

Location of hand dug well water sources

Serial Number	Sample Number	Location
1	H1	Osuwem
2	H2	Asutsiare
3	H3	Ayikumah
4	H4	Dodowa
5	H5	Prapram,
6	H6	Ningo
7	H7	Dawa
8	H8	Osuwem
9	H9	Asutsiare
10	H10	Ayikumah
11	H11	Dodowa
12	H12	Prapram,
13	H13	Ningo
14	H14	Dawa
15	H15	Osuwem

APPENDIX 8

Location of River Water Source

Serial Number	Sample Number	Location
1	R1	Asutsiare
2	R2	Asutsiare
3	R3	Asutsiare

APPENDIX 9

Location of Canal Water Source

Serial Number	Sample Number	Location
1	C1	Asutsiare
2	C2	Asutsiare
3	C3	Asutsiare

APPENDIX 10

Disease risk levels of water sources in the rainy and dry seasons

Disease risk levels of water sources in the rainy season

Water sources	Conformity with WHO guidelines	Low Risk	Intermediate Risk	High Risk	Total
Bore hole	4	4	0	0	8
Canal	0	0	3	0	3
Dam	0	1	13	1	15
Hand dug well	5	10	0	0	15
River	3	0	0	0	3
Stream	7	0	10	0	17

Dieseas risk levels of water sources in the dry season

Water sources	Conformity with WHO guidelines	Low Risk	Intermediate Risk	High Risk	Total
Bore hole	2	6	0	0	8
Canal	0	0	3	0	3
Dam	0	0	15	0	15
Hand dug well	3	12	0	0	15
River	0		3	0	3
Stream	1	3	13	0	17

INTERPRETATION

E. COLI COUNT PER 100ML	RISK LEVEL
0	In conformity with WHO guidelines
1-10	Low Risk
11-100	Intermediate Risk
>100	High Risk

APPENDIX 11

Results of Multiple Antibiotic Resistances (MRA) Index of Resistant *E. coli* Isolates

SR	Isolate Number	Season Isolated	No. of antibiotics to which the Isolate was resistant (a)	MAR Index (a/b)
1	01	Dry	2	0.14
2	02	Rainy	3	0.21*
3	04	Dry	2	0.14
4	05	Rainy	4	0.29*
5	06	Rainy	5	0.36*
6	10	Dry	2	0.14
7	11	Rainy	3	0.21*
8	12	Dry	4	0.29*
9	15	Dry	2	0.14
10	17	Dry	3	0.21*
11	20	Dry	2	0.14
12	22	Rainy	2	0.14
13	27	Rainy	3	0.21*
14	29	Dry	6	0.43*
15	30	Dry	2	0.14
16	31	Dry	4	0.29*
17	33	Dry	3	0.21*
18	35	Dry	2	0.14
19	36	Dry	5	0.36*
20	41	Dry	2	0.14
21	43	Rainy	3	0.21*
22	44	Dry	2	0.14
23	45	Rainy	4	0.29*
24	46	Dry	3	0.21*

* Higher than MAR index value (>0.2) b = 14 (number of antibiotics tested)

APPENDIX 11 : Continued Results of Multiple Antibiotic Resistances (MRA) Index of Resistant *E. coli* Isolates

SR	Isolate Number	Season Isolated	No. of antibiotics to which the Isolate was resistant (a)	MAR Index (a/b)
25	49	Dry	2	0.14
26	50	Dry	2	0.14
27	51	Rainy	4	0.29*
28	54	Dry	10	0.71*
29	56	Rainy	2	0.14
30	58	Rainy	3	0.21*
31	60	Dry	7	0.50*
32	62	Rainy	8	0.57*
33	63	Rainy	3	0.21*
34	65	Rainy	4	0.29*
35	67	Dry	5	0.36*
36	70	Rainy	3	0.21*
37	72	Dry	4	0.29*
38	74	Rainy	3	0.21*
39	76	Dry	2	0.14
40	79	Dry	2	0.14
41	82	Rainy	9	0.64*
42	83	Rainy	2	0.14
43	85	Rainy	4	0.29*
44	87	Dry	2	0.14
45	89	Rainy	3	0.21*
46	93	Dry	7	0.50*
47	95	Dry	2	0.14
48	97	Dry	3	0.21*

* Higher than MAR index value (>0.2) , b = 14 (number of antibiotics tested)

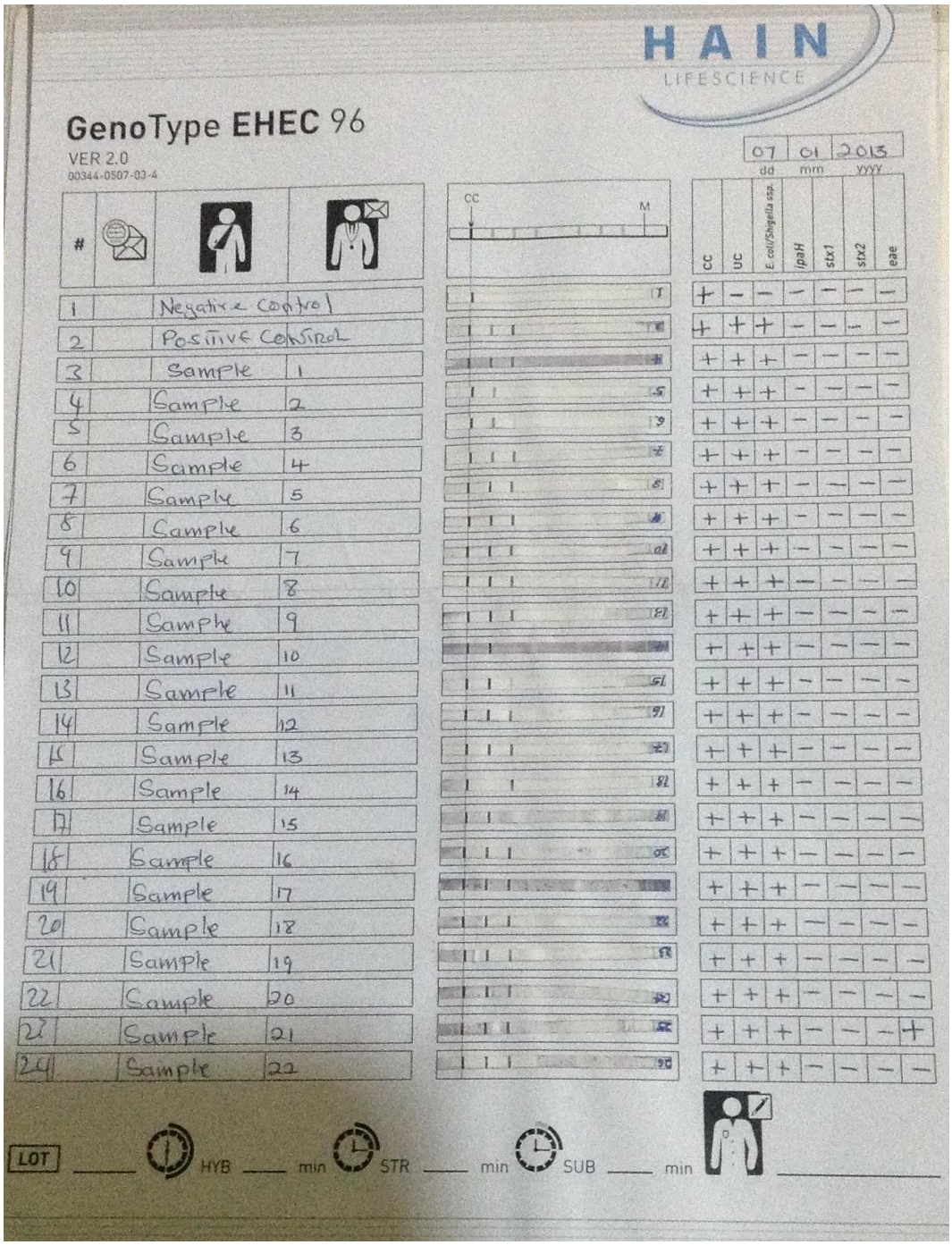
APPENDIX 12

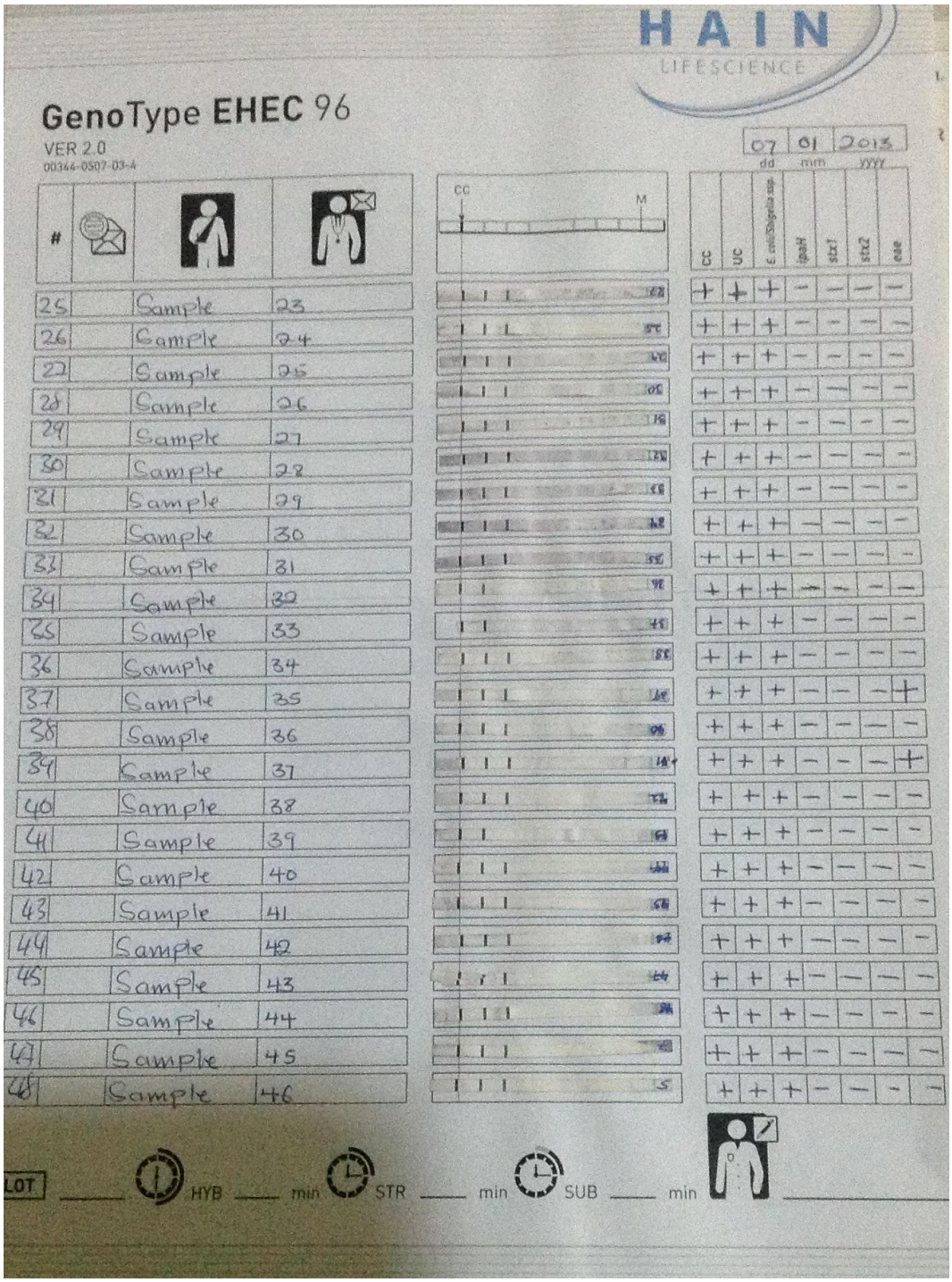
Radiation sensitivity and corresponding Multiple Antibiotic Resistances (MRA) index of selected isolates of multiple resistant e *E. coli* Isolates.

SR	Isolate Number	D_{10} (KGy)	MAR Index (a/b)
1	15	0.33	0.14
2	22	0.05	0.14
3	31	0.22	0.29
4	20	0.43	0.14
5	36	0.33	0.36
6	44	0.25	0.14
7	46	0.36	0.21
8	51	0.37	0.29
9	58	0.34	0.21
10	43	0.50	0.21
11	33	0.48	0.21
12	29	0.33	0.43
13	17	0.25	0.21
14	10	0.36	0.14
15	05	0.33	0.29
16	01	0.05	0.14
17	12	0.22	0.29
18	41	0.33	0.14
19	49	0.25	0.14
20	72	0.33	0.29
21	65	0.25	0.29
22	62	0.36	0.57
23	54	0.48	0.71
24	06	0.37	0.36
25	35	0.34	0.14
26	02	0.50	0.21
27	04	0.37	0.14
28	11	0.22	0.21
29	27	0.43	0.21

APPENDIX 13

Figure A1: PCR strips after hybridization





HAIN LIFESCIENCE

GenoType EHEC 96

VER 2.0

00344-0507-03-4

07 (dd) 01 (mm) 2013 (yyyy)

#		
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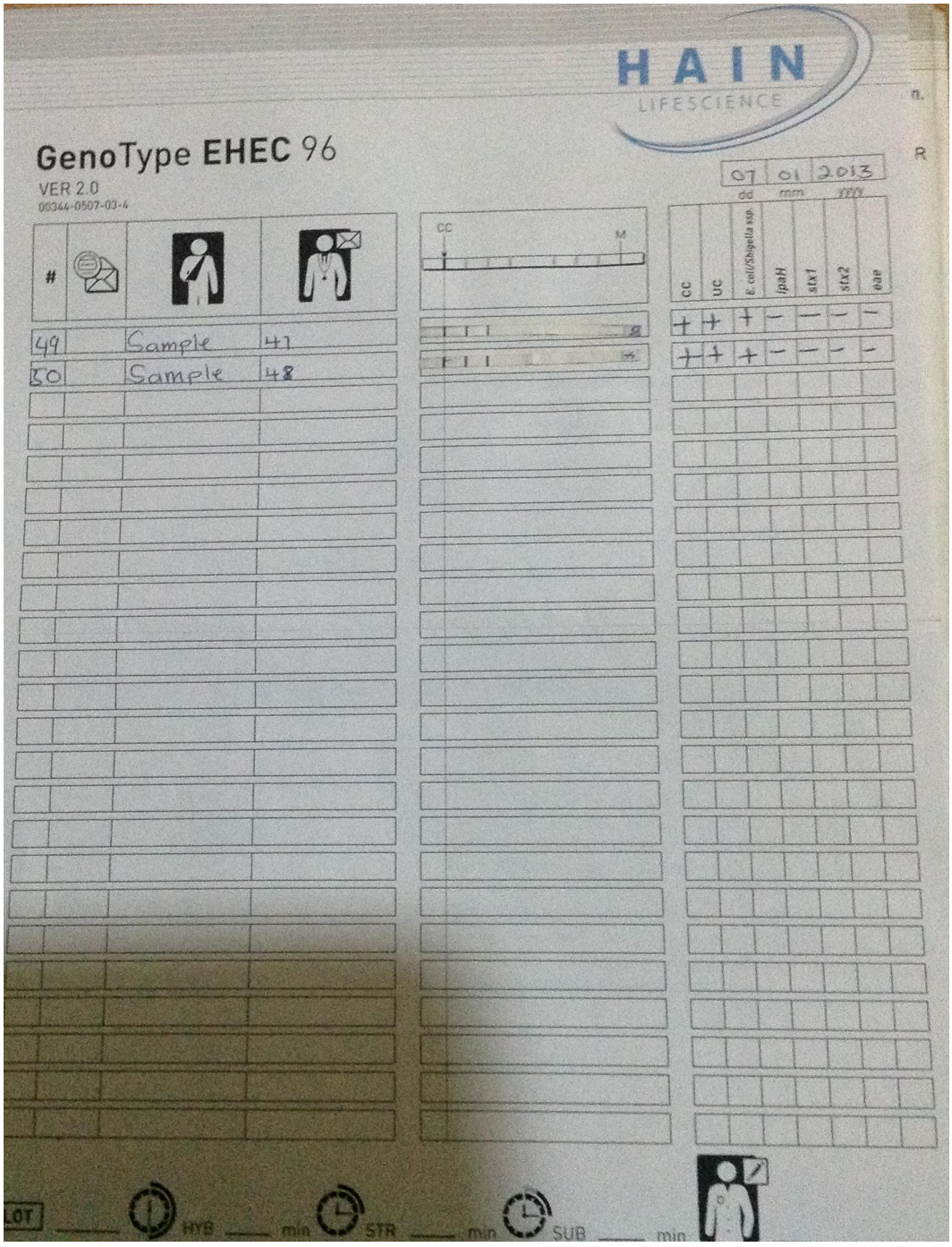
26	Sample	24
27	Sample	25
28	Sample	26
29	Sample	27
30	Sample	28
31	Sample	29
32	Sample	30
33	Sample	31
34	Sample	32
35	Sample	33
36	Sample	34
37	Sample	35
38	Sample	36
39	Sample	37
40	Sample	38
41	Sample	39
42	Sample	40
43	Sample	41
44	Sample	42
45	Sample	43
46	Sample	44
47	Sample	45
48	Sample	46

CC M

CC	UC	E. coli/Shigella spp.	rfbO	stx1	stx2	eae
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	+
+	+	+	−	−	−	−
+	+	+	−	−	−	+
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−
+	+	+	−	−	−	−

LOT _____ HYB ____ min STR ____ min SUB ____ min


HAIN
LIFESCIENCE
GenoType EHEC 96
VER 2.0
00344-0507-03-4
07 01 2013
dd mm yyyy
CC
M
cc uc E. coli/Shigella ssp. ipaH stx1 stx2 eae
49 Sample 47
50 Sample 48
LOT
HYB min
STR min
SUB min

APPENDIX 14

Figure A 2 A picture of borehole from the sampling site

APPENDIX 15

Figure A 3 A picture of a stream from the sampling site

APPENDIX 16

Figure A4. A Picture of hand-dug well from the sampling site