KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

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GROUNDWATER QUALITY IN THREE PERI-URBAN COMMUNITIES IN KUMASI AND THE EFFECTIVENESS OF SOME ANTI-DIARRHOEAL AGENTS AGAINST ISOLATED ORGANISMS

BY

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By

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DECLARATION

I hereby declare that this submission is my own work towards the MPHARM and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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LIST OF ABBREVIATIONS

A.M. (am) – Ante Meridiem

ADH – Arginine dihydrolase

AIDS – Acquired Immune Deficiency Syndrome

AMY - Amygdalin

AP1LBH – Borehole from Aprade

AP1RPT – Overhead polytank from Aprade

AP2LW – First well from Aprade

AP2RW – Second well from Aprade

APHA - American Public Health Association

API 20E – Analytical Profile Index for the identification of enterobacteria

ARA - Arabinose

AWWA - American Water Works Association

BNF - British National Formulary

BP – British Pharmacopoeia

CFU – Colony Forming Units

CIT - Citrate

Co-Trimoxazole - Sulfamethoxazole BP and Trimethoprim BP

CWSA - Community Water and Sanitation Agency

DANIDA - Danish International Development Agency

DO1LBH – First borehole from Domeabra

DO1RBH – Second borehole from Domeabra

DO2RBH – Third borehole from Domeabra

DO3RBH – Forth borehole from Domeabra

EMB - Eosin Methylene Blue agar

EMS – Emergency Medicine Secrets

FC - Fa[redacted]Coliform

FS - Faecal Streptococcal

GEL - Gelatin

GLU - Glucose

GSB - Ghana Standard Board

GWCL - Ghana Water Company Limited

GWSC - Ghana Water and Sewerage Corporation

H_2S – Hydrogen Sulphide

HIV – Human Immunodeficiency Virus

IFCR - International Federation of Red Cross and Red Crescent Societies

IND - Indole

INO - Inositol

ISO - International Organisation for Standardisation

IWMI - International Water Management Institute

KNUST – Kwame Nkrumah University of Science and Technology

Lau sul. – Lauryl Sulphate agar

LDC – Lysine decarboxylase

Log Conc. – Log of Concentration

MaMDG - Mini Atlas of Millennium Development Goals

MAN- Manitol

ME1W - Well from Mesewam

MEL - Melibiose

Metrolex F - Metronidazole BP and Furazolidone BP

mg/ml - Milligram per millilitre

MIC - Minimum Inhibitory Concentration

MPN - Most Probable Number

MUD - 4-methylumbelliferyl-β-D-glucoside

MUG - 4-methylumbelliferyl-β-D-glucuronide

NGO – Non-Governmental Organisation

NIT - Nitrate Reduction Test

NTU – Nephelometric Turbidimetric Unit (Unit for Turbidity)

°C – Degree Celsius

ODC – Ornithine decarboxylase

°H - Hazen Unit (Unit for colour)

ONPG - o-nitrophenyl-β-D-galactopyranoside

ONPG – β-galactosidase

P. M. (pm) – Post Meridiem

pH - Hydrogen Ion Concentration

PNPG - p-nitrophenol-β-D-glucuronide

RHA - Rhamnose

SAC - Sucrose

Sl&B – Slanetz and Bartley's agar

SOR - Sorbitol

STG - Standard Treatment Guidelines

STG - Standard Treatment Guidelines

TCU - True Colour Units

TDA - Tryptophan deaminase

TDS - Total Dissolved Solid

U. S. A. - United States of America

UN – United Nations

UNESCO – United Nations Educational, Scientific, and Cultural Organisation

URE - Urease

USEPA - United States Environmental Protection Agency

VP – Voges-Proskauer

WHO - World Health Organisation

WPSF - Water Pollution Control Federation

XGAL - 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

XGLUC - 5-bromo-4-chloro-3-indolyl-β-D-glucuronide

ZOI - Zone of Inhibition

µS/cm - microsiemens per centimetre (Unit for Conductivity)

Dedicated to

The Blessed Virgin Mary, Star of the Sea

And

My mother, Sarah Monica Louis Otchere

ABSTRACT

The aim of the research was to assess the physical and bacteriological quality of water used in three peri-urban communities in Kumasi, Ghana, and to determine the Minimum Inhibitory Concentration of ten antibiotics sold on the Ghanaian market against diarrhoea causing organisms isolated from the water samples.

The Pour Plate, Membrane Filtration and inoculation into various liquid media were employed in examining nine (9) sources of drinking water used by the three communities for the presence of indicators of faecal contaminants (total coliforms and faecal coliforms) over a period of six (6) months. The samples were also analysed for pH, turbidity, conductivity, total dissolved solids and colour.

The samples showed different levels of contamination with enterococci, other gram positive and gram negative bacteria and fungi. Twenty randomly selected isolates from the membrane filtration method were identified using the API 20E system of identification and consisted of *Escherichia coli* 30%, *Enterobacter sakazakii* 20%, *Enterobacter cloacae* 15%, *Citrobacter diverus* 15%, *Salmonella spp* 10% and *Serratia marcescens* 10%.

From the MIC determination, *Escherichia coli* exhibited the highest susceptibility whilst *Streptococci faecalis* exhibited the least susceptibility. There was no significant difference between the MIC values produced by combination drugs and the values from single-component drugs. The samples that were analysed were of variable physical and bacteriological safety and quality. Most of the drugs used in this research had high MIC values against *E. coli*.

CHAPTER ONE

INTRODUCTION

1.1 GENERAL INTRODUCTION

About 20% of the world's population still lack access to safe drinking water (UN, 2006). Although there is some progress in the provision of safe drinking water, the progress is uneven, and many poor countries, especially in Sub-Saharan Africa, lag behind (MaMDG, 2005). If these countries are to meet the United Nations' Millennium Development Goal of halving the proportion of people without access to safe drinking water by the year 2015, then much more needs to be accomplished (Ansong and Biney, 2007).

So far, only 12% of developing nations met the deadline of introducing an effective water strategy by 2005, and most of these countries are in Southern Asia where it is reported that more than 1.2 billion people have gained access to safe drinking water since 1990 (Adams, 2006).

The quality of water used in an area, especially for drinking is of great importance because of the wide variety of water-borne diseases, especially, those of acute onset like diarrhoea. Worldwide, more than 1.5 million children under the age of five die each year from lack of access to safe drinking water (UN, 2004). Because of the importance of water, governments and various non-governmental organizations like the WHO, UNESCO, World Bank, World Vision, DANIDA and many others continue to do their best to ensure that water used by people is up to the WHO standard.

The pathogens responsible for causing water-borne diseases are numerous and include bacteria, viruses, protozoa and helminths. Bacterial contamination of water has resulted in many cases of infectious diseases. Examples of such cases include the severe cholera outbreak in Latin America in which over 9000 people died between January and December 1991 and the recent outbreak in Ghana in October 2005 where of the 1,587 cases reported, 23 people in Kumasi and its environs died (Rodriguez, 1994; Asamoah, 2006).

Some of the reasons for the poor quality of water used in some homes are inadequate equipment for the supply of pipe-borne water, low educational level of populace, lack of time for relatively cheaper methods of water treatment and the high cost of quality treatment materials. These reasons have resulted in higher incidents of water-borne diseases (Wegelin and Gubler, 1990).

Some hydrologists believe that by the end of 2025, about one third of the population of the developing world will face severe water shortage (Van Koppen et al, 1997). The only way water supply can be controlled to meet demand, whether for natural processes or for human need, is through storage. On land, surface water is stored in water courses, lakes, rivers, streams and springs and in other water bodies and in the frozen form as ice and snow. When water infiltrates the ground, it is stored in aquifers.

One of the advantages of storing water underground is that it can be kept for years, with little or no evaporation loss so that it can be used in drought years. Again, the storage point can be near or directly under the point of use and is immediately available on demand through pumping (Obeng and Tchannerl, 1989).

Another advantage is that because the water slowly percolates into the aquifer, it is usually purified of biological pollutants and can serve as the best source of drinking water especially, for rural areas of developing countries, where water treatment facilities are not available.

There are however, some disadvantages associated with the use of underground water, the first being the rapid decline in the level of underground water in many areas. In certain areas in India, Bangladish and West Bengal underground water overuse has caused fluoride and arsenic contamination of drinking water supply. In coastal regions, overdraft of underground water has resulted in high levels of salinity in the water making it unfit for human consumption. Another disadvantage is the cost involved in drilling and pumping to extract underground water for use (IWMI, 1999).

Underground water is gradually becoming an increasingly popular resource, and it is emerging as a formidable poverty reduction tool because it can be delivered directly to poorer communities far more cheaply, quickly and easier than the pipe-laying method. Fresh water is essential for human survival. The quantity, quality and presence of pathogens in water have a direct impact on human health (MaMDG, 2005; IWMI, 1999).

The water need of a people is an integrated part of their livelihood. Water is used for the day to day activities which include domestic purposes such as cooking, drinking, bathing and washing, farming on a large scale and gardening. It is also used for livestock, fisheries and other aquaculture, aforestation, food processing, butchery and constructional works. Water is also used for small businesses, and ceremonial and cultural practices. But in most cases and at most places, the peoples' need for water is not fully met by the service provider.

Access to increased quantities of water for cooking and drinking, combined with improved hygienic behaviour has been found to decrease significantly, diseases of faeco-oral origin. The most important factor to address for all water intended for drinking purposes is the quality and not the quantity (Medema et al, 2000).

1.2 THE HYDROLOGICAL CYCLE

The movement of water and water vapour around, over and through the Earth is called the water or hydrological cycle and it is driven by solar energy. As water moves through the cycle, it changes phases between liquid, solid, and gas. Water moves from one compartment of the hydrosphere to another by the physical processes of evaporation, condensation, precipitation and infiltration (Telford et al, 1994).

Evaporation is the transformation of water from liquid to gas phases as it moves from the ground or water bodies into the overlying atmosphere. Approximately 90% of atmospheric water comes from evaporation, while the remaining 10% is from transpiration from plants. Condensation is the transformation of water vapour to liquid water droplets in the air, producing clouds and fog (Telford et al, 1994).

Precipitation is the process whereby condensed water vapour falls to the Earth's surface. Most precipitation occurs as rain, but also includes snow, dew and fog. Infiltration or percolation is the flow of water from the ground surface into the ground. Once infiltrated, the water becomes soil moisture or groundwater. The percentage of precipitation that soaks into the ground is influenced by factors such as soil and rock type, soil saturation, vegetation, slope of the land and the climate (Telford et al, 1994).

1.3 PROSPECTING FOR UNDERGROUND WATER

Underground water is water located beneath the ground surface in pore spaces and in the fractures of geological formations. As water moves downwards, it fills the spaces between grains of sand and cracks and pores in rocks. It eventually collects above a water tight layer of rocks and the ground becomes saturated resulting in an aquifer. The depth at which soil pore spaces become fully saturated with water is called the water table (Telford et al, 1994).

Prospectors for underground water depend on using what can be seen at the surface to predict what is underground. They also rely on using certain geophysical methods to obtain reliable evidence of materials underground and drilling test holes (Telford et al, 1994).

The process of prospecting for underground water starts with assessing available background data. This is followed by field reconnaissance which involves inspection of all existing water supplies in the community, and inspection of facilities such as toilets, refuse dumps and cemeteries that might be potential sources of pollution. If the necessary data is satisfactory, various geographical methods may be employed to draw a geological map of the area and when funds are enough, drilling is done (Telford et al, 1994).

1.4 THE SITUATION IN GHANA

Since the hosting of the World Water Day in Ghana in 2005, a lot of Ghanaians have come to appreciate the important role water plays in the lives of individuals and in the development of a nation. The government has also become more conscious in its duty in ensuring that the WHO goal of providing good drinking water for all by the year 2025 will be realized in Ghana (Schiffer, 2006; Owusu, 2007).

Ghana has a total area of 238,540km^2 with undulating topography and a warm, humid climate. The mean annual rainfall of the country is estimated at 1,187mm. Mean annual temperatures range from 26.1°C near the coast to 28.9°C in the extreme north. Annual potential open water evaporation has been estimated as ranging between 1,350 mm in the south to about 2,000 mm in the north (AQUASTAT, 2005).

The country's population is about 21.4 million (2004), of which 54 percent is rural. The annual population growth rate is 1.7 percent. Population density is 90 inhabitants per square kilometre nationwide, with a variation from 26 inhabitants per km^2 in the Northern Region to 896 inhabitants per km^2 in the Greater Accra Region (Water Aid, 2003). In 2002, 79 percent of the total population had access to improved drinking water sources. This coverage was 93 percent in urban areas and 68 percent in rural areas (MaMDG, 2005).

1.4.1 Water resources

The three main river systems that drain the country are the Volta river system (Oti, Daka, White and Black Volta, Pru, Sene and Afram rivers), the South Western river system (Bia, Tano, Ankobra and Pra rivers) and the Coastal river system (Ochi-Nakwa, Ochi Amissah, Ayensu, Densu and the Tordzie rivers) (Water Aid, 2003; AQUASTAT, 2005).

1.4.2 Groundwater in Ghana: Groundwater occurs mainly in the Voltaian formation and is located in the south-western and in the extreme north east parts of the country and the Cenozoic and Mesozoic sediments which occur mainly in the extreme south east and western parts of the country (Water Aid, 2003; AQUASTAT, 2005).

1.4.3 Wetlands: Wetlands constitute about 10 percent of Ghana's total land area. There are three main types; marine or coastal, inland and human-made wetlands. These wetlands are very productive and their resources have been traditionally used by local populations as a source of the basic necessities of life, ranging from building materials, hunting and fishing, to sources of water for humans and livestock (Water Aid, 2003, AQUASTAT, 2005).

1.4.4 Water use

In the United States of America, an estimated 100 million people rely on groundwater as their source of drinking water. Approximately one-third of all public supplies and 95 percent of all rural domestic supplies are from groundwater sources (USEPA, 1996). In Asia, groundwater provides half of the drinking water, and in Europe 98 percent in Denmark and 96 percent in Austria rely on underground water (Waller, 1982; Molden, 1997).

The main consumptive water uses in Ghana are for irrigation, domestic and industrial purposes. Groundwater is usually abstracted from boreholes and wells for most rural areas. Some borehole supplies are also tapped to supplement urban water supplies.

Water quality in the country is generally good, especially for irrigation purposes. However, in the Akomadan and Kumsai areas, there have been reports of heavy pollution (Water Aid, 2003). Use of highly polluted water for irrigation places both growers and consumers at a risk of bacterial and helminth infections (Yoder, 1983).

Water found along the coast is usually contaminated with fluoride, chloride, dissolved salts and hardness tends to increase in drinking water as one moves from the mountain to the coast (Water Aid, 2003).

The projected future annual water demand by 2020 is about a 130 percent increase in present water use. Groundwater abstraction is projected to increase by 69 percent in order to meet the water demand in 2020 (AQUASTAT, 2005).

The requirement of water in the Kumasi metropolis is estimated at about 225,000m^3 per day but the current supply that is obtained from the Barekese and Owabi dams is less than 50 percent (CWSA, 2001). This makes it even more difficult for regular water supply to most of the peri-urban areas that are springing up in the metropolis. Due to the lack of pipe-borne water supply to these areas, most peri-urban communities rely on underground water to meet their daily water need. The underground water is obtained through the construction of boreholes and wells.

In the Kumasi metropolis, some residents of certain areas have to queue for long hours before a bucket of water can be obtained for use and sometimes a bucket of water is bought at a very high cost. People living at places like Atonsu, Kenyasi, Pankrono, Anloga and Asokore Mampomg usually face severe water crisis. Most communities in these areas have therefore resorted to the digging of wells for water. These wells yield water of various physical, chemical and bacteriological quality (Obiri-Danso et al, 2003; Kyei-Baffour et al, 2005).

A research on Hand-filled-hand-tied water sold on the streets of Kumasi showed various degrees of contamination with total coliforms, faecal coliforms and enterococci (Obiri-Danso et al, 2003). Some of the sellers use water from wells for such businesses.

1.5 WATER CONTAMINATING FACTORS

The sanitary level of the community, human and animal waste disposal methods, the distance between the water source and septic tanks, refuse dumps and cemeteries, and the occupation of the population are a few of the factors that might lead to a possible contamination of the water source. The use of inappropriate water-fetching devices by consumers can also contaminate well water (Hill, 1999).

In the home or domestic setup, the quality of water is the single most important concern and has led to the use of various means of improving the quality of water used in the home (Van der Hoek et al, 2001). The conventional method of using chemicals (chlorination) is quite expensive and hence other relatively cheaper and affordable point-of-use treatment methods such as boiling or filtration may be resorted to.

Even where the treatment facilities deliver the required quality, as in urban areas with municipal supply, water might get polluted during transmission along leaky and under-pressurized pipes, at the point of collection, during transportation in containers that might not be clean and during storage in houses. Point-of-use treatment methods should therefore be accompanied by education and behavioural change (The Network, 2005).

1.6 POLLUTANTS OF UNDERGROUND WATER

Contaminants of underground water can be either natural or human-induced. Naturally occurring contaminants are present in the rocks and sediments that the water dissolves as it percolates and mainly consists of minerals and metals such as iron and manganese. Human-induced pollution can be from industrial discharges, leaking underground fuel tanks and toxic chemical spills (Hill, 1999).

The most common water-quality problem in rural water supplies is bacterial contamination from septic tanks and landfills. Degradation of groundwater supplies also occurs as a result of poor waste-disposal practices and poor land management.

Microbiological contaminants found in ground water include bacteria, viruses, fungi and helminthes. Pathogens transmitted through drinking water are mostly of faecal origin and are therefore known as enteric pathogens. Coliform bacteria are mainly from the intestines of humans and other warm-blooded animals and are hence used as an indicator for the presence of pathogenic bacteria, viruses, and parasites from domestic sewage, animal waste and plant or soil material. These organisms can cause polio, cholera, typhoid fever, dysentery, and infectious hepatitis (Ashbolt, 2003).

Some inorganic contaminants found in ground water are chloride, copper, fluoride, iron, lead, nitrate, sodium, and zinc. Organic contaminants include compounds which enter the environment during the processing of plastics, crude oil, pharmaceuticals and preservatives. Radiological contaminants in ground water consist mainly of gross alpha-particles, Radium-226 and 228, and Beta and photon particles (Luanne, 1997).

Many of the groundwater pollutants are colourless, odourless and tasteless. The physical properties of an aquifer, such as thickness, rock or sediment type and location, play a large part in determining whether contaminants from the land surface will reach the ground water. The risk of contamination is greater for unconfined aquifers than for confined aquifers because unconfined aquifers usually are nearer to the land surface and lack an overlying confining layer to impede the movement of contaminants (Luanne, 1997).

1.7 WATER RELATED DISEASES

Water-related diseases are classified into three groups depending on their pathways of transmission. These are:

a) Faeco-orally transmitted diseases: These are diseases which are transmitted if people ingest faecally contaminated water or food. Usually, the main symptom is diarrhoea. Examples are cholera, typhoid, dysentery, poliomyelitis and hepatitis A.

b) Water-based and vector-borne diseases: These diseases are transmitted through vectors or intermediate hosts which spend some or all of their lives in water. They include malaria, guinea worm, river blindness, schistosomiasis, yellow fever and filariasis.

c) Water-washed diseases: These are disease conditions that generally improve by increased availability of water for bathing, regardless of quality. Examples are eye and skin infections (Fewtrell et al, 2006).

Infectious diarrhoea makes the largest single contribution to the burden of disease associated with unsafe water, sanitation and hygiene (Medema et al, 2004). Drinking water is a major source of microbial disease causing organisms (pathogens), especially, in developing regions. The World Health Organisation estimates that about 1.1 billion people globally drink unsafe water. It also reports that 88% of all diarrhoea diseases in the world is due to unsafe water, sanitation and hygiene and this causes approximately 3.1% of annual deaths involving 1.7 million people. The report further goes on to say that ninety percent of the deaths are in children under the age of five years and all these deaths occur in developing countries (WHO, 2002c; UN, 2005). In North America, it is estimated that up to 30% of gastrointestinal diseases are caused through water. Developing regions have a higher rate of endemic gastrointestinal tract diseases (Ashbolt, 2003).

1.8 PHYSICAL EXAMINATION OF POTABLE WATER

1.8.1 Turbidity: Turbidity in water is caused by the presence of suspended matter such as clay, silt, and fine particles of organic and inorganic matter. Water of high turbidity may not adversely affect health but may cause the need for additional treatment. Following rainfall, variations in ground-water turbidity may be an indicator of surface contamination (Grenberg et al, 1995). Turbidity is measured visually or nephelometrically. Both the WHO and the Ghana Standards Board state 5NTU as the limit for potable water (GSB, 1998; WHO, 1993).

1.8.2 Conductivity: This is the numerical expression of the ability of an aqueous solution to carry an electric current. The value is recorded in millisiemens per meter (mS/m). Conductivity of water increases with storage due to the absorption of atmospheric carbon dioxide and ammonia. The WHO and Ghana Standards Board's limit for conductivity of potable water is a maximum of 400µs/cm (Greenberg et al, 1995; GSB, 1998; WHO, 1993).

1.8.3 Total Dissolved Solids (TDS): This refers to suspended or dissolved matter in water. Water with high dissolved solid generally is of inferior palatability. A limit of 500mg dissolved solids per litre is desirable for drinking water (WHO, 1993; GSB, 1998).

1.8.4 Colour: Colour can be caused by decaying leaves, organic matter, copper, lead and iron. It indicates inadequate treatment and high treatment demand. Colour can be determined by the Visual Comparison, Tristimulus Filter and Spectrophotometric methods. According to the WHO, the colour of portable water should not exceed 50 Hazen Units (WHO, 1993). The Ghana Standards Board however sets the limit at 15 true colour units (TCU), where 15 TCU means 15 Hazen units after filtration (GSB, 1998).

1.8.5 Hydrogen Ion Concentration (pH): pH indicates, by numerical expression, the degree to which water is alkaline or acidic. It is represented on a scale of 0-14 where 0 is the most acidic, 14 is the most alkaline, and 7 is neutral. High pH causes a bitter taste, water pipes and water-using appliances become encrusted and this decreases the effectiveness of disinfection, causing the need for additional chlorine. Low-pH water corrodes or dissolves metals and other substances. Potable water should have a pH range of 6.5 to 8.5 (WHO, 1993; GSB, 1998).

1.8.6 Taste/Odour: Many sensations ascribed to the sense of taste actually are odours. In its pure form, water does not produce odour. It is therefore recognized as a quality factor affecting the acceptability of drinking water. Certain odours may be indicative of organic or non-organic contaminants that originate from municipal or industrial waste discharges or from natural sources. The sensory organs are the devices used for the Threshold Taste/Odour Tests. Taste and odour should not be objectionable to most consumers (WHO, 1993; GSB, 1998).

1.9 MICROBIOLOGICAL EXAMINATION OF POTABLE WATER

There is a wide range of microorganisms of interest in water quality testing. A fundamental limiting factor in the assessment of microbial quality of waters, and especially drinking water, is often the very low number of each organism present. Therefore, most microbiological procedure consists of concentration, enrichment, detection and quantification (Ashbolt et al, 2001; Brown, 2005).

1.9.1 ANALYSIS OF MICROORGANISMS

1.9.1.1 Bacteria: Bacteria are usually concentrated using the membrane filtration method and detected and enumerated by selective growth on agar and counting of colony forming units.

1.9.1.2 Viruses: The analysis of viruses involves the concentration of the viruses using the adsorption-elution method. Detection and enumeration is done using the cell culture or cytopathic effect and the counting of plaque forming units (Erickson and Dufour, 1986).

1.9.1.3 Parasitic protozoa: Such organisms are concentrated using either the cartridge filtration method or the immunomagnetic separation methods (IMS). They are then detected and enumerated by Immunological staining or by counting the fluorescent cysts (Erickson and Dufour, 1986).

1.9.2 RECOVERY OF TARGET MICROORGANISMS

Traditional approaches to the isolation of microbial indicators have relied on various agar plate and liquid media methods. The basic Pour Plate technique has a maximum sample volume of about 1ml whereas the Spread Plate technique uses 0.1 or 0.2ml samples (Grendel et al, 1995). For larger volume processing and rapid throughput, the Membrane Filtration technique is preferred if the sample is not very turbid since turbidity will interfere with the filtration process (ISO, 2000b).

Liquid cultivation techniques, either for the detection of the target organism (presence or absence test) or quantitatively, using Multiple Tube Techniques and Most Probable Number (MPN) calculations, allow flexible sample volume range and the handling of turbid samples (Greenberg et al, 1995).

In liquid cultivation techniques, small volumes of sample dilutions or up to ten litre samples can be used. The detection of target microorganisms by non-cultivation methods is also presented for enteric viruses and parasitic protozoa (WHO, 1993).

1.9.2.1 Recovery of Bacteria

Bacteria are generally recovered on 47 millimetre diameter membrane filters with porosities of 0.22 to 0.45 micrometre (Brown, 2005). Membrane filters may be incubated on solid media, pads soaked in liquid media or as a MPN system in enrichment broth.

1.9.2.2 Recovery of Parasitic Protozoa

Cysts of protozoan parasites can be recovered on similar membranes but with larger surfaces (up to 293mm diameter) and porosities as high as 2 micrometer. For convenience, however, various cartridge filters are generally preferred to recover protozoan cysts from up to 100 litre water samples even in the presence of some turbidity (Ashbolt et al, 2001).

1.9.2.3 Recovery of Viruses

A number of techniques have been described for the recovery of viruses by approaches based on the filtration of test water through filter media to which the phages or viruses adsorb. The phages or viruses are afterwards released from the filter media into a small volume suitable for quantitative plaque assays or presence or absence testing. This method is not used routinely and only done upon special request (e.g. for legal purposes) or when very much needed. It is also expensive (WHO, 1993).

1.9.3 PRE-ENRICHMENT AND ENRICHMENT TECHNIQUES

Detection and enumeration of index and indicator parameters rather than the search for specific pathogenic bacteria is used in the routine bacteriological analysis of water (ISO, 2003). Nonetheless, under special circumstances the search for pathogenic bacteria, instead of indicator organisms, may be necessary, for instance during an epidemic or when evaluating new water resources (WHO, 1993).

Typically the number of pathogenic microorganisms is low and their recovery is low because they are in a stressed condition (water). The chances of detecting pathogenic bacteria are therefore greater by using a pre-enrichment step prior to enrichment and selective plating (LeChevallier et al, 1982). This allows environmentally stressed organisms to recover and grow before selective pressures are applied.

Generally, pre-enrichment media contain no antibiotics or other selective agents and this allows the growth of most microorganisms in the sample. Subsequent inoculation into enrichment media selects the pathogen of interest, which can be detected by plating onto solid selective media (LeChevallier et al, 1982).

1.9.4 DETECTION, IDENTIFICATION AND QUANTIFICATION OF MICROORGANISMS

In the detection, identification and quantification of target organisms some approaches are solely based on a single technique whereas other strategies take advantage of a combination of different methods. For example, to identify *Escherichia coli* reliance can be placed on a one-day-cultivation on chromogenic media.

Alternatively, in a much faster approach, short pre-cultivation on an artificial medium can be combined with labeling using fluorescent probes, microscopy, and laser scanning techniques (ISO, 2000a-b).

The traditional cultivation techniques are usually sensitive but the identification is often not as reliable as might be desired. Methods based on molecular biology can also be used.

1.10 CHROMOGENIC MEDIA-BASED DETECTON METHODS

Chromogenic media are culture media without harsh selective agents, but contain specific enzyme substrates that allow significant improvements in the recovery and identification of target bacteria. This method is based on a colour reaction produced between the target organism and the enzyme substrate (Manafi, 1996).

Table 1.10.1 Examples of chromogenic substrates for the detection of indicator bacteria

Bacteria	Chromogenic substance	Enzyme tested
Coliform bacteria	o-nitrophenyl-β-D-galactopyranoside (ONPG) 6-bromo-2-naphtyl-β-D-galactopyranoside 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (XGAL)	β-D-galactosidase (E.C.3.2.1.23)
E. coli	5-bromo-4-chloro-3-indolyl-β-D-glucuronide (XGLUC) 4-methylumbelliferyl-β-D-glucuronide (MUG) p-nitrophenol-β-D-glucuronide (PNPG)	β-D-glucuronidase (GUD) (E.C.3.2.1.31)
Enterococci	4-methylumbelliferyl-β-D-glucoside (MUD) Indoxyl-β-D-glucoside	β-D-glucosidase (β-GLU) (E.C.3.2.21)

The use of chromogenic media has the advantages of being simple, convenient, easy to use and generally results in fewer errors than traditional cultivation-based methods (WHO, 1993; ISO, 2000a,b; LeChevallier et al, 1982).

1.11 COLIFORM DETECTION IN POTABLE WATER

Microbiological examination of water is used to determine sanitary quality. The various methods that are employed are intended to indicate the degree of contamination with waste (Harley and Prescott, 1990). Tests for detection and enumeration of indicator organisms rather than pathogens are used. The coliform group of organisms comprises all aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas formation within 48hours at 35°C (Brown, 2005).

Coliforms are the principal indicators of suitability of water for domestic, industrial and other use. Coliform group density is a criterion of the degree of pollution and sanitary quality. Two standard methods, the Multiple-Tube Fermentation method and the Membrane Filtration method are used for the detection of bacteria of the coliform group. A third less popular method, the Pour Plate method may be used depending on a series of factors. Statistical comparisons of results obtained by the Multiple Tube Method and the Membrane Filter technique show that the membrane filter is more precise (Greenberg et al, 1995; ISO, 2000c).

The ability of coliform bacteria group in the intestines of humans and other warm blooded organisms to produce gas from lactose in a suitable culture medium at 45°C is used to define faecal coliforms from other coliforms (Oxoid Manual, 1979; Scharlau Manual, 2006).

1.11.1 Multiple-Tube Fermentation Method

This standard test for the coliform group is also known as the Most Probable Number (MPN) technique. It is a qualitative approach and is determined by the use of Lauryl Sulphate tryptose or lactose fermentation broth. Formation of gas in any of the tubes within 48 hours constitutes a positive presumptive reaction.

Results of the examination or replicate tubes and dilutions are reported in terms of the Most Probable Number. This number, which is based on certain probability formula, is an estimate of the mean density of coliforms in the sample (Greenberg et al, 1995).

Disadvantages are that for greater accuracy, a lot of tubes have to be prepared. Again, the samples have to be shaken very well to produce a random dispersion of the bacteria. The main advantage is that the method can be used for turbid water samples, for salt or brackish water, mud, sediments and sludge (Greenberg et al, 1995).

1.11.2 Membrane Filtration Method

The quantitative, Membrane Filtration technique, is highly reproductive, can be used to test relatively large volumes of samples and yields definite results more rapidly. The Membrane Filtration method permits testing large volumes of low turbidity water and is extremely useful in monitoring drinking water. It is however limited in testing water high in turbidity and in non-coliform bacteria. The standard volume to be filtered is 100ml. This volume of the sample is filtered through a sterile grided membrane filter under pressure from a vacuum pump. The membrane filter is then placed on the appropriate media and incubated (WHO, 1993; Payment et al, 2001).

Coliform density is reported as total coliform per 100ml or as faecal coliform per 100ml. Low coliform estimates may be caused by the presence of high numbers of non-coliforms or the presence of toxic substances. The membrane filter technique can be used for the examination of saline waters but not waste waters that have received only primary treatment followed by chlorination.

Other disadvantages include contamination if culture media plates are prepared and stored for too long, and the possibility of variable recovery depending on the quality of membrane filters used (Greenberg et al, 1995).

1.11.3 Pour Plate / Spread Plate Technique

This method is not generally used for drinking water unless evidence shows that it will yield reliable results. The Pour Plate method, formerly known as the Standard Plate Count has several disadvantages that limits the recovery of the maximum number of organisms since organisms tend to grow inside the media used. Incubation at 44°C to 46°C may cause heat shock to stressed bacteria and the medium may decrease recovery of starved bacteria.

Volumes between 0.1 and 1ml are added to the appropriate molten agar, poured into sterile plates and incubated. The number of colonies produced is multiplied by the dilution factor if applicable to obtain the correct figure. Results are also expressed in number of colonies formed per 100ml.

A modified version involving a standardised rod may be used to spread a known volume of sample onto the surface of a medium for incubation. This method, known as the Spread Plate method has the advantage of eliminating heat shock. Additionally, all colonies will be on the surface of the agar where they can be seen and counted readily and distinguished from particulates of air bubbles. It also requires less time and space than the pour plate method but it is limited by the very small amount of water sample that has to be used. Spreading the sample evenly on the surface of the agar can also be a problem (Greenberg et al, 1995; WHO, 1993).

1.11.4 Test for Faecal Streptococci

The term faecal streptococci and Lancefield group D are used synonymously to mean streptococci that occur in the faeces of humans and other warm-blooded animals (Harley and Prescott, 1990). When this group is used as an indicator of faecal contamination, the *Streptococcus faecalis, Streptococcus faecalis* subspecie *liquefaciens, Streptococcus faecalis* subspecie *zymogenes, Streptococcus faecium, Streptococcus bovis* and *Streptococcus equinus* are the species and subspecies implied (Hill, 1999; Mackie and McCartney, 1978).

The normal habitat of faecal streptococci is the intestines of humans and animals; thus these organisms when present in water are indicators of faecal pollution. Faecal streptococci have short survival times outside their natural habitat; thus, their presence in water indicates very recent contamination (Harley and Prescott, 1990). Faecal streptococci are tested for either by using the Multiple Tube technique, Membrane Filtration or Pour Plate method (Greenberg et al, 1995; WHO, 2003).

The type of faecal pollution, if any, can be established by means of a faecal coliform (FC) count (indicative of human pollution) and faecal streptococcal (FS) count (indicative of pollution from other animal sources). The ratio of faecal coliforms to faecal streptococci (FC/FS) is interpreted as follows: 0.7-4.4 indicates human and animal pollution, >4.4 indicates human pollution and <0.4 indicates poultry and livestock pollution (Harley and Prescott, 1990).

1.12 STANDARDS OR LIMITS FOR WATER QUALITY

In the United States of America, the standard set for potable water is a limit of 1 coliform per 100ml and an action limit of 4 coliforms per 100ml (USEPA, 1996, Harley and Prescott, 1990). The WHO states that any water sample intended for drinking should not have coliforms present in any 100ml sample (WHO, 1993, WHO, 1997). The Ghana Standards Board sets the limit for all water intended for drinking as "When 100ml sample of drinking water is tested by appropriate standard methods, *Escherichia coli* or thermotolerant coliforms shall not be detected" (GSB, 1998).

1.13 IDENTIFICATION OF BACTERIA

Various tests have to be carried out to correctly identify an unknown organism. Staining, morphology, motility, culture data and biochemical reactions might be used (Brown, 2005). When enough information has been gathered, a taxonomic key which enables one to identify the organism is consulted.

Some of the tests used to determine the morphology of organisms are the gram stain, acid fast stain, motility and cellular arrangement and endospore stain. The cultural characteristics of an organism pertain to its macroscopic appearance on different kinds of media. Tests used to determine the cultural characteristics of organisms include inoculation on nutrient agar slants, inoculation in nutrient broth, thioglycolate media and gelatin (Brown, 2005, Harley and Prescott, 1990).

Tests used to determine the physiological characteristics include oxidation and fermentation tests including durham-tube sugar fermentations, mixed acid fermentations, butanediol fermentation, catalase production, oxidase production and nitrate reduction. The Durham-tube sugar fermentation is used to test the ability of the organism to ferment various sugars (glucose, sucrose, mannitol, inositol, lactose, etc) with the production of gas. The mixed acid test involves the Methyl-Red test. Butanediol fermentation requires performing the Voges-Proskauer test. The catalase test is performed to determine whether the organisms are aerobes or otherwise. The oxidase test assays for the presence of cytochrome oxidase. Nitrate reduction is used to assay for the presence of nitrite (Brown, 2005, Harley and Prescott, 1990).

Certain hydrolytic reactions such as starch, casein, fat, tryptophan and urea hydrolysis also need to be performed to help further in the identification of the organisms. Finally, several additional physiological tests that include tests for hydrogen sulphide production, citrate utilisation, phenylalanine deaminization, indole and urease production and litmus milk reactions might be of great help.

Once all the data have been recorded, a manual of bacterial identification, like the Bergy's Manual of Systematic Bacteriology, is consulted and the organism is duly identified by its genus and species (Brown, 2005, Harley and Prescott, 1990).

Going through the above system is very tedious and requires tremendous amount of media, glassware and time. These disadvantages were overcome with the introduction of miniaturised multitest systems.

Miniaturised systems have the advantages of requiring minimum media preparation, simplicity of performance, reliability, rapid and uniform results. The miniature systems consist of a plastic tube or strip that contains many different media to be inoculated and incubated. To facilitate rapid identification, these systems utilise numerical coding systems that can be applied to charts or computer programmes. The tests are usually for organisms in certain categories.

For example, there are miniature tests for the identification of gram-negative, oxidative-negative bacteria (enterobacteriaceae), gram-negative, oxidative-positive bacteria, Staphylococci and gram negative rods. Examples of miniature tests are the API, Enterotube II and Oxi/Ferm Tube II systems of identification (Brown, 2005, Harley and Prescott, 1990).

1.14 API 20E SYSTEM OF MICROBIAL IDENTIFICATION

The API 20E System is a miniature version of conventional tests that are used for identification of members of the family enterobacteriaceae and other gram-negative bacteria. It was developed by Analytab Products, New York (Brown, 2005). The system utilises a plastic strip with 20 separate compartments. Each compartment consists of a depression or a cupule and a small tube that contains a specific dehydrated medium. The system has a capacity of 23 biochemical tests (API 20E ref.).

To inoculate each compartment, a saline suspension of the unknown organism is made, then with the help of a pasteur pipette, each compartment is filled with the bacterial suspension. The cupule receives the suspension and allows it to flow into the tube of medium.

The dehydrated medium is thus reconstituted by the saline suspension. To provide anaerobic conditions for some of the compartments, sterile mineral oil is added to them.

After incubation for 18 to 24hours, the reactions are recorded, test reagents are added to some compartments and test results are tabulated. A 7 or 9 digit profile is computed and using the Analytical Profile Index, one is able to determine the name of the organism (Brown, 2005; Harley and Prescott, 1990; API 20E ref.).

1.15 USE OF ANTIBIOTICS IN THE TREATMENT OF DIARRHOEA

The principal bacteria pathogens associated with acute gastrointestinal illness from drinking contaminated water are various Salmenella and Shigella species, *Escherichia coli, Caphylobacter jejuni, Vibrio cholerae, Aeromonas hydrophila* and *Yersinia enterocolitica* (Ashbolt, 2003; Luanne, 1997). Enterobacter and Citrobacter species have also been associated with gastroenteritis (Mackie and McCartney, 1978).

The pathogenicity of invasive bacteria is generally the result of toxins that are produced by these bacteria. These toxins work through various mechanisms to disrupt the ionic balance of the cell membrane which causes the cells of the small intestines to secrete more water than can be absorbed by the large intestines, resulting in diarrhoea (EMS, 2000).

The first line of treatment in acute diarrhoea, as in gastro-enteritis, is prevention of fluid and electrolyte depletion (BNF, 2006). This is particularly important in infants and children under 5years of age.

Antibacterial drugs are generally unnecessary in simple diarrhoea but when it is severe or when there is systemic bacterial infection there is the need for appropriate treatment (BNF, 2006, STG, 2004). Antibiotics that are used for the management of diarrhoea include macrolides like erythromycin, quinolones like ciprofloxacin, cephalosporins like cefotaxim and others like chloramphenicol, trimethoprim and metronidazole (BNF, 2006).

Products of combinations of the above have also been produced that are effective for the management of diarrhoea. Examples are the Sulphamethoxazole-Trimethoprim combination (Co-trimoxazole), Metronidazole-Furazolidone combination (Metrolex F) and the Ciprofloxacin-Tinidazole combination (BNF, 2006; STG, 2004).

On the Ghanaian market, various drugs are available for the management of diarrhoea. Ten (10) of such products were used in this research.

1.16 AIM OF THE RESEARCH

The aim of the research is to determine the bacteriological quality and physical properties of the water used for drinking in three peri-urban communities in Kumasi.

The research also aims to determine the Minimum Inhibitory Concentration of some common antibiotics sold on the Ghanaian market for the management of diarrhoea against diarrhoea-causing organisms present in drinking water.

1.17 SCOPE OF THE RESEARCH

Activities to be undertaken include:

- Development, distribution and analysis of questionnaires to opinion leaders, Health Care centres and some users of the water source in the communities.
- Determination of the pH, turbidity, and conductivity, total dissolved substances and colour of the samples.
- Isolation and enumeration of total and faecal coliforms using the Membrane Filtration technique.
- Identification of isolated organisms using API 20E system if identification.
- Determination of Minimum Inhibitory Concentrations using the Cup Plate method.

CHAPTER TWO

MATERIALS AND METHODS

2.0 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Glassware

Test tubes, petri dishes, volumetric flasks, measuring cylinders, sample bottles, beakers were thoroughly cleaned with detergent and dried. Where necessary, it was sterilized by hot air at 160°C for two hours.

2.1.2 Plastic ware

Sterile disposable syringes (Hongda Med) of volumes 2ml, 5ml and 10ml were used for precise measurement of volumes.

2.1.3 Other Materials

Forceps, platinum loop, cork borer No. 6, etc. were thoroughly cleaned with detergent and dried and where necessary, was sterilized by hot air at 160°C for two hours.

2.2 INSTRUMENTS

Autoclave- Express Pressure Steam sterilizer (Arnold and Sons Ltd, Basildon)

Balance- FEL Service analytical and precision balance (Model TL2472)

Colony counter- Gerber Instruments AG Type 06, 1100

Hot Air oven- Gallenkamp Hot Air oven BS Size 1 Model OV-330

Incubators- Gallenkamp Plus II incubator, Gallenkamp Compensat incubators.

Laminar horizontal flow table- SKAN AG (Postfach CH-4009 Basel)

Membrane filter paper-

Microscope- Reichert Neovar 40

Nessleriser- 2150 Loviband

pH meter- WTW pH 323, WTW 82365

Thermometer- 75mm Immersion thermometer (-10 to 110 °C)

Turbidmeter- Nephla EU DR LANGE

Water bath- New Brunswick Scientific Reciprocal water shaker

Membrane Filtration setup with Vacuum pump. The membrane filter paper of the following specification - diameter 47mm, pore size – 0.45um, absorbent, white and gridded. (Sartorius AG) was used for the filtration.

2.3 CULTURE MEDIA

- Cetrimide agar (Scharlau 01-160)
- Eosin Methylene Blue agar (Scharlau 01-068)
- MacConkey agar No. 2 (Oxoid CM 0109)
- Mannitol salt agar (Scharlau 01-116)
- Nutrient agar (Oxoid CM0003)
- Nutrient broth (Oxoid CM0001)
- Slanetz Bartley agar (Scharlau 01-178)
- Tryptose Lauryl Sulphate broth (Scharlau 02-108)

2.4 METHODS

2.4.1 COMMUNITIES OF STUDY

Questionnaires were designed and given to opinion leaders and to the various health posts (Appendix IV, V and VII) in the communities under study with the aim of knowing more about the communities (Varkevisser et al, 1991; Kumekpor, 2002). Informal interviews of landlords and landladies who owned wells were also conducted to obtain required information (Appendix VI) (Kumekpor, 2002). Some data about the communities was sourced from the Ghana Water Company Limited (GWCL), the Community Water and Sanitation Agency and the Ashanti Regional Statistics office. The communities were also observed personally for their way of life (Appendix III) (Kumekpor, 2002).

2.4.2 CODING OF SAMPLES

A combination of alphabets and numerals indicating the name of the community, the location of the water source and the type of water supply was used to code the samples for consistency, easy labelling and quick reference (Kumekpor, 2002; Varkevisser et al, 1991).

Table 2.4.2.1 Interpretation of codes used to label samples

Code	Interpretation
ME1W	Well from Mesewam
AP1LBH	Borehole from Aprade, 1 Left
AP1RPT	Poly tank from Aprade, 1 Right
AP2LW	Well from Aprade, 2 Left
AP2RW	Well from Aprade, 2 Right
DO1LBH	Borehole from Domeabra, 1 Left
DO1RBH	Borehole from Domeabra, 1 Right
DO2RBH	Borehole from Domeabra, 2 Right
DO3RBH	Borehole from Domeabra, 3 Right

2.4.3 SAMPLING METHOD

The samples were collected before sunrise (Greenberg et al, 1995). The 1500ml sampling bottles were thoroughly washed and disinfected with alcohol. A rope was attached to the bottle and immersed in the well and allowed to fill with water. Care was taken so that the bottle did not touch the sides of the well. Once the bottle was full, it was pulled out of the well and corked firmly, ensuring that there was enough air space in the bottle, at least 3cm, at the top of the bottle. For the sampling from boreholes and poly tank sources, the water was pumped and allowed to run for about 3 minutes before collection.

The corked bottles were appropriately labelled and transported to the laboratory in a cool box that would maintain the temperature of the samples (Greenberg et al, 1995; Cochran, 1977; WHO, 1993).

2.4.4 DETERMINATION OF PHYSICAL PROPERTIES

2.4.4.1 Total Dissolved Solids (TDS)

About 100ml of the sample was poured into a sterilized beaker. The conductimeter was placed in the water and programmed to read the TDS. The value was recorded once the reading had stabilized.

2.4.4.2 Conductivity

About 100ml of the sample was poured into a sterilized beaker. The conductimeter was placed in the water. The value for the conductivity was recorded once the reading had stabilized.

2.4.4.3 Turbidity

A volume of the sample was poured into the turbidity cuvette and the turbidity read off from the digital display panel. The reading was recorded three times and the average of the results recorded as the turbidity of the sample.

2.4.4.4 Colour

Two nessler tubes were filled to just about full, one with the sample and the other, with sterile distilled water as the reference. They were placed into the nessleriser and the hue rotated until the sample had the same colour as the reference. Where the colour of the sample was not matching, the sample was diluted with sterile distilled water and the process repeated. The value of the shade of hue that was identical with the sample and reference was recorded and where necessary, multiplied by the dilution factor.

2.4.4.5 pH

About 100ml of the sample was poured into a sterile beaker. The pH meter was rinsed with sterile distilled water and used to read the pH of the sample. When the pH reading had stabilized, the value displayed on the panel was recorded.

2.5 DETERMINATION OF MICROBIOLOGICAL PROPERTIES

2.5.1 Preparation of Culture Media

See Appendix I

2.5.2 Pour Plate Method

Mannitol Salt agar (20ml) was melted and stabilized at 45°C for 15 minutes. Using a sterile syringe, 1ml of the samples was drawn aseptically into a sterile petri dish. The stabilised media was poured into the petri dish containing the sample, swirled clockwise gently to mix and allowed to solidify. The petri dish was inverted and incubated at 37°C for 24 hours. For each sample, two plates were prepared.

The above was repeated using Bismuth Sulphite agar, Eosin Methylene Blue agar and Cetrimide agar.

2.5.3 Inoculation of water samples into Lauryl Sulphate broth

One ml quantities of the various samples was added to 10ml Lauryl Sulphate broth containing inverted durham tubes and incubated at 37°C for 24 hours. The tubes were observed for growth (turbidity) and gas production. The tubes were also observed under ultra violet rays of wave length 365 nanometers for fluorescence.

The tubes were tested for indole production by the addition of Kovac's reagent. The test was recorded as positive if the reagent layer turned cherry red after 2 minutes.

2.5.4 Membrane filtration

Using sterile forceps, a sterile membrane filter paper was placed (with the grid side up) on the receptacle of the filtration unit. The funnel unit was carefully placed over the receptacle and locked into place. 100ml portions of the sample were filtered under partial vacuum.

With the filter paper still in place, the funnel was rinsed with about 30ml sterile distilled water. The funnel was lifted and the filter paper immediately removed using sterile forceps and placed on the agar plate in a petri dish, with the grided portion upwards, in a rolling motion to avoid entrapment of air (Greenberg et al, 1995).

Lauryl Sulphate broth was solidified with 1.5% agar agar (Difco, 1992).

Each sample was filtered in triplicates. The petri dishes were inverted and incubated at 37°C for 48 hours. The colonies that were formed were counted with the aid of a colony counter and recorded as total coliforms. Samples were filtered using the above procedure and incubated on Slanetz and Bartley's agar at 44°C for 48 hours. The colonies were counted and recorded as faecal coliforms.

2.6 ISOLATION OF ORGANISMS

The colonies that were formed on the various selective media were fished out depending on their colour and growth morphology and inoculated into nutrient broth and incubated at 37°C for 24 hours. Agar slants of the various organisms were prepared and stored for identification and subsequent use.

2.7 STAINING

2.7.1 Simple Staining

A loopful of culture from the agar slant was fixed on a slide. The slide was flooded with Methylene Blue solution for 30seconds and then washed with water. The slide was blotted dry and the organism observed under the microscope for its morphology (Brown, 2005).

2.7.2 Gram Staining

A loopful of the culture was fixed on a slide. Ammonium Oxalate Crystal Violet solution was applied for 20seconds and washed with water. Lugol's Iodine solution was applied for 30seconds, then decolorized with 95% Ethyl Alcohol for 5seconds and washed with water. It was counterstained with 0.5% Safranin for 30seconds, washed with water and observed under the microscope for its reaction to Gram's stain (Brown, 2005).

From the pattern of growth, simple staining and gram staining, the organisms that had the same features were considered as identical and confirmed using the API 20E test kit.

2.8 IDENTIFICATION OF ORGANISMS

2.8.1 API Identification

A total of 20 isolates were randomly selected from the agar slants performed and identified as follows: a loopful of bacteria was transferred into a test tube containing 5ml of sterile 0.85% saline and shaken to disperse the organism thoroughly. The flap of the incubation tray was labelled with the known characteristics of the organism. About 5ml water was added to the base of the tray to provide a humid atmosphere during incubation. One API strip was removed and placed in the incubation tray and the remaining strips resealed immediately.

Drawing the suspension of organisms into a 5ml disposable syringe, the API test strip was tilted and inoculated as follows: the microtubes marked ADH, LDC, ODC, H_2S and URE were slightly under filled. The microtubes marked CIT, VP and GEL were filled to the brim. The rest of the microtubes were filled halfway. After inoculation, the microtubes marked ADH, LDC, ODC, H2S and URE were completely filled with sterile paraffin oil (Sterilised at 160 ^{0}C for two hours, Harley and Prescott, 1990), to create anaerobic conditions. The incubation tray was covered and incubated at 36^{o}C for 18hours.

After 18 hours, the results for ONPG, ADH, LDC, ODC, CIT, H2S, URE, GEL, GLU, MAN, INO, SOR, RHA, SAC, MEL, AMY and ARA were recorded. One drop of TDA solution (10% Ferric chloride) was added to the TDA microtube and the results recorded. One drop of James solution (Kovac's reagent) was added to the IND microtube and the results recorded. Finally, one drop each of VP1 (α-naphthol solution) and VP2 (40% KOH) solutions were added to the VP microtube. This was allowed to stand for about 10 minutes before the results were recorded.

For nitrate reduction test, one drop each of NIT 1 (0.8% sulfanilic acid) and NIT 2 (0.5% N, N-dimethyl-α-naphthyylamine) solutions were added to the GLU test microtube. The color change was recorded after 3 minutes. Negative tests were confirmed by the addition of zinc metal. To test for catalase, one drop of 1.5% hydrogen peroxide was added to the MAN, INO and SOR microtubes and the tubes that had bubbles were recorded as positive.

After all the reactions have been recorded on the report sheet, the seven-digit profile number was identified as follows: Within each test section, the numbers to the positive tests were added and the sum written in the circle provided at the end of the section. The unknown organism was identified by looking up the profile number in the API 20E Index Booklet (API 20E ref).

Once the organism had been identified, the agar slant from which the inoculum was prepared was appropriately labelled and stored for use.

2.9 STANDARDIZATION OF TEST ORGANISMS

The efficacy of a drug is influenced by the inoculum size of the micro organism. It was therefore considered necessary to ascertain that a standard inoculum size is used throughout the MIC determination (Davies et al, 1980; Denyer and Hugo, 1991).

A straightened platinum loop was used to pick isolated colonies from the stock plates and inoculated into separate 10ml nutrient broths. These were labelled appropriately and incubated at 37°C for 24 hours. One loopful quantities of the cultures was serially diluted (10^2 and 10^4 folds) using sterile distilled water. 1ml quantity of each diluted suspension was inoculated into appropriately labelled nutrient agar and incubated at 37°C for 24 hours.

The total colonies formed were counted and multiplied by the dilution factor for that particular dilution, thus determining the number of viable cells by the Pour Plate Method of bacterial enumeration. The number of loopfuls needed to produce approximately 3×10^6 colonies was calculated and those loopfuls transferred into one ml sterile nutrient broth for dilution. Volumes of the one ml that would yield approximately 3×10^6 CFU were then drawn using sterile syringes and used for the MIC determination.

Of all the organisms that were identified, the ones that are potential diarrhoea-causing were selected, standardized and used.

2.10 MIC DETERMINATION USING THE CUP PLATE METHOD

2.10.1 Preparation of Test Solutions

One tablet containing 200mg Metronidazole BP was powdered and added to enough sterile water to make 100ml. Out of this initial stock solution, three other lower concentrations were prepared. The above was repeated using Ciprofloxacin BP, Erythromycin BP, Co-trimoxazole (Sulfamethoxazole BP + Trimethoprim BP) and Metrolex F (Metronidazole BP + Furazolidone BP) tablets.

The content of one capsule of Tetracycline, equivalent to Tetracycline BP, 250mg, was added to enough sterile water to make 100ml. Out of this initial stock solution, three other lower concentrations were prepared. The above procedure was repeated using Chloramphenicol BP capsule.

Metronidazole suspension (2 ml) equivalent to 80mg Metronidazole BP was added to enough sterile water to make 100ml. Out of this initial stock solution, three other lower concentrations were prepared. The above procedure was repeated using suspensions of Metrolex F (Metronidazole BP + Furazolidone BP) and Co-trimoxazole (Sulfamethoxazole BP + Trimethoprim BP).

2.10.2 Antibacterial Activity

Twenty millilitres (20 ml) of nutrient agar was melted and stabilized at 45°C in a water bath. It was aseptically seeded with the calculated volume of the 24 hour broth cultures of the identified organism containing 3×10^6 CFU/ml and poured into a sterile petri dish and allowed to set. A flame-sterilized cork borer No. 6 (10mm diameter) was used to bore 4 equidistant holes in the set agar. These were filled to ¾th full with the different concentrations of the antibiotics.

The plates were pre-incubated at room temperature for 30 minutes to allow adequate diffusion of the antibiotics and then incubated at 37°C for 24 hours. The Zones of Inhibition (in millimetres) were measured (from the edge of the cup to the start of growth) and recorded. A straight line graph of log concentration against Zone of Inhibition was plotted and the antilog of the x-intercept calculated as the Minimum Inhibitory Concentration of the drug against that particular organism.

CHAPTER THREE

RESULTS

3.0 RESULTS

3.1 COMMUNITIES OF STUDY

Answers to the questionnaires given to Opinion leaders (Appendix IV), Healthcare providers (Appendix V) and some selected homes and well owners (Appendix VI) were summarised and recorded under 3.1.1 (for Aprade), 3.1.2 (for Mesewam) and 3.1.3 (for Domeabra). Results to the Guidelines for Personal Observation (Appendix III) were summarised and recorded under 3.1.1.1. (for Aprade), 3.1.2.1 (for Mesewam) and 3.1.3.1 (for Domeabra).

3.1.1 APRADE

This is a peri urban community of Kumasi that falls under the Asokwa district with a population of about 3000 (Statistical Unit, 2006). It is about 3km from the Accra-Kumasi highway. Trading is the major occupation and about 90% of the inhabitants are Christians. Although the community has no tourist attraction, it used to be popular for its fetish shrine and its numerous traditional health attendants (with only two in active operation now). The community has one Municipal Assembly basic school and four private basic schools. They have two public toilets and two refuse dumps. They have three Licensed Chemical Shops, one having been in existence for over 10 years and the other two under five years.

Aprade has one borehole, three wells that are well patronized by members of the community and numerous individually dug wells in houses at the new site of the community. One of the

wells which is highly patronized by the populace has the water connected into an overhead polytank. A bucket of water costs between one and two Ghana pesewas for the polytank and the borehole and free for the wells. They also have one stream (Subin) which they use for washing and building purposes.

The most common disease in the community is Malaria (Anifori, E., Personal Communication, 2007) and this was attributed to the poor sanitation in the community – choked gutters, weedy surroundings, indiscriminate disposal of rubbish, etc. Semiannually, the community receives talks on various health conditions like proper eye care, malaria, typhoid fever, diabetes and tuberculosis from various groups the recognized ones being the Emena Hospital and Micro Care, an NGO.

3.1.1.1 PERSONAL OBSERVATION

The community wakes up between 4:30 and 5am and by 5:30am; most household chores have been done. Children and adult women are the main people who fetch water for the household in the mornings. The community is quiet when the children are in school and gets vibrant again when they are out of classes either for break or at closing. In the afternoon, it is usually the children who fetch water. Activities in the community are at its peak in the evening after 6pm when most people have returned from their works. People continue to fetch water till about 9:00pm when activities start dwindling.

A lot of water is fetched on weekends and some women even wash clothing around the wells and the borehole. The turbidity of the borehole does not change with the quantity of water fetched although the force with which the water flows decreases and more strokes will have to be made in order to fill one bucket.

For the wells, the turbidity of the water changes depending on the season with the water being more turbid during the wet season and when the water is very low during the dry season. One rubber container with a rope attached is used for drawing water and it is left on the well when not in use. The wells are de-silted and washed once a year.

3.1.2 MESEWAM

This is a small farming community with old buildings and very neat surroundings. It also falls under the Asokwa district and has a population of about 500 inhabitants (Statistical Unit, 2006). It is about 5km off the Accra-Kumasi highway. They have two churches, one well, one public toilet and one refuse dump. They do not have a school. They have a community clinic which has been in existence for the past 11 years. The major reported cases are dog and snake bites and malaria (Mesewam Clinic archives).

The community benefits regularly from health educational talks on HIV/AIDS, Malaria and Tuberculosis from the Atonso Agogo Government and Emena Hospitals.

3.1.2.1 PERSONAL OBSERVATION

Mesewam is a very neat community with well kept streets. The community is very quiet during the day and only alive during the early hours of the day and late afternoons when the school children and their parents have returned from school and work respectively. Although they have electricity, the community wakes up quite late – around 5am.

Each household has its own container for fetching water and they usually come in households to fetch water from the well. Although the well is very deep (30 feet), the water becomes turbid after very few draws and as such only adults do the drawing so as to do it carefully. The well is de-silted and washed once a year, usually in the month of March, just before the start of the rainy season.

3.1.3 DOMEABRA

Domeabra, a farming community about 10km from the Accra-Kumasi highway has a population of about 2000 inhabitants (Statistical Unit, 2006). The community has 6 churches, 4 basic schools, two public toilets and two refuse dumps. There are two licensed chemical shops, with one of them closed almost all the time and 4 known herbalists. Since they do not have a clinic or hospital, the members visit the two health posts (hospital and clinic) at Apromase, a community about 2km from Domeabra.

The community has 4 boreholes and a stream, Akokronari. The stream water is used mainly for agricultural purposes. The community has regular monthly health meetings organised by the Ministry of Health - Community Health for nursing mothers and occasional health talks by private groups on issues like eye care among others.

3.1.3.1 PERSONAL OBSERVATION

The community wakes up between 5 and 5:30am because the boreholes are usually unlocked at 5:30am. Children and adult women are the main people who fetch water for the household in the mornings. In the afternoon, children are the main people who go fetching water and this continues till about 9:30pm. The boreholes are locked at 10:30pm. A bucket of water costs one Ghana pesewa. The Unit Committee keeps the money and uses it for repairs when the need arises, which is often – about three times in a year.

On Saturdays, the women and some children do their washing near the boreholes. The main problem faced is when the link is broken and the boreholes have to be repaired and this can take over a month depending on the amount of money already available.

3.2 PHYSICAL PROPERTIES OF WATER SAMPLES

The tables below (3.2.1-3.2.6) show the results obtained after analysing the water samples for Total Dissolved solids, Conductivity, Turbidity, Colour and pH.

Table 3.2.1 Physical Properties of water sampled in October 2007

Source (Limits)	TDS/mg/ml (500)	Conductivity/µs/cm (400)	Turbidity/NTU (5)	Colour/ °H (15/50)	pH (6.5-8.5)
ME1W	177.0	182.65	33.83	<150	5.19
AP1LBH	60.0	62.00	0.33	<5	5.23
AP1RPT	181.5	185.15	0.33	<5	5.14
AP2LW	125.5	128.00	9.10	<40	5.37
AP2RW	59.0	61.05	2.12	<20	4.51
DO1LBH	101.5	104.30	0.07	<5	4.71
DO1RBH	75.0	76.15	0.11	<5	5.30
DO2RBH	66.8	70.30	0.17	<5	5.55
DO3RBH	260.5	264.50	0.03	<5	5.14

Table 3.2.2 Physical Properties of water sampled in November 2007

Source (Limits)	TDS/mg/ml (500)	Conductivity/µs/cm (400)	Turbidity/NTU (5)	Colour/ °H (15/50)	pH (6.5-8.5)
ME1W	182.0	186.3	37.33	<150	5.38
AP1LBH	60.0	62.3	0.350	<5	5.00
AP1RPT	183.0	186.3	0.310	<5	5.30
AP2LW	125.0	128	10.00	<50	5.48
AP2RW	59.0	60.1	2.35	<20	4.38
DO1LBH	104.2	106.6	0.116	<5	4.83
DO1RBH	75.0	76.7	0.033	<5	5.13
DO2RBH	64.5	67.8	0.170	<5	5.17
DO3RBH	255.0	260.0	0.020	<5	5.06

Table 3.2.3 Physical Properties of water sampled in December 2007

Source (Limits)	TDS/mg/ml (500)	Conductivity/µs/cm (400)	Turbidity/NTU (5)	Colour/ °H (15/50)	pH (6.5-8.5)
ME1W	172.0	179	30.33	<120	5.00
AP1LBH	60.0	61.7	0.300	<5	5.45
AP1RPT	180.0	184	0.340	<5	4.98
AP2LW	126.0	128	8.200	<30	5.25
AP2RW	59.0	62	1.890	<20	4.64
DO1LBH	98.7	102	0.024	<5	4.58
DO1RBH	75.0	75.6	0.183	<5	5.46
DO2RBH	69.0	72.8	0.171	<5	5.92
DO3RBH	266.0	269	0.030	<5	5.21

Table 3.2.4 Physical Properties of water sampled in January 2008

Source (Limits)	TDS/mg/ml (500)	Conductivity/µs/cm (400)	Turbidity/NTU (5)	Colour/ °H (15/50)	pH (6.5-8.5)
ME1W	167	170.6	38.2	<150	5.11
AP1LBH	62	63.4	0.08	<10	4.85
AP1RPT	247	252	0.08	<10	5.31
AP2LW	124	126.1	8.97	<50	5.12
AP2RW	73	76.6	3.35	<30	4.71
DO1LBH	107	110.1	0.19	<10	4.87
DO1RBH	86	90.0	0.81	<5	5.12
DO2RBH	64	65.7	0.11	<10	5.06
DO3RBH	248	253.0	0.05	<10	5.21

Table 3.2.5 Physical Properties of water sampled in February 2008

Source (Limits)	TDS/mg/ml (500)	Conductivity/µs/cm (400)	Turbidity/NTU (5)	Colour/ °H (15/50)	pH (6.5-8.5)
ME1W	167	170.2	17.09	<100	5.12
AP1LBH	63	65.4	0.16	<10	4.90
AP1RPT	247	253.0	0.17	<10	5.43
AP2LW	115	117.0	1.40	<50	5.02
AP2RW	82	84.0	1.22	<30	4.81
DO1LBH	105	106.6	0.37	<10	5.15
DO1RBH	71	72.3	0.13	<10	5.11
DO2RBH	67	68.7	0.12	<10	5.41
DO3RBH	248	253.0	0.16	<10	4.95

Table 3.2.6 Physical Properties of water sampled in March 2008

Source (Limits)	TDS/mg/ml (500)	Conductivity/µs/cm (400)	Turbidity/NTU (5)	Colour/ °H (15/50)	pH (6.5-8.5)
ME1W	167.0	170.40	27.65	<120.00	5.12
AP1LBH	62.5	64.40	0.12	<10.00	4.88
AP1RPT	247.0	252.50	0.13	<10.00	5.37
AP2LW	119.5	121.55	5.19	<50.00	5.07
AP2RW	77.5	80.30	2.29	<30.00	4.76
DO1LBH	106.0	108.35	0.28	<10.00	5.01
DO1RBH	78.5	81.15	0.47	<10.00	5.12
DO2RBH	65.5	67.20	0.12	<10.00	5.24
DO3RBH	248.0	253.00	0.11	<10.00	5.08

Over the six month study period, all the samples had lower pH values. Me1w and Ap2lw, both well samples, had higher turbidity and colour values. All the samples, however, had Total Dissolved Solids and conductivity values within the stipulated WHO and GSB limits.

3.3 BACTERIOLOGICAL PROPERTIES OF WATER SAMPLE

3.3.1 Inoculation into Lauryl Sulphate broth

Results on the growth in Lauryl sulphate broth, fluorescence under UV radiation and indole tests conducted on the sample are tabulated below (Tables 3.3.1.1 - 3.3.1.6):

Table 3.3.1.1 Inoculation into Lauryl Sulphate broth of water sampled in October 2007

Source	Growth	Gas	Fluorescence - 365NM	Indole test
ME1W	+++	++	Present	Positive
AP1LBH	+	+	Present	Positive
AP1RPT	+	-	Absent	Negative
AP2LW	++	+	Present	Positive
AP2RW	++	+	Present	Positive
DO1LBH	+	-	Absent	Negative
DO1RBH	+	-	Absent	Negative
DO2RBH	+	-	Absent	Negative
DO3RBH	-	-	Absent	Negative

+++ = Profuse growth/gas production ++ = Moderate growth/gas production

+ = Little growth/gas production - = No growth/gas production

Table 3.3.1.2 Inoculation into Lauryl Sulphate broth of water sampled in November 2007

Source	Growth	Gas	Fluorescence - 365NM	Indole test
ME1W	+++	++	Present	Positive
AP1LBH	+	+	Present	Positive
AP1RPT	+	-	Absent	Negative
AP2LW	+	+	Present	Positive
AP2RW	+	+	Present	Positive
DO1LBH	+	-	Absent	Negative
DO1RBH	-	-	Absent	Negative
DO2RBH	+	-	Absent	Negative
DO3RBH	-	-	Absent	Negative

Table 3.3.1.3 Inoculation into Lauryl Sulphate broth of water sampled in December 2007

Source	Growth	Gas	Fluorescence - 365NM	Indole test
ME1W	++	++	Present	Positive
AP1LBH	+	+	Absent	Positive
AP1RPT	+	+	Present	Negative
AP2LW	++	+	Present	Positive
AP2RW	+	+	Present	Positive
DO1LBH	-	-	Absent	Negative
DO1RBH	-	-	Absent	Negative
DO2RBH	-	-	Absent	Negative
DO3RBH	-	-	Absent	Negative

Table 3.3.1.4 Inoculation into Lauryl Sulphate broth of water sampled in January 2008

Source	Growth	Gas	Fluorescence - 365NM	Indole test
ME1W	++	+	Present	Positive
AP1LBH	-	-	Absent	Negative
AP1RPT	-	-	Absent	Negative
AP2LW	++	+	Present	Positive
AP2RW	++	+	Present	Positive
DO1LBH	+	-	Absent	Negative
DO1RBH	+	-	Absent	Negative
DO2RBH	-	-	Absent	Negative
DO3RBH	-	-	Absent	Negative

Table 3.3.1.5 Inoculation into Lauryl Sulphate broth of water sampled in February 2008

Source	Growth	Gas	Fluorescence - 365NM	Indole test
ME1W	++	++	Present	Positive
AP1LBH	+	-	Absent	Negative
AP1RPT	+	-	Absent	Negative
AP2LW	++	++	Present	Positive
AP2RW	++	+	Present	Positive
DO1LBH	+	-	Absent	Negative
DO1RBH	-	-	Absent	Negative
DO2RBH	-	-	Absent	Negative
DO3RBH	-	-	Absent	Negative

Table 3.3.1.6 Inoculation into Lauryl Sulphate broth of water sampled in March 2008

Source	Growth	Gas	Fluorescence - 365NM	Indole test
ME1W	++	+	Present	Positive
AP1LBH	+	-	Absent	Negative
AP1RPT	+	-	Absent	Negative
AP2LW	+	+	Present	Positive
AP2RW	+	+	Present	Positive
DO1LBH	+	-	Absent	Negative
DO1RBH	-	-	Absent	Negative
DO2RBH	+	-	Absent	Negative
DO3RBH	-	-	Absent	Negative

The general results of the samples taken over the study period showed that the well samples (Me1w, Ap2lw and Ap2rw), had growth, produced gas, had fluorescence and were indole positive, confirming the presence of *E. coli*. Ap1rpt and Do1lbh were on most occasions contaminated with coliforms other than *E. Coli*. Do1rbh and Do3rbh were the least contaminated sources.

3.3.2 Membrane Filtration

3.3.2.1 Growth on Lauryl Sulphate Agar Limit 0CFU/100ml

The tables below, (3.3.2.1 - 3.3.2.6), represent the CFU (coliforms) after 100ml samples were filtered and incubated on Lauryl sulphate broth solidified with 1.5% agar powder.

Table 3.3.2.1.1Colony Forming Units (CFU) on LSA for water sampled in October 2007

Source	CFU1 /100ml	CFU2 /100ml	CFU3 /100ml	MEAN CFU	SD
ME1W	908	700	753	787	108
AP1LBH	147	154	169	156	12
AP1RPT	127	164	152	147	19
AP2LW	553	455	470	493	53
AP2RW	356	311	368	345	30
DO1LBH	131	108	146	128	19
DO1RBH	17	10	37	21	14
DO2RBH	96	73	84	84	11
DO3RBH	57	46	69	57	11

Table 3.3.2.1.2Colony Forming Units (CFU) on LSA for water sampled in November 2007

Source	CFU1 /100ml	CFU2 /100ml	CFU3 /100ml	MEAN CFU	SD
ME1W	945	750	864	853	98
AP1LBH	119	181	142	147	31
AP1RPT	133	146	192	157	31
AP2LW	579	495	612	562	60
AP2RW	412	138	216	255	141
DO1LBH	131	108	96	112	18
DO1RBH	17	10	37	21	14
DO2RBH	101	54	80	78	24
DO3RBH	68	42	74	61	17

Table 3.3.2.1.3Colony Forming Units (CFU) on LSA for water sampled in December 2007

Source	CFU1 /100ml	CFU2 /100ml	CFU3 /100ml	MEAN CFU	SD
ME1W	870	650	642	721	129
AP1LBH	174	126	196	165	36
AP1RPT	120	181	112	138	38
AP2LW	527	414	328	423	100
AP2RW	300	484	520	435	118
DO1LBH	131	108	196	145	46
DO1RBH	17	10	37	21	14
DO2RBH	90	92	87	90	3
DO3RBH	45	50	63	53	9

Table 3.3.2.1.4Colony Forming Units (CFU) on LSA for water sampled in January 2008

Source	CFU1 /100ml	CFU2 /100ml	CFU3 /100ml	MEAN CFU	SD
ME1W	543	502	412	486	67
AP1LBH	22	30	26	26	4
AP1RPT	82	64	70	72	9
AP2LW	208	365	187	253	97
AP2RW	193	148	198	180	28
DO1LBH	12	14	10	12	2
DO1RBH	14	9	12	12	3
DO2RBH	60	52	50	54	5
DO3RBH	10	14	15	13	2

Table 3.3.2.1.5Colony Forming Units (CFU) on LSA for water sampled in February 2008

Source	CFU1 /100ml	CFU2 /100ml	CFU3 /100ml	MEAN CFU	SD
ME1W	500	458	512	490	28
AP1LBH	24	25	22	24	2
AP1RPT	39	48	55	47	8
AP2LW	222	267	230	240	24
AP2RW	104	153	162	140	31
DO1LBH	20	14	9	14	6
DO1RBH	6	8	3	6	3
DO2RBH	32	41	44	39	6
DO3RBH	8	12	18	13	5

Table 3.3.2.1.6Colony Forming Units (CFU) on LSA for water sampled in March 2008

Source	CFU1 /100ml	CFU2 /100ml	CFU3 /100ml	MEAN CFU	SD
ME1W	522	480	462	488	31
AP1LBH	23	28	24	25	2
AP1RPT	34	28	27	30	4
AP2LW	215	316	209	247	60
AP2RW	122	102	124	116	12
DO1LBH	16	14	10	13	3
DO1RBH	8	5	7	6	2
DO2RBH	20	19	27	22	4
DO3RBH	6	11	12	10	3

Generally, all the samples were contaminated with coliforms but the samples taken from well sources had higher values, indicating more contamination than samples from other sources.

3.3.2.2 Growth on Slanetz and Bartley's Agar (Sl&B) Limit 0CFU/100ml

The tables below, (3.3.2.2.1 - 3.3.2.2.6), represent the CFU (faecal streptococci) after 100ml samples were filtered and incubated on Slanetz and Bartley's agar.

Table 3.3.2.2.1Colony Forming Units (CFU) on Sl&B for water sampled in October 2007

Source	CFU1 /100ml	CFU2 /100ml	CFU3 /100ml	MEAN CFU	SD
ME1W	91	60	89	80	17
AP1LBH	6	9	11	9	3
AP1RPT	3	4	2	3	1
AP2LW	35	34	43	37	5
AP2RW	24	26	35	28	6
DO1LBH	7	5	6	6	1
DO1RBH	10	9	7	8	1
DO2RBH	6	6	1	4	3
DO3RBH	2	3	4	3	1

Table 3.3.2.2.2Colony Forming Units (CFU) on Sl&B for water sampled in November 2007

Source	CFU1 /100ml	CFU2 /100ml	CFU3 /100ml	MEAN CFU	SD
ME1W	102	58	94	85	23
AP1LBH	7	12	12	10	3
AP1RPT	2	0	1	1	1
AP2LW	40	26	31	32	7
AP2RW	28	37	37	34	5
DO1LBH	5	0	1	2	3
DO1RBH	15	9	8	11	4
DO2RBH	1	3	1	2	1
DO3RBH	2	1	4	2	2

Table 3.3.2.2.3Colony Forming Units (CFU) on Sl&B for water sampled in December 2007

Source	CFU1 /100ml	CFU2 /100ml	CFU3 /100ml	MEAN CFU	SD
ME1W	79	62	83	75	11
AP1LBH	5	6	10	7	3
AP1RPT	4	8	2	5	3
AP2LW	29	42	55	42	13
AP2RW	20	14	33	22	10
DO1LBH	9	10	11	10	1
DO1RBH	4	8	6	6	2
DO2RBH	11	9	0	7	6
DO3RBH	2	4	4	3	1

It can be seen that all the samples analysed on the months of October, November and December 2007 were contaminated with faecal coliforms the well samples were more contaminated.

Table 3.3.2.2.4Colony Forming Units (CFU) on Sl&B for water sampled in January 2008

Source	CFU1 /100ml	CFU2 /100ml	CFU3 /100ml	MEAN CFU	SD
ME1W	97	31	75	68	34
AP1LBH	0	0	0	0	0
AP1RPT	0	0	0	0	0
AP2LW	14	26	11	17	8
AP2RW	23	14	14	17	5
DO1LBH	0	0	0	0	0
DO1RBH	0	0	0	0	0
DO2RBH	0	0	0	0	0
DO3RBH	0	0	0	0	0

Table 3.3.2.2.5Colony Forming Units (CFU) on SI&B for water sampled in February 2008

Source	CFU1 /100ml	CFU2 /100ml	CFU3 /100ml	MEAN CFU	SD
ME1W	58	56	62	59	10
AP1LBH	1	0	1	0.67	1
AP1RPT	0	0	0	0	0
AP2LW	12	18	16	15	3
AP2RW	10	12	8	10	2
DO1LBH	1	1	0	0.67	1
DO1RBH	1	1	0	0.67	1
DO2RBH	0	0	0	0	0
DO3RBH	0	0	0	0	0

Table 3.3.2.2.6Colony Forming Units (CFU) on SI&B for water sampled in March 2008

Source	CFU1 /100ml	CFU2 /100ml	CFU3 /100ml	MEAN CFU	SD
ME1W	50	54	66	57	12
AP1LBH	1	0	0	0	0
AP1RPT	0	0	0	0	0
AP2LW	27	43	29	33	9
AP2RW	24	17	15	19	5
DO1LBH	1	1	0	0	0
DO1RBH	1	0	0	0	0
DO2RBH	0	0	0	0	0
DO3RBH	0	0	0	0	0

For samples taken in January, February and March 2008, almost all of them were not contaminated with the exception of the well samples Me1w, Ap2lw and Ap2rw.

3.3.3 Average Colony Forming Units (CFU)

Table 3.3.3.1 Average CFU/100ml on LSA and Sl&B for October, November and December 2007

	OCTOBER		NOVEMBER		DECEMBER	
Source	LAU SUL	SL& B	LAU SUL	SL& B	LAU SUL	SL & B
ME1W	787	80	853	85	721	75
AP1LBH	156	9	147	10	165	7
AP1RPT	147	3	157	1	138	5
AP2LW	493	37	562	32	423	42
AP2RW	345	28	255	34	435	22
DO1LBH	128	6	112	2	145	10
DO1RBH	21	8	21	11	21	6
DO2RBH	84	4	78	2	90	7
DO3RBH	57	3	61	2	53	3

Table 3.3.2 Average CFU/100ml on LSA and Sl&B for January, February and March 2008

	JANUARY		FEBRUARY		MARCH	
Source	LAU SUL	SL& B	LAU SUL	SL& B	LAU SUL	SL & B
ME1W	486	68	490	59	488	57
AP1LBH	26	0	24	0.67	25	0
AP1RPT	72	0	47	0	30	0
AP2LW	253	17	240	15	247	33
AP2RW	180	17	140	10	116	19
DO1LBH	12	0	14	0.67	13	0
DO1RBH	12	0	6	0.67	6	0
DO2RBH	54	0	39	0	22	0
DO3RBH	13	0	13	0	10	0

3.4 POUR PLATE METHOD

Inoculation of 1ml samples into some selective media gave the growth patterns below:

Table 3.4.1 General growth pattern of various samples on selective media by Pour Plate

Source	Media			
	Mannitol Salt Agar	Bismuth Sulphite Agar	EMB Agar	Cetrimide Agar
ME1W	Large pink and yellowish colonies.	Numerous black colonies surrounded by silverish metallic sheen	Dark violet colonies with black centres and green metallic glow, pink colonies with blueish-purple centres.	No growth
AP1LBH	Few Large yellowish coloured colonies	One black colony with a metallic sheen	Purple pinkish colonies	No growth
AP1RPT	Numerous yellowish colonies. Whole media turned yellow	No growth	Purple pinkish colonies	No growth
AP2LW	Numerous large yellowish coloured colonies	Numerous tiny black colonies with a metallic sheen	Large pinkish-purple coloured colonies	No growth
AP2RW	Few discrete yellow colonies	Few black round colonies with and without metallic sheens	Purple-pinkish colonies	No growth
DO1LBH	Few yellow coloured colonies	No growth	Few purple – pink coloured colonies	No growth
DO1RBH	Yellow coloured colonies observed	No growth	Few purple-pink colonies observed	No growth
DO2RBH	Few yellow coloured colonies	No growth	Few purple-pink coloured colonies	No growth
DO3RBH	Large yellowish colonies	No growth	Few purple coloured colonies	No growth

3.5 MICROSCOPY

Simple and gram stains performed revealed that the samples were contaminated with both gram positive and gram negative organisms.

Table 3.5.1 Simple and gram stain slides observed under microscope

Media	Colonies selected	Observation/Inference
Mannitol Salt	Yellow colonies	Gram positive tetracocci
	Pink colonies	Gram positive diplo and tetra cocci
Lauryl Sulphate	Cream colonies	Gram negative streptococci/bacilli
	Yellow colonies	Gram negative cocci
Bismuth Sulphite	Black colonies	Gram negative streptobacilli
Slanetz and Bartleys	Red colonies	Gram positive streptococci
Cetrimide agar	Red colonies	Gram negative bacilli
	Cream colonies	Gram negative coccobacilli
Mac. Agar No. 2	Deep red colonies	Gram negative bacilli
	Less red colonies	Gram negative cocci
EMB	Green colonies	Gram negative cocci
	Pink colonies	Gram negative cocci
	Cotton-like growth	Gram positive cocci

3.6 API 20E IDENTIFICATION OF ORGANISMS

Table 3.6.1 Organisms identified using API 20E Test Kit

Media		Identification code	Organism
Lauryl Sulphate	Cream colonies	51445721	*Escherichia coli*
	Yellow colonies	32067730	*Enterobacter sakazakii*
Bismuth Sulphite	Black colonies	63077631	*Salmonella* spp.
Cetrimide agar	Red colonies	53077211	*Serratia marcescens*
Mac. Agar No. 2	Red colonies	33057631	*Enterobacter cloacae*
EMB	Green colonies	33445631	*Citrobacter diverus*

3.7 IDENTIFICATION OF OTHER ORGANISMS

Characteristic growth on selective media, simple and gram staining of other colonies revealed the presence of the organisms below listed:

Table 3.7.1 Other Organisms detected

Media		Identification
Slanetz and Bartleys	Red colonies	*Streptococci faecalis* (Enterococci)
EMB	Cotton-like growth	*Candida albicans*
Mannitol Salt	Yellow colonies	*Staphylococcus aureus* (pyogens)
	Pink colonies	Presumptive non pathogenic staphylococcus

3.8 Standardization of Organisms

Using the dilution factor and the colony forming units produced, the volume of suspension of organism standardised to produce approximately 3×10^6 CFU was calculated as below:

Table 3.8.1 Standardisation of Organisms

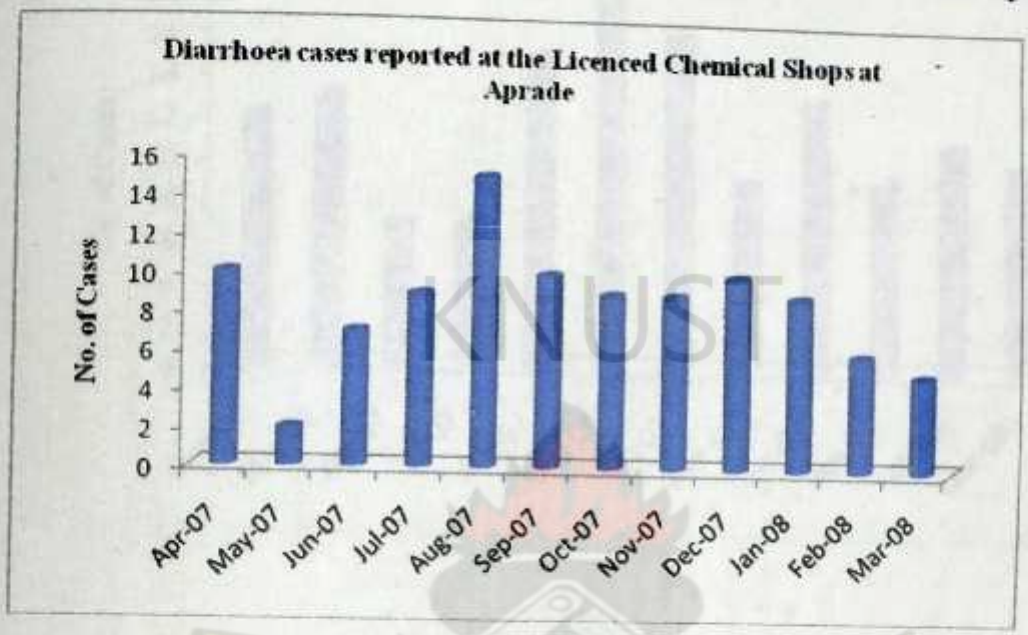
Organism	Dilution factor	CFU	Loopfuls used in 1ml nutrient broth	Volume used in MIC determination(ml)
Escherichia coli	10^4	203	2	0.74
Enterobacter sakazakii	10^4	194	2	0.77
Salmonella spp.	10^4	180	2	0.83
Streptococci faecalis	10^4	254	2	0.59

3.9 DIARRHOEA CASES

Figures 3.9.1.1, 3.9.2.1 and 3.9.3.1 represent the total number of diarrhoea cases in children under 5years reported at the various health centres of the communities of study.

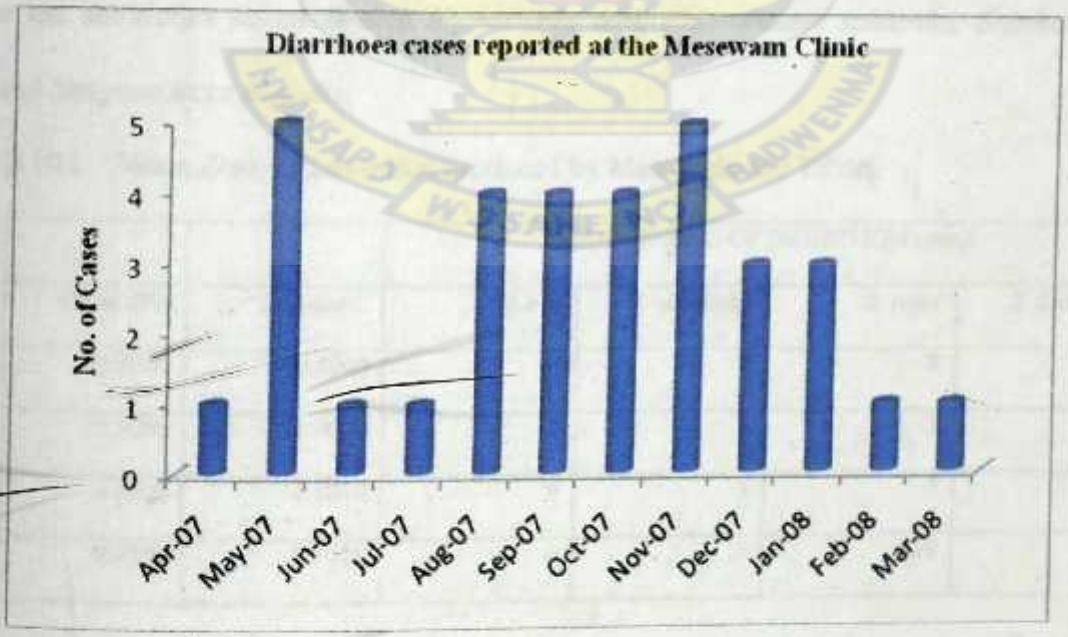
3.9.1 Aprade

Figure3.9.1.1 Reported cases of diarrhoea from April 2007 to March 2008- Aprade



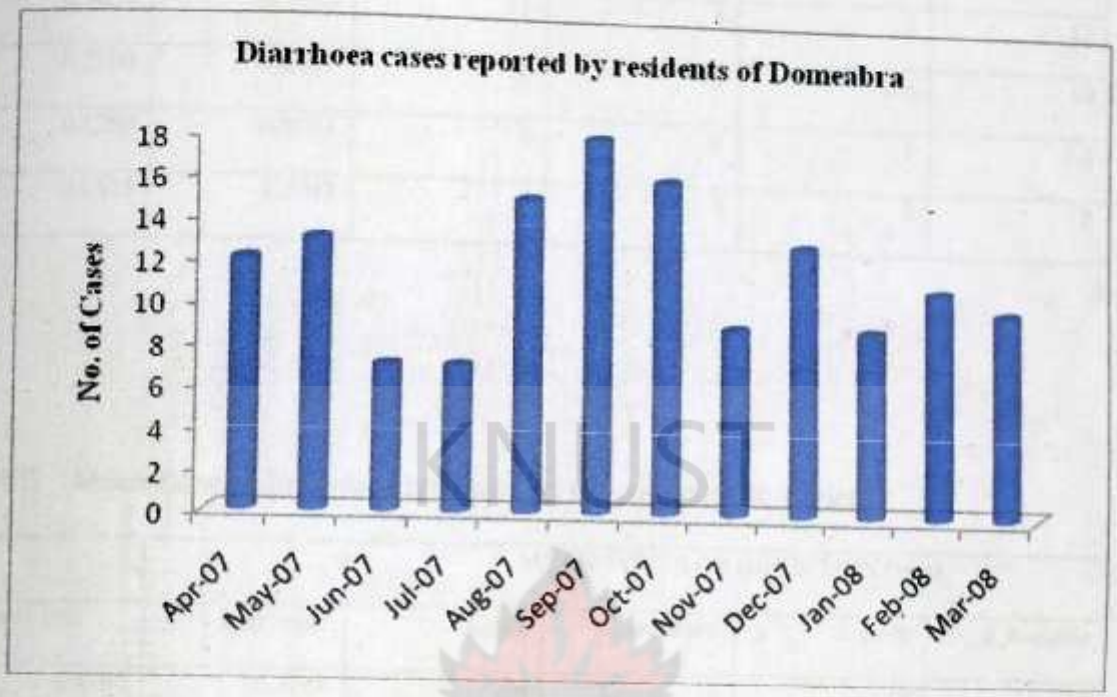
3.9.2 Mesewam

Figure 3.9.2.1 Reported cases of diarrhoea from April 2007 to March 2008 –Mesewam



3.9.3 Domeabra

Figure 3.9.3.1 Reported cases of diarrhoea from April 2007 to March 2008- Domeabra



3.10 RESULTS OF ANTIMICROBIAL TESTS

The tables below (Table 3.10.1 – 3.10.10) indicated the mean Zones of Inhibition exhibited by the ten antibiotics tested against *Escherichia coli, Enterobacter sakazakii, Salmonella typhi* and *Streptococcus faecalis*.

Table 3.10.1 Mean Zone of Inhibition produced by Metronidazole Tablet.

		MEAN ZONES OF INHIBITION (mm)			
Conc. (%)	Log conc.	*E.coli*	*E. sakazakii*	*S. typhi*	*S. faecallis*
0.2000	-0.6990	3	5	8	5
0.1000	-1.0000	1	5	3	3
0.0500	-1.3010	0	3	3	0
0.0250	-1.6021	0	3	0	0

Table 3.10.2 Mean Zone of Inhibition produced by Metrolex F Tablet.

		MEAN ZONES OF INHIBITION (mm)			
Conc. (%)	Log conc.	*E.coli*	*E. sakazakii*	*S. typhi*	*S. faecallis*
0.5000	-0.3010	11	8	6	12
0.2500	-0.6021	10	7	6	10
0.1250	-0.9031	9	6	5	8
0.0625	-1.2041	8	5	3	8

Table 3.10.3 Mean Zone of Inhibition produced by Co-trimoxazole Tablet.

		MEAN ZONES OF INHIBITION (mm)			
Conc. (%)	Log conc.	*E.coli*	*E. sakazakii*	*S. typhi*	*S. faecallis*
0.4800	-0.3188	10	16	16	17
0.2400	-0.6198	10	15	15	15
0.1200	-0.9208	9	13	14	13
0.0600	-1.2218	4	13	13	10

Table 3.10.4 Mean Zone of Inhibition produced by Ciprofloxacin Tablet.

		MEAN ZONES OF INHIBITION (mm)			
Conc. (%)	Log conc.	*E.coli*	*E. sakazakii*	*S. typhi*	*S. faecallis*
0.5000	-0.3010	18	18	14	22
0.2500	-0.6021	17	17	13	21
0.1250	-0.9031	17	17	10	17
0.0625	-1.2041	16	15	10	13

Table 3.10.5 Mean Zone of Inhibition produced by Erythromycin Tablet.

		MEAN ZONES OF INHIBITION (mm)			
Conc. (%)	Log conc.	*E.coli*	*E. sakazakii*	*S. typhi*	*S. faecallis*
0.25000	-0.6021	10	11	15	13
0.12500	-0.9031	10	10	13	12
0.06250	-1.2041	9	9	13	10
0.03125	-1.5051	9	9	11	10

Table 3.10.6 Mean Zone of Inhibition produced by Chloramphenicol capsule.

		MEAN ZONES OF INHIBITION (mm)			
Conc. (%)	Log conc.	*E.coli*	*E. sakazakii*	*S. typhi*	*S. faecallis*
0.25000	-0.6021	12	14	13	13
0.12500	-0.9031	10	13	10	11
0.06250	-1.2041	10	13	9	8
0.03125	-1.5051	8	10	8	8

Table 3.10.7 Mean Zone of Inhibition produced by Tetracycline Capsule.

		MEAN ZONES OF INHIBITION (mm)			
Conc. (%)	Log conc.	*E.coli*	*E. sakazakii*	*S. typhi*	*S. faecallis*
0.25000	-0.6021	12	12	13	12
0.12500	-0.9031	10	11	12	10
0.06250	-1.2041	10	10	11	8
0.03125	-1.5051	8	9	9	7

Table 3.10.8 Mean Zone of Inhibition produced by Metronidazole Suspension.

		MEAN ZONES OF INHIBITION (mm)			
Conc. (%)	Log conc.	*E.coli*	*E. sakazakii*	*S. typhi*	*S. faecallis*
0.0800	-1.0969	11	9	8	3
0.0400	-1.3979	10	8	8	2
0.0200	-1.6990	9	8	7	0
0.0100	-2.0000	9	7	6	0

Table 3.10.9 Mean Zone of Inhibition produced by Metrolex F Suspension.

		MEAN ZONES OF INHIBITION (mm)			
Conc. (%)	Log conc.	*E.coli*	*E. sakazakii*	*S. typhi*	*S. faecallis*
0.0360	-1.4437	11	5	13	9
0.0180	-1.7447	8	4	11	8
0.0090	-2.0458	7	3	10	3
0.0045	-2.3468	5	3	8	3

Table 3.10.10 Mean Zone of Inhibition produced by Co-trimoxazole Suspension.

		MEAN ZONES OF INHIBITION (mm)			
Conc. (%)	Log conc.	*E.coli*	*E. sakazakii*	*S. typhi*	*S. faecallis*
0.0480	-1.3188	15	16	15	18
0.0240	-1.6198	15	15	11	16
0.0120	-1.9208	14	13	5	15
0.0060	-2.2218	13	13	0	14

3.11 Graphs of Minimum Inhibitory Concentration (MIC)

Values of the mean Zones of Inhibition were plotted against the log concentrations of the antibiotics used. The MICs of the antibiotics were obtained from the antilog of the intercepts on the x-axes.

Figure 3.11.1 Graph of Mean Zone of Inhibition against log Concentration (Metronidazole Tablet)

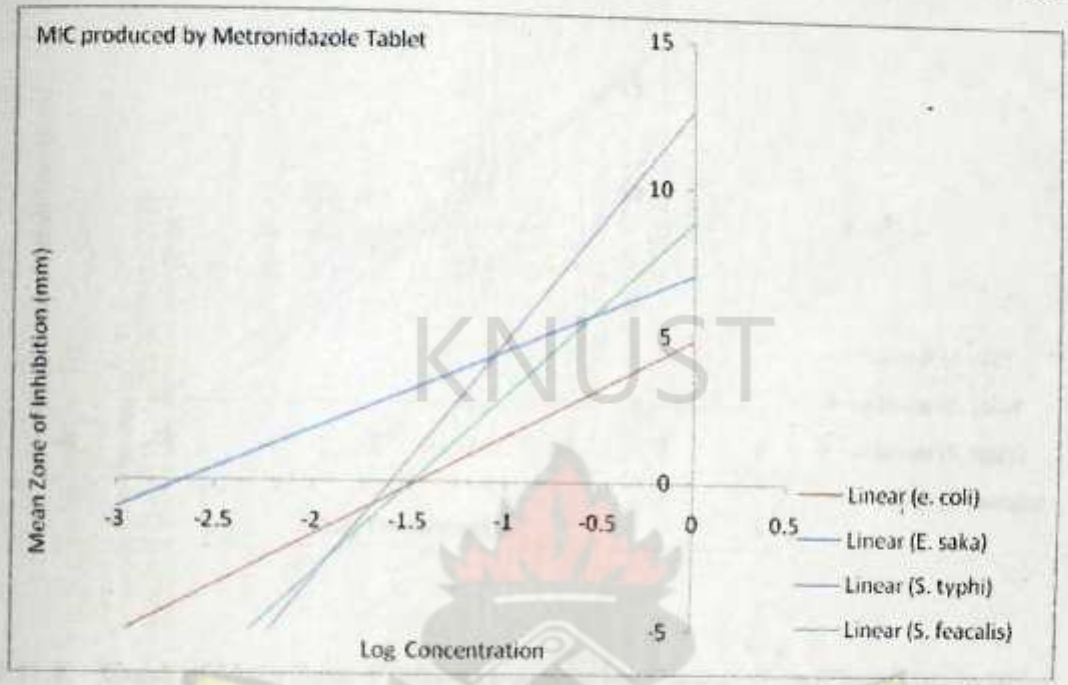


Figure 3.11.2 Graph of Mean Zone of Inhibition against log Concentration (Metrolex F tablet)

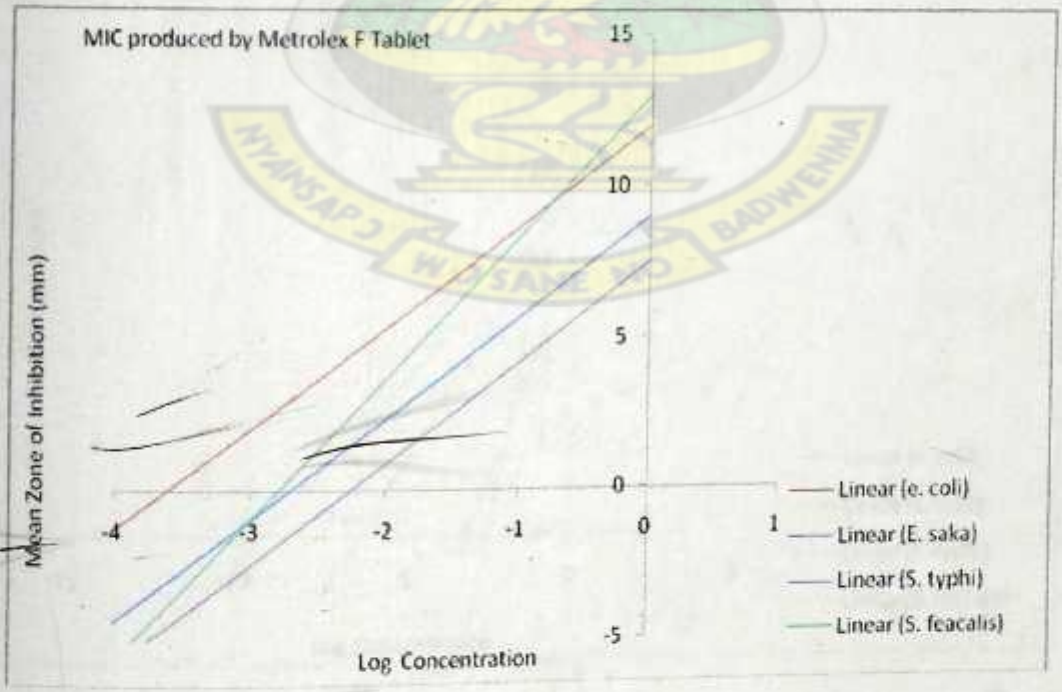


Figure 3.11.3 **Graph of Mean Zone of Inhibition against log Concentration (Co-trimoxazole tablet)**

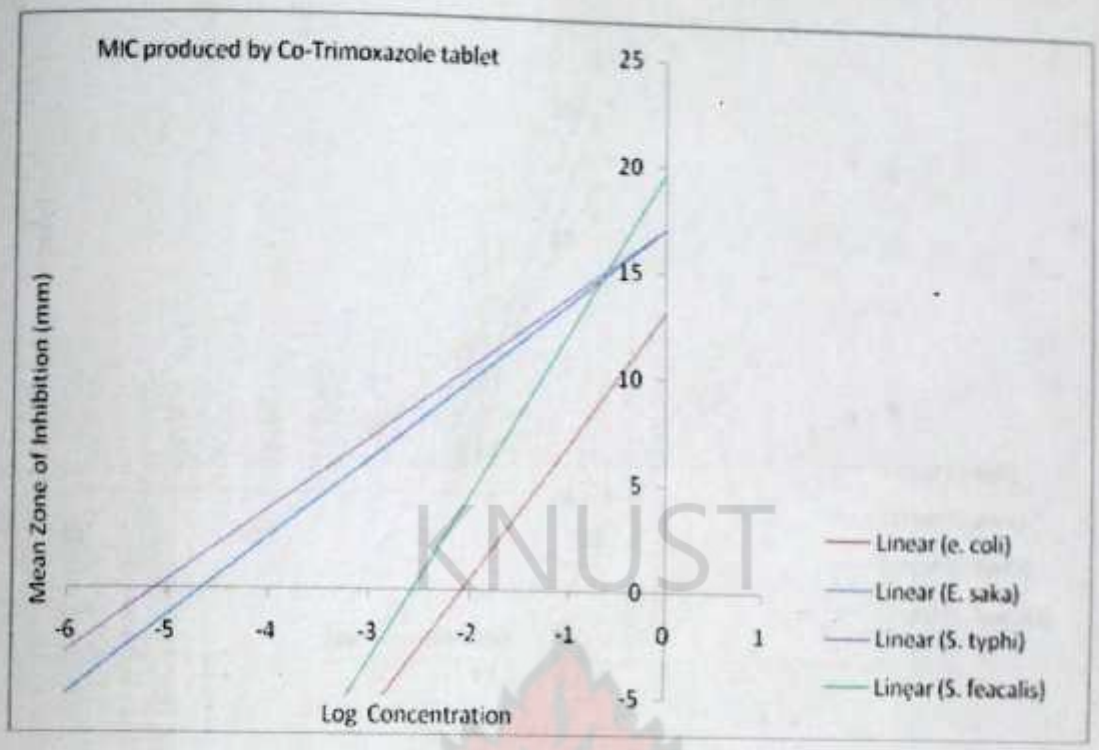


Figure 3.11.4 **Graph of Mean Zone of Inhibition against log Concentration (Ciprofloxacin tablet)**

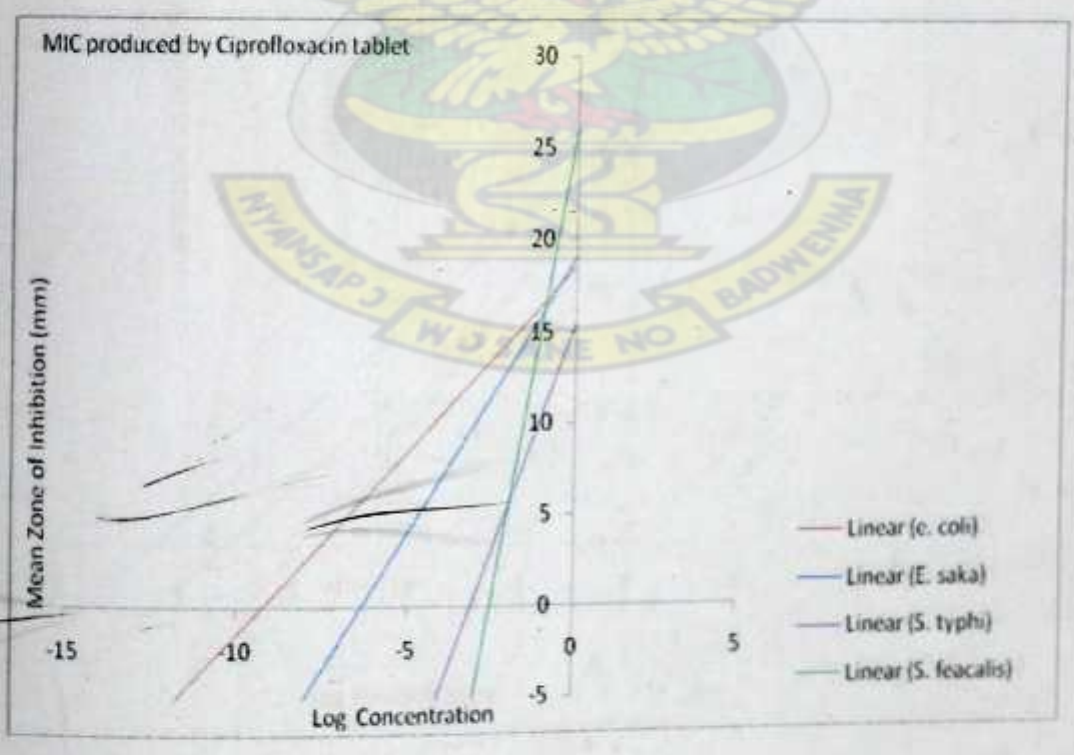


Figure 3.11.5 Graph of Mean Zone of Inhibition against log Concentration (Erythromycin tab)

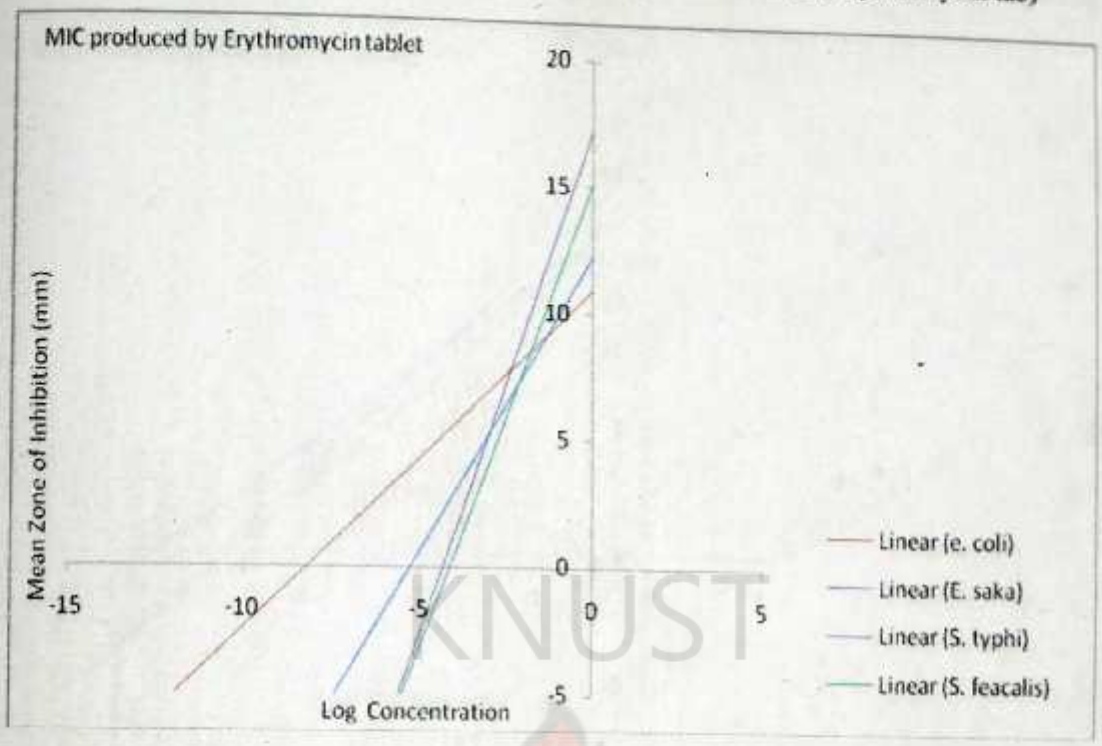


Figure 3.11.6 Graph of Mean Zone of Inhibition against log Concentration (Chloramphenicol capsule)

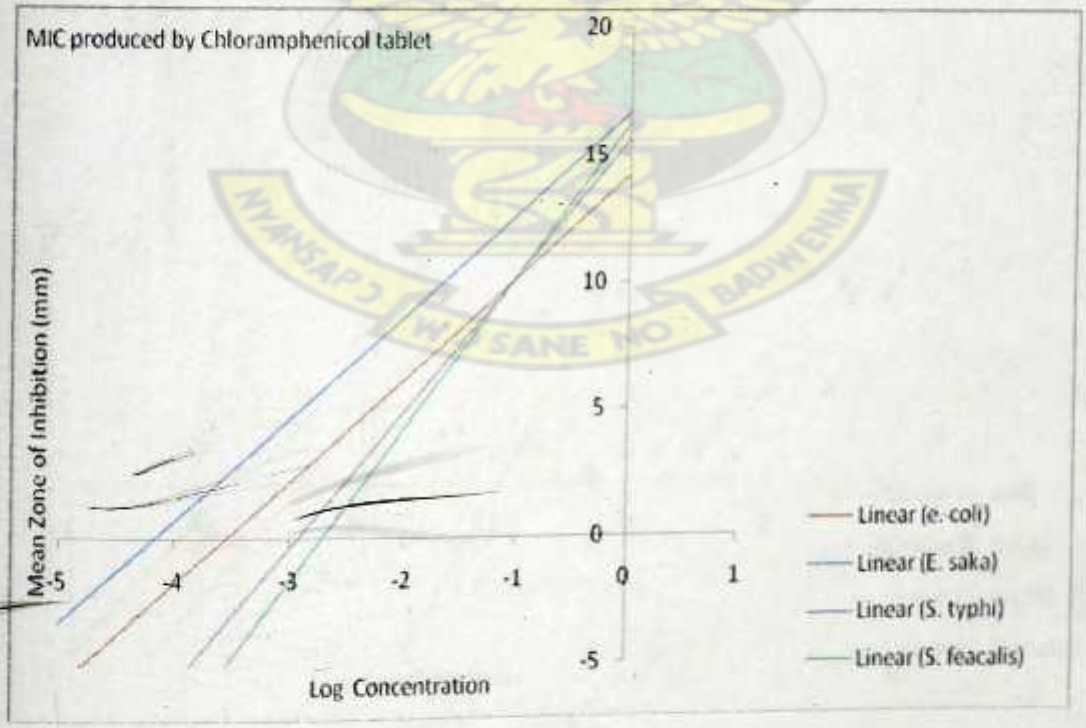


Figure 3.11.7 Graph of Mean Zone of Inhibition against log Concentration(Tetracycline capsule)

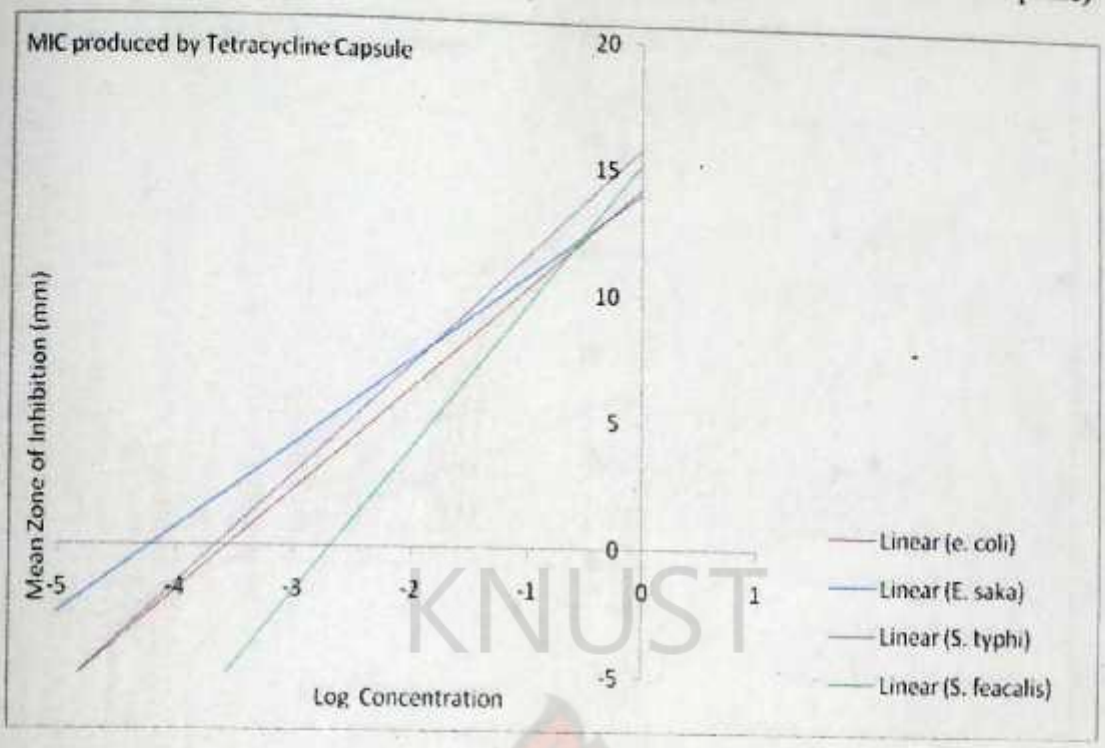


Figure 3.11.8 Graph of Mean Zone of Inhibition against log Concentration (Metronidazole suspension)

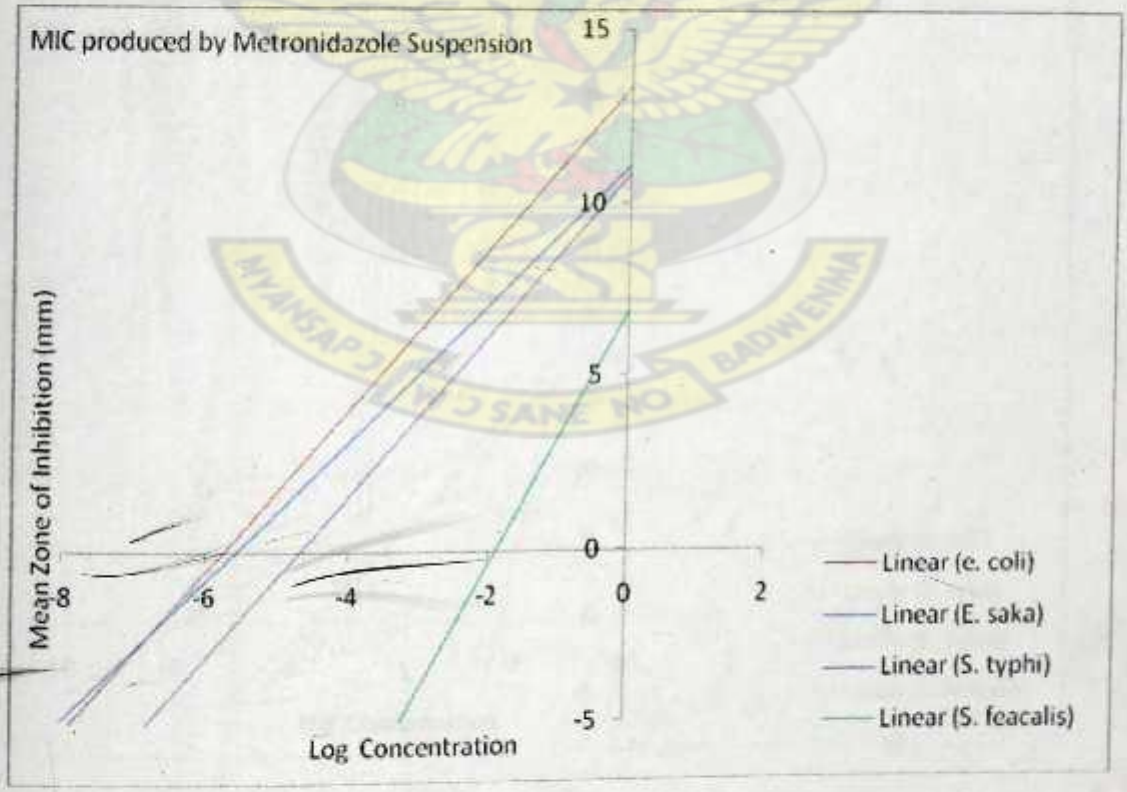


Figure 3.11.9 Graph of Mean Zone of Inhibition against log Concentration (Metrolex F Suspension)

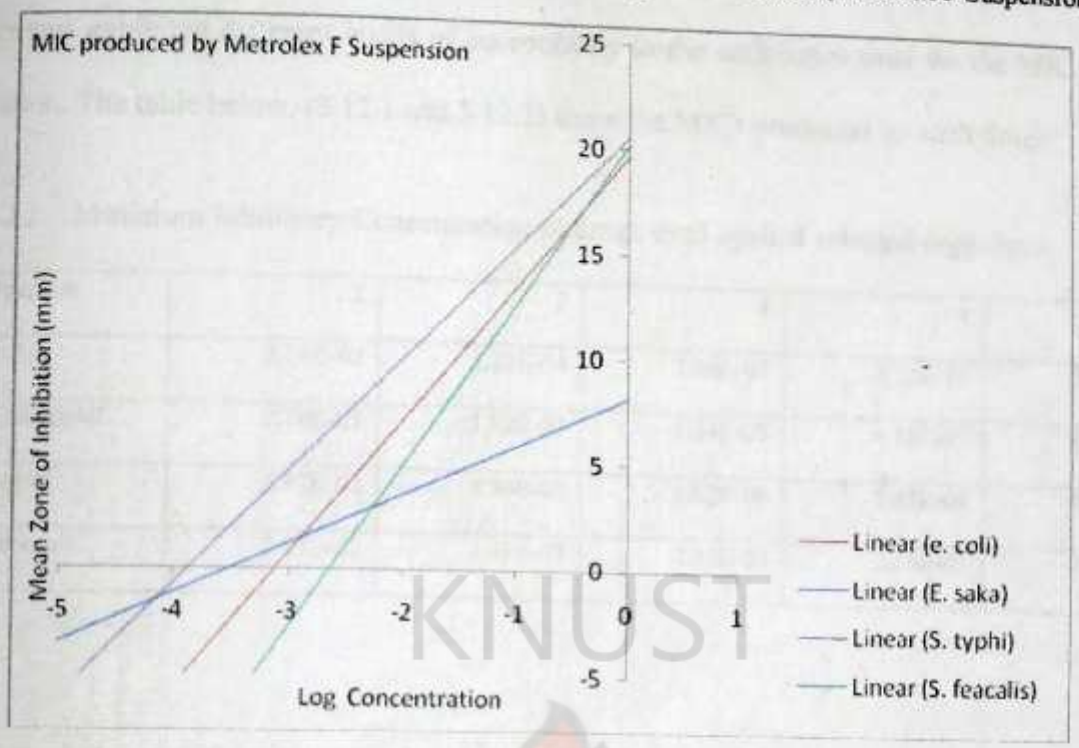
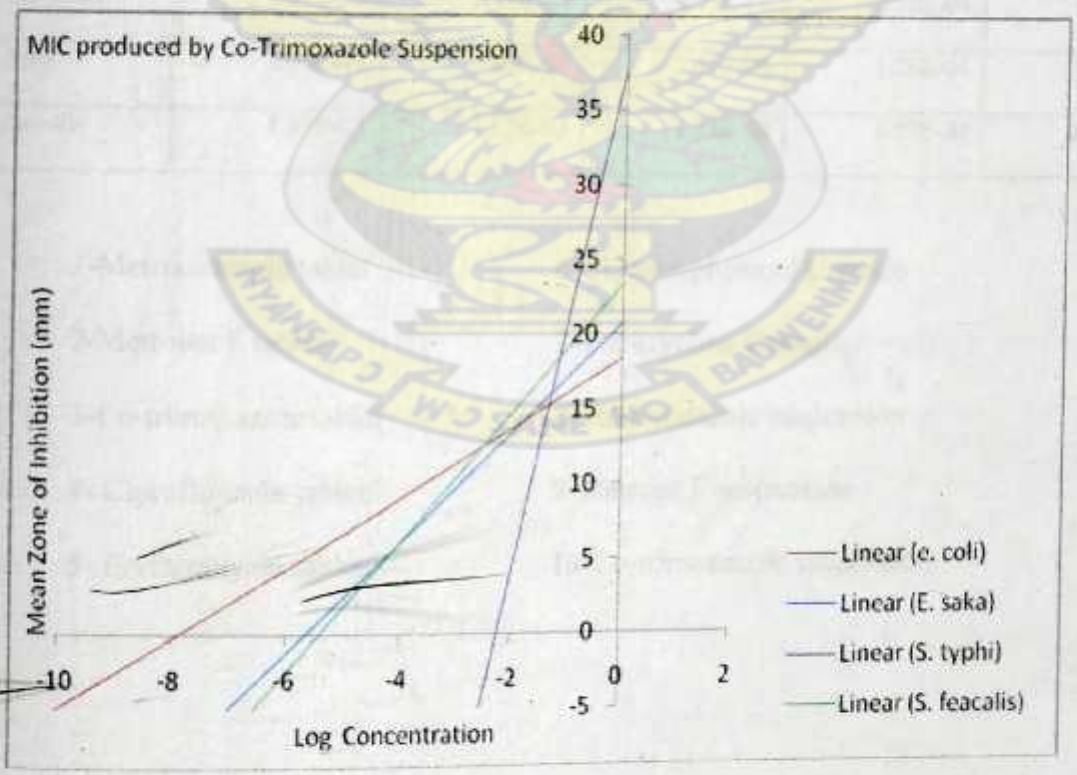


Figure 3.11.10 Graph of Mean Zone of Inhibition against log Concentration (Co-trimoxazole suspension)



3.12 Values of Minimum Inhibitory Concentration

The organisms exhibited different levels of suceptibility to the antibiotics used for the MIC determination. The table below, (3.12.1 and 3.12.2) show the MICs produced by each drug.

Table 3.12.1 Minimum Inhibitory Concentration of drugs used against selected organisms

Organism	1	2	3	4	5
E. coli	3.16E-02	2.23E-04	7.50E-03	7.25E-10	5.30E-09
E. sakazakii	2.10E-03	1.82E-03	1.54E-05	4.18E-07	6.72E-06
S. typhi	2.47E-02	4.96E-03	5.62E-06	7.88E-04	4.89E-05
S. faecalis	2.91E-02	1.49E-03	2.05E-03	2.59E-03	7.28E-05

Table 3.12.2 Minimum Inhibitory Concentration of drugs used against selected organisms

Organism	6	7	8	9	10
E. coli	2.34E-04	2.67E-04	1.93E-06	7.32E-04	1.10E-08
E. sakazakii	4.83E-05	6.16E-05	2.75E-06	2.25E-04	1.37E-06
S. typhi	1.09E-03	2.09E-04	2.47E-05	1.26E-04	2.35E-06
S. faecalis	1.83E-03	1.67E-03	1.23E-02	1.97E-03	5.82E-03

1-Metronidazole tablet

2-Metrolex F tablet

3-Co-trimoxazole tablet

4- Ciprofloxacin tablet

5- Erythromycin tablet

6-Chloramphenicol Capsule

7-Tetracycline Capsule

8-Metronidazole suspension

9-Metroex F suspension

10-Co-trimoxazole suspension

CHAPTER FOUR

DISCUSSION

4.0 DISCUSSION

Water quality has become a big issue today because of rapid urban expansion, development and growth in the country's population (IWMI, 2003). For the millions of peri-urban and rural populations in developing countries, diarrhoeal diseases continue to be one of the major causes of morbidity and mortality and in such population; the lack of safe water perpetuates poverty (Keller, 1998; Luanne, 2000).

Sources of water used by some Ghanaians have been shown to be contaminated not only with microbial indicators of faecal pollution, but they also had varied metal and pH levels and these pose a risk to the health of consumers (Obiri-Danso et al, 2002; Obiri-Danso et al, 2004; Kyei-Bafour et al, 2005; Edoh et al, 2004).

The use of antibiotics for the treatment of infectious diarrhoea is on the increase (Lutterodt et al, 1998) and the extensive misuse of antibiotics has resulted in microbial resistance to drugs and the resulting difficulty in the treatment of infectious diseases (Amoah et al, 2004). Records in some countries in Africa, Asia and South America indicate that the use of trimethoprim, ampicillin and tetracycline for the treatment of diarrhoea is largely ineffective (Adebolu and Oladimeji, 2005; Bergan et al, 1996).

4.1 Physical Analysis

All the samples that were analysed had Total Dissolved Solid (TDS) and Conductivity values that were within the standard values set by both the WHO and the Ghana Standards Board. The least values obtained were 59mg/ml for TDS from AP2RW (second well in Aprade) in October, November and December 2007 and 60.1µS/cm for conductivity from the same source in November 2007. The highest values were 266mg/ml for TDS from DO3RBH (third borehole in Domeabra) sampled in December 2007 and 269µS/cm for conductivity from the same source in the same month.

Over 20% of the samples (11 out of the 54 samples) had turbidity values above the standard. All those samples were sourced directly from wells. The Mesewam well had the highest turbidity values (between 17 and 38.2NTU). With the exception of the February sampling, all the wells had relatively very high turbidity values. This could be attributed to the fact that between January and February 08, there was no rainfall. Since rainfall is known to affect turbidity, it stands to reason that the turbidity values will be lower when there is lesser rainfall (Greenberg et al, 1995).

Studies conducted by Obeng and Tschannerl have shown that wells that have properly designed heads which rises sufficiently above the ground and those with narrow mouths are less contaminated than those with none. The studies also showed that covering the well mouth whenever the well is not in use and discouraging people who fetched from the well from standing on the well and from washing close to the well makes the water less contaminated (Obeng and Tschannerl, 1989). These are a few of the reasons that could be attributed to the samples from AP2RW (second well in Aprade), a well, having turbidity

values within the desired range. The values were 1.22NTU as the least and 3.35NTU as the highest.

The WHO sets 50°H (Hazen Unit) as the maximum permissible limit for colour of water intended for drinking (WHO, 1997), whiles the Ghana Standards Board sets a limit of 15°H (GSB, 1998).

Judging by the WHO standard, all the samples had acceptable values with the exception of the samples picked from the Mesewam well. These samples had values above 100°H throughout the whole period of the study.

If however, the Ghanaian standard is used, then all the well samples had unacceptable values with the lowest being 20°H from AP2RW (second well in Aprade) in the months of October, November and December and the highest value of 150°H from the Mesewam sample in the months of October, November and January. Colour in water is caused by a large range of substances and invariably calls for some basic treatment before use. The water drawn from the wells need to be treated, at least, by filtering, before use.

From the results obtained, it could be seen that the samples taken from bore holes and poly tank sources generally had acceptable values for turbidity and colour. This could be due to the fact that water from these sources are less contaminated than those form open wells (CWSA, 2004). Also in cases where water pumps are installed as in poly tanks, cloudy water will clear after being pumped and allowed to stand. Most pumps also use filters resulting in cleaner water (CWSA, 2004).

All the samples that were collected over the six-month period did not meet the requirement for pH. All the values were below the required range of 6.5-8.5. The values obtained were 4.38 for the least and 5.92 for the highest. Water of such pH is acidic and unfit for human consumption (WHO, 1997; GSB, 1998). Such water when stored in metallic containers will gradually corrode the metals and in the long run, adversely affect the health of the individuals who have been consuming it over the period (USEPA, 2006b).

Water of low pH has to be treated with lime to raise the pH to a level that is acceptable for human consumption (USEPA, 2006a,b).

Generally, the physical properties (TDS, Conductivity, Turbidity and Colour) of the samples collected during the dry season- January, February and March were better than the values obtained when the samples were collected in the wet season- October, November and December. This could be attributed to likely contamination caused by turbulence, springing, runoffs and rising of the water table during the wet season (Badrakh et al, 2007).

4.2 Bacteriological Analysis

The growth of organisms in Lauryl Sulphate broth is used in the detection of coliforms in water. The high nutrient quality and the presence of phosphate buffer ensure rapid growth and increased gas production (Scharlau Manual, 2006). Gas production is the only significant criterion and it indicates the presence of coliforms. The presence of *Escherichia coli* is indicated by fluorescence under a long wavelength ultra violet lamp (Oxoid Manual, 1979). This is confirmed by a positive indole test. The presence of gas but absence of fluorescence indicates the presence coliforms other than *Escherichia coli* (Oxoid Manual, 1979).

From the results, it was noticed that all the tubes that produced gas also had fluorescence at 365nm with the exception of the samples obtained from AP1LBH (borehole from Aprade) and AP1RPT (polytank source from Aprade) in the month of December. The AP1LBH (borehole from Aprade) tube produced gas but did not have fluorescence at 365nm and yet gave a positive indole response. The AP1RPT (polytank source from Aprade) tube produced gas and had fluorescence at 365nm but was negative for indole production. These deviations could not be accounted for satisfactorily.

It could be deduced from the inoculation into Lauryl Sulphate broth that 38 out of the 54 samples, representing 70.4%, contained various organisms that could grow in Lauryl Sulphate broth. 22 (57.9%) of those samples produced gas, 21 (55.3%) produced fluorescence and 19 (86.4%) of the samples that produced gas contained *Escherichia coli*. Only one of the sample representing 4.5%, produced gas but did not contain *Escherichia coli*. 16 (29.2%) of the samples had growth but neither produced gas, fluorescence nor were they indole positive. These samples were most likely contaminated with organisms other than *Escherichia coli* (Oxoid Manual, 1979; Sharlau Manual, 2006).

Growth and gas production was present in all the samples taken directly from wells. The samples from boreholes were the least contaminated. It could again be seen from the results that the samples collected during the rainy season were more contaminated than those sampled during the dry season. The sampling from the poly tank source had almost the same values throughout the six-month period.

4.2.1 Quantitative Assay

For the quantitative assay, the pattern observed on the Lauryl Sulphate agar was similar to the growth pattern observed in the Lauryl Sulphate broth. None of the samples passed the required standard of absence of total or faecal coliforms in any 100ml sample. 60% (15 of the 27) of the samples collected and tested during January, February and March however, had neither total nor faecal coliforms.

Some schools of thought suggest that for ground water used in poorer or developing countries, the WHO should be less strict on the total absence of coliforms and allow a maximum permissible level of 10CFU/100ml of total coliforms, 3CFU/100ml of *E. coli* and 1CFU/100ml of faecal streptococci when determined by the Most Probable Number or Membrane Filtration method (Chaidez et al, 2006; Edoh et al, 2005). If such literature is agreed to, then, 5.6% (3/54) of the samples had acceptable values of total coliforms and 33.3% (18/54) had acceptable values of faecal streptococci. All of these "acceptable" samples if the suggested limit is used are samples sourced during the dry season.

Going strictly by the WHO and the Ghana Standards Board limits, none of the samples had acceptable value of total coliforms with the exception of 55.6% (15 samples) that were sampled during January, February and March from bore hole and poly tank sources.

4.2.2 API Identification

The 20 randomly selected isolates from the agar slants identified using the API 20E test kit consisted of the following bacteria; *Escherichia coli* 30%, *Enterobacter sakazakii* 20%, *Enterobacter cloacae* 15%, *Citrobacter diverus* 15%, *Salmonella spp* 10% and *Serratia marcescens* 10%.

Slanetz and Bartley's agar is used exclusively for the identification of enterococci (faecal streptococci). The presence of Sodium azide inhibits the growth of gram-negative bacteria. When incubated at 44°C, all red colonies are accepted as faecal streptococci (Scharlau Manual, 2006; Oxoid Manual, 1979; Maier and Lightfoot, 1998). The colonies that were observed on the media were thus, recorded as faecal streptococci – *Streptococci faecalis*.

Of the 54 samples collected, 72.2% (39) were contaminated with faecal streptococci. Only 27.8% (15) of the samples were not contaminated by faecal streptococci and these were from the boreholes and poly tank samples collected in January, February and March.

Although the graphs on the diarrhoea cases reported at the various health centres (Figures 3.13.1.1-3.1) show a pattern of an increase in reported cases of diarrhoea during periods when the sampled water were more contaminated and vice versa, the data cannot be used solely to conclude since it was obtained over a period of only one year and some members of the communities under study sought healthcare elsewhere other than in their vicinity. Studies have however shown that an improvement in water quality greatly reduces the occurrence of diarrhoea in a given population (Esrey et al, 1991; WHO, 2002)

4.3 Minimum Inhibitory Concentration Test

The sensitivity of *E. coli* and some other diarrhoea-causing organisms isolated from drinking water to some commonly used antibiotics has been studied in some communities in the Volta region of Ghana (Amoah et al, 2004) and in some other countries (Adebolu and Oladimaji, 2005, Bergan et al, 1996; Chaidez et al, 2006) but not much information is available on samples from Kumasi, Ghana. There are also reports showing various levels of sensitivity of diarrhoea-causing organisms to antibiotics with some level of resistance by *E. coli* and Salmonella species to Ampicillin and Chloramphenicol (Amoah et al, 2003).

From the results obtained from the MIC determination, the organisms showed various degrees of susceptibility to the drugs used. *Escherichia coli* exhibited the highest susceptibility to 50% of the drugs used. This was followed by *Enterobacter sakazakii* with 30% and Salmonella spp with 20%. *Streptococci faecalis* was the one organism that showed the least susceptibility to all the drugs used for the study.

Drugs with different mechanisms of actions are combined for their additive and synergistic effects (Rang et al, 2003) but there was no significant difference between the Minimum Inhibition Concentrations values produced by the combination drugs used (Metroex F and Co-trimoxazole) and the other single-component drugs. Some single-component drugs had higher MIC values than the combination drugs when used against the same organism.

The highest Minimum Inhibitory Concentration was produced by Ciprofloxacin tablet against *Escherichia coli* and the least by Metronidazole tablet against the same organism.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The samples that were analysed were of variable physical and bacteriological safety and quality. All the samples that were analysed had Total Dissolved Solid (TDS) and Conductivity values that were within the standard values set by both the WHO and the Ghana Standards Board. Samples from wells had higher turbidity, colour and coliform values than samples collected from borehole or poly tank sources.

All the samples that were analysed did not meet the WHO or Ghana Standards Board requirement for pH. The pH values were very low.

The Total Dissolved Solid, Conductivity, Turbidity and Colour of the samples collected during the dry season- January, February and March were better than the values obtained when the samples were collected in the wet season- October, November and December.

More than half of the samples (70.4%) were contaminated with coliforms, 35.2% with *Escherichia coli* and 72.2% with *Streptococci faecalis*. Other contaminants of the samples were identified as *Enterobacter sakazakii*, *Enterobacter cloacae*, *Citrobacter diverus*, *Salmonella spp*, *Serratia marcescens*, *Candida albicans*, *Staphylococcus aureus* (pyogens), and Presumptive non pathogenic staphylococci.

The contaminants consisted of both gram-positive and gram-negative bacteria, and fungi. The organisms used for the Minimum Inhibitory Concentration determination showed various degrees of susceptibility to the drugs used.

Escherichia coli exhibited the highest susceptibility followed by *Enterobacter sakazakii* and then Salmonella specie. *Streptococcus faecalis* was the least susceptibile.

There was no significant difference between the MICs produced by combination drugs (Metroex F and Co-trimoxazole) and the MIC produced by single-agent drugs.

5.2 RECOMMENDATIONS

- Water monitoring programmes should be organized regularly for the inspection of sanitary and hygienic aspects of raw water sources, particularly for pathogens.
- There is the need to create awareness on the quality of water used in the communities used for this research and its possible impact on the life of the people in the community through the organisation of Health workshops and talks.
- More research should be carried out to know how best the people of the community can use their water.
- Private water supplies should be tested regularly to detect contamination problems early.
- The household water treatment and safe storage which includes basic treatment techniques like boiling, filtration, solar, ultra violet and chemical disinfection, flocculation and safe storage methods like storing water in narrow-mouthed, screened and covered containers should be encouraged. Keeping water covered in homes for some days before use and using well-washed cups or containers to fetch stored water are also known to be of help in minimising the spread of diarrhoea (Obiri-Danso et al, 2002b). It calls for education and a change in hygienic behaviour.

In as much as the above are highly recommended, it should not be an alternative to the provision of potable water but rather, a complement to infrastructural improvement.

APPENDICES

APPENDIX I - PREPARATION OF CULTURE MEDIA

A liter (1000ml) each of the following media contained the following constituents

Tryptose Lauryl Sulphate broth (02-108)

Tryptose	20.00
Sodium Lauryl Sulphate	0.10
Lactose	5.00
Dipotassium phosphate	2.75
Monopotassium phosphate	2.75
Sodium Chloride	5.0

35.6g was dissolved in 1 litre of distilled water and distributed into test tubes fitted with inverted Durham tubes (for gas) and sterilized at 121 ^{0}C for 15 minutes.

For the preparation of the agar, 1.5 % w/v agar powder was added to the above and brought to boil. It was then sterilized at 121 ^{0}C for 15 minutes.

CDH agar powder (Agar agar)

1.5% was added to the liquid media for gel formation.

Eosin Methylene Blue agar (01-068)

Peptone	10
Lactose	10
Dipotassium hydrogen phosphate	2
Yellowish Eosin	0.40
Methylene Blue	0.065
Agar	15

37.5g was added to distilled water, brought to boil and distributed into test tubes and firmly corked. They were sterilised at 121 ^{0}C for 15 minutes, mixed well and poured into sterile petri dishes.

MacConkey Agar No. 2 (CM 109)

Peptone	20
Lactose	10
Bile salts No. 2	1.5
Sodium Chloride	5
Neutral Red	0.05
Crystal violet	0.001
Agar	15

51.5g was suspended in 1 litre of distilled water and brought to boil, distributed into appropriate containers and sterilized by autoclaving at 121 ^{0}C for 15 minutes.

Mannitol Salt agar (CM 0085)

Lab-lemco Powder	1.0
Peptone	10
Mannitol	10
Sodium Chloride	75
Phenol red	0.025
Agar	15

111g was suspended in 1 litre of distilled water and brought to boil to dissolve completely. It was distributed into test tubes, corked firmly and sterilized by autoclaving at 121 ^{0}C for 15 minutes.

Cetrimide agar (USP, EP – CM 0579)

Gelatin peptone	20
Magnesium Chloride	1.4
Potassium sulphate	10
Cetrimide	0.3
Agar	13.6

45.3 g was suspended in 1 litre of distilled water. 10 ml of glycerol was added and brought to boil. It was distributed into test tubes, corked firmly and sterilized by autoclaving at 121 ^{0}C for 15 minutes.

Slanetz Bartley Agar (01-178)

Tryptose	20
Yeast extract	5
Dextrose	2
Potassium phosphate	4
Sodium azide	0.4
TTC	0.1
Agar	12

43.5 g was suspended in distilled water and brought to boil. It was cooled to 50 C and distributed into sterile petri dishes immediately.

Bismuth Sulphite agar (CM 0201)

Peptone	5.0
Lab-Lemco Powder	5.0
Glucose	5.0
Di-sodium phosphate	4.0
Ferrous sulphate	0.3
Bismuth Sulphite indicator	0.8
Brilliant green	0.016

40g was suspended in 1 litre distilled water and heated till the agar dissolved. It was cooled to 50 ^{0}C and poured thickly into sterile petri dishes and allowed to solidify.

Nutrient agar (CM 003)

Meat extract	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
Agar	15.0

28 g of powder was suspended in 1 litre of distilled water and boil. It was distributed into test tubes, corked firmly and sterilized by autoclaving at 121 ^{0}C for 15 minutes.

Nutrient broth (CM 001)

Meat extract	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0

13 g of powder was dissolved in 1 litre of distilled water. It was distributed into test tubes, corked firmly and sterilized by autoclaving at 121 ^{0}C for 15 minute.

APPENDIX II

BATCH NUMBERS AND CODES OF DRUGS USED FOR DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

Name	Dosage Form	Expiry Date	Manufacturing Company	Batch/Lot Number	Code Number
Metronidazole	Tablet	07/10	Phyto-Riker	L08584	-
Metrolex F	Tablet	06/11	LUE Limited	ES75	MH/DRUGS/KD-19
Co-trimoxazole	Tablet	08/10	Phyto-Riker	L10654	-
Ciprofloxacin	Tablet	08/10	Phyto-Riker	K09157	-
Erythromycin	Tablet	04/11	Phyto-Riker	L05454	-
Chloramphenicol	Capsule	06/10	Ernest Chemist	0106G	-
Tetracycline	Capsule	02/11	Ernest Chemist	0105H	-
Metronidazole	Suspension	08/10	Phyto-Riker	09E413	074
Metrolex F	Suspension	12/10	LUE Limited	179707	MH/DRUGS/767
Co-trimoxazole	Suspension	12/10	Phyto-Riker	01F451	075

APPENDIX III

GUIDELINES FOR OBSERVATION OF COMMUNITIES

What time do they come to fetch water?

Morning

Afternoon

Evening

Who usually comes to fetch the water?

How is the water fetched - With own container or with a general container

Who draws or pumps the water?

How long does it take to fill a normal 35 litres bucket?

Does the water change in turbidity after drawing or pumping?

What are the possible sources of contamination?

Water pouring back into well

Stagnant water around well

Cracks around well

Dipping of hand into water after fetching

Refuse dumps nearby

KVIP, Pit latrines or Septic tanks nearby

How active is the community on weekends?

Is there washing around the water source?

APPENDIX IV

QUESTIONNAIRE FOR OPINION LEADERS

1. Name of Community: .....
2. District: .....
3. Population: .....
4. Major Occupation: .....
5. Major Religion: .....
6. Tourist attractions available in the community: .....

   .....
7. Type of Health Care institution(s) available:

Type of institution	Number	Length of existence in the community
(1)Hospital		
(2) Clinic		
(3) Pharmacy		
(4) Lin. Chemical Shop		
(5) Traditional Medicine Attendants		
8. What are the most common diseases in your community and why?

   .....

   .....

   .....
9. What are the usual ways of getting information in this community?

   Rumours [ ] Friends [ ] Gong-gong beater [ ]
10. How often do health educational programs take place?

    Monthly [ ] Quarterly [ ] Semi-annually [ ] Annually [ ]
11. State the organization that undertakes such educational programs
12. Who normally gives talk in this community oh health issues?

    Health Workers [ ] Teachers [ ] Preachers [ ] Information Services [ ] Town Council [ ] Drug peddlers [ ] Chemical sellers [ ]
13. What issues are normally discussed?

    Malaria [ ] Typhoid [ ] Cholera [ ] Diarrhoea [ ] Others
14. How often do you receive this information?

    Weekly [ ] Monthly [ ] Quarterly [ ] Semi-annually [ ] Annually [ ]

15. Where do you normally receive the information? .....

16. How have such educational programmes been of help to you and your family?.....

.....

17. If we want to educate you on diarrhoea, what information would you like to know?

.....

.....

18. Who are those who need information on diarrhoea and why?

.....

.....

.....

APPENDIX V

QUESTIONNAIRE FOR HEALTH CARE PROVIDERS

1. Name of Community.....
2. District: .....
3. Name of institution: .....
4. Type of institution: (1)Hospital [ ] (2) Clinic [ ]
   (3) Pharmacy [ ] (4) Lin. Chemical Shop [ ]
   (5) Traditional Medicine Attendants [ ]
5. For how long has the institution been in existence?
6. In-charge:

Specialist	[ ]	Pharmacist	[ ]	Medical Doctor	[ ]
Disp. Tech.	[ ]	Medical Assistant	[ ]	Counter Asst.	[ ]
Nurse	[ ]	Herbalist	[ ]	Midwife	[ ]

7. Average number of patients who visit the facility daily

   In the dry season

   In the wet season

7 Common ailments

	Very often	quite often	rarely	never
Diarrhoea	[ ]	[ ]	[ ]	[ ]
Cholera	[ ]	[ ]	[ ]	[ ]
Dysentery	[ ]	[ ]	[ ]	[ ]
Typhoid fever	[ ]	[ ]	[ ]	[ ]

Others (Please specify) .....

.....

8 What are the most common diseases in the community and why?

.....

.....

.....

9 What are the usual ways of getting information to people of the community?

Rumours [ ] Friends [ ] Gong-gong beater [ ]

10 How often do health educational programs take place?

Monthly [ ] Quarterly [ ] Semi-annually [ ] Annually [ ]

11 Which organization organises such educational programs

12 Who normally gives talk in this community on health issues?

Health Workers [ ] Teachers [ ] Preachers [ ] Information Services [ ] Town Council [ ] Drug peddlers [ ] Chemical sellers [ ]

13 What issues are normally discussed?

Malaria [ ] Typhoid [ ] Cholera [ ] Diarrhoea [ ] Others

14 Where does the community receive the information? .....

15 If a group wants to educate the community on diarrhoea, what information would you like them to know? .....

.....

16 Who are those who need information on diarrhoea and why?

.....

.....

APPENDIX VI

QUESTIONNAIRE FOR SOME SELECTED HOMES AND WELL OWNERS

1. Community: .....
2. Occupation: .....
3. Sex: Male [ ] Female [ ]
4. Marital statue: Married [ ] Single [ ] Divorced [ ]
5. Source of water supply

   Well [ ] Bore hole [ ] Pipe borne water [ ] Rain water [ ]

   Stream [ ] Sachet water [ ] Other (Please specify)
6. How much does it cost? .....
7. Do you sometimes use well water? Yes [ ] No [ ]
8. If yes, how often?

   Everyday [ ] Very often [ ] Quite often [ ] Rarely [ ] Never [ ]
9. What do you use the well water for?

   Drinking [ ] Cooking [ ] Washing [ ] Bathing [ ] Other (Please specify)
10. Do you treat the well water before

	Yes	No
Drinking	[ ]	[ ]
Cooking	[ ]	[ ]
Washing	[ ]	[ ]
Bathing	[ ]	[ ]

11. What treatment method do you use? (Please tick)

    Set aside for sometime and allow to set [ ] Boiling [ ] Filtering [ ]

    Addition of chemicals [ ]
12. How do you store your water?

    In a cooler [ ] In a metal container [ ]

    In a plastic container [ ] Other (Please specify)
13. Does your water change in colour after storage? Yes [ ] No [ ]
14. If yes, state .....
15. Does your water change in taste after storage? Yes [ ] No [ ]
16. If yes, state.....
17. Does your water change in odour after storage? Yes [ ] No [ ]
18. If yes, state .....

19. Do you access any health facility in your community? Yes [ ] No [ ]

20. If NO, state .....

21. If YES, what health facility do you have access to? .....

22. Who normally gives talk in this community oh health issues?

Health Workers [ ] Teachers [ ] Preachers [ ] Information Services [ ] Town Council [ ] Drug peddlers [ ] Chemical sellers [ ]

23. What issues are normally discussed?

Malaria [ ] Typhoid [ ] Cholera [ ] Diarrhoea [ ] Others

24. How often do you receive this information?

Weekly [ ] Monthly [ ] Quarterly [ ] Semi-annually [ ] Annually [ ]

25. Where do you normally receive the information? .....

26. How have such educational programmes been of help to you and your family?.....

.....

27. Supposing we want to educate you on diarrhoea, what information would you like to know? .....

.....

28. Who are those who need information on diarrhoea and why?

.....

.....

.....

APPENDIX VII

FORMS GIVEN TO LICENSED CHEMICAL SHOPS TO FILL ON PATIENTS WITH COMPLAINTS OF DIARRHOEA

1. Name of Community: ..... Date: .....
2. Type of Health Facility: .....
3. Age of Patient: .....
4. Sex of Patient: .....
5. Complaint:

   .....

   .....
6. History:

   .....

   .....
7. Drugs given: (1)

   (2)

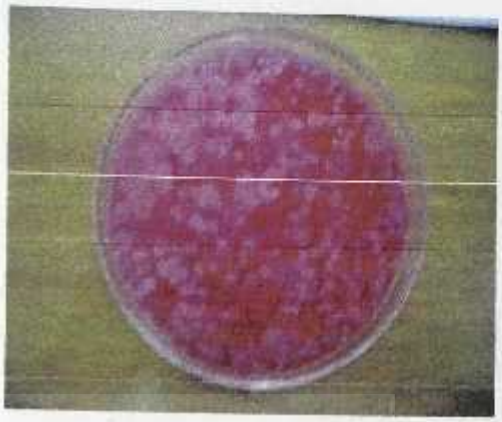
   (3)

   (4)

APPENDIX VIII

SOME RESULTS OF THE POUR PLATE METHOD

Candida albicans growth on EMB agar



Presumptive non-pathogenic staphylococci (pink colonies) on Mannitol Salt agar

Candida albicans growth on EMB agar

Staphylococcus aureus growing on mannitol Salt agar (Media turned yellow)

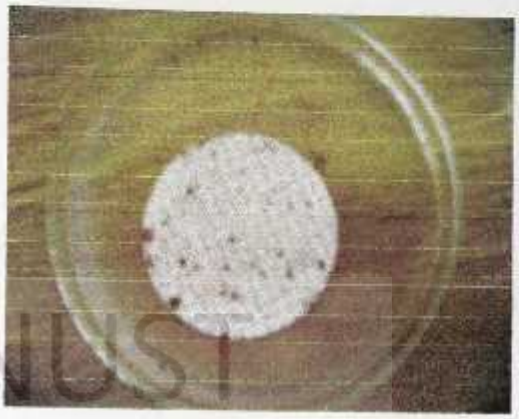
Citrobacter diverus colonies (bright green) on EMB agar

Salmonella species growing on Bismuth Sulphite agar.

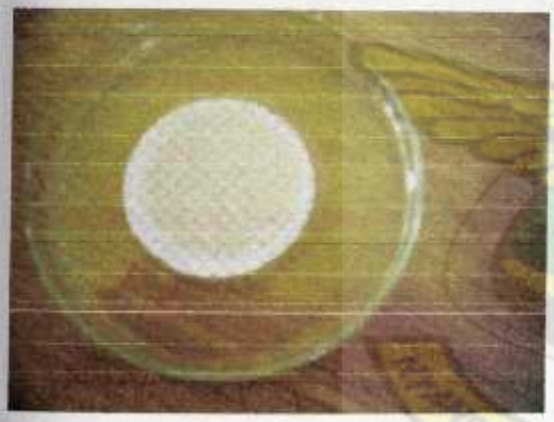
APPENDIX IX

SOME RESULTS OF THE MEMBRANE FILTRATION METHOD

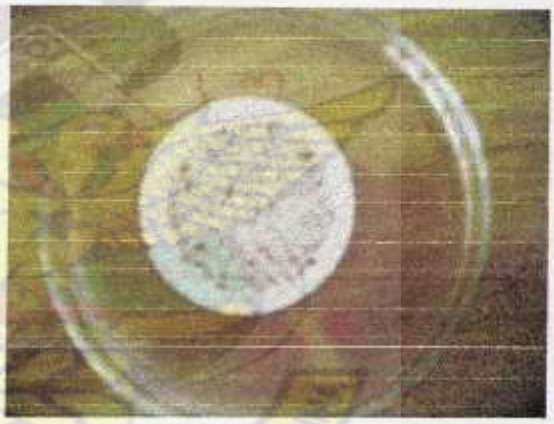
Total coliform colonies growing on Lauryl Sulphate agar.



Faecall coliform colonies growing on Slanetz and Bartley's agar.



Total coliform colonies growing on Lauryl Sulphate agar.



Faecall coliform colonies growing on Slanetz and Bartley's agar

APPENDIX X

SOME RESULTS OF THE API 20E IDENTIFICATION ON THE TEST KIT

6 Salf. Black

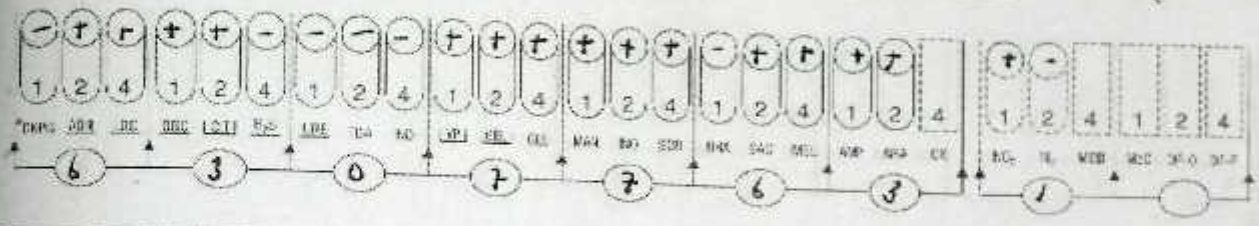
api® 20 E

CE

REF :

Origine / Source / Herkunft /
Origen / Origem / Προέλευση /
Ursprung / Oprindelse / Pochodzenie :

BIOMÉRIEUX



Autres tests / Other tests / Andere Tests /
Otras pruebas / Altri test / Outros testes /
Άλλες εξετάσεις / Andra tester /
Andre tests / Inne testy :

Ident. / Ταυτοποίηση :
Salmonella spp

Lac Salf. Yellow

api® 20 E

CE

REF :

Origine / Source / Herkunft /
Origen / Origem / Προέλευση /
Ursprung / Oprindelse / Pochodzenie :

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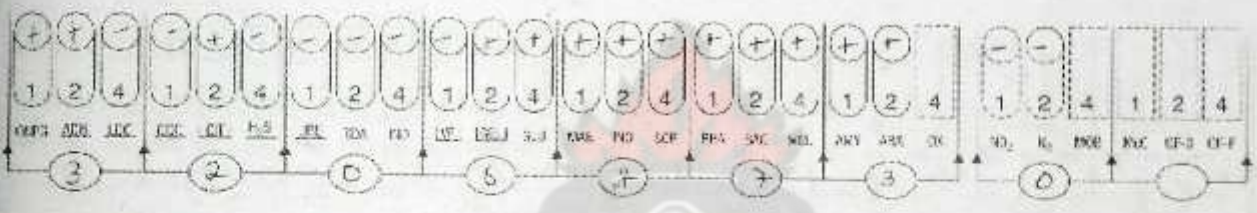

Autres tests / Other tests / Andere Tests /
Otras pruebas / Altri test / Outros testes /
Άλλες εξετάσεις / Andra tester /
Andre tests / Inne testy :

Ident. / Ταυτοποίηση :
Enterobacteriaceae

Lac Salf. Green

api® 20 E

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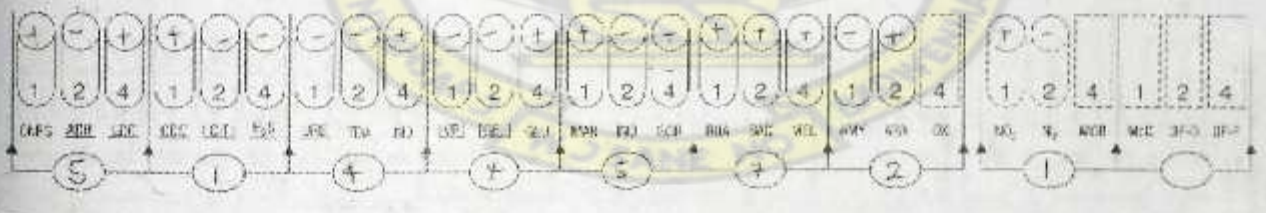
REF :

Origine / Source / Herkunft /
Origen / Origem / Προέλευση /
Ursprung / Oprindelse / Pochodzenie :

BIOMÉRIEUX



Autres tests / Other tests / Andere Tests /
Otras pruebas / Altri test / Outros testes /
Άλλες εξετάσεις / Andra tester /
Andre tests / Inne testy :

Ident. / Ταυτοποίηση :
E. coli

Ma 2

api® 20 E — CE — BIOMÉRIEUX

REF :

Origine / Source / Herkunft / Origen / Origem / Προέλευση / Ursprung / Oprindelse / Pochodzenie :

ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX	NO2	N2	MOB	McC	OF-O	OF-F
+	+	-	+	+	-	-	-	-	+	-	+	+	+	+	-	+	+	+	+		+					
1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4

Code: 3 3 0 5 7 6 3 1

Autres tests / Other tests / Andere Tests / Otras pruebas / Altri test / Outros testes / Άλλες εξετάσεις / Andra tester / Andre tests / Inne testy :

Ident. / Ταυτοποίηση : Entrobacter cloacae

Emb - agar

api® 20 E — CE — BIOMÉRIEUX

REF :

Origine / Source / Herkunft / Origen / Origem / Προέλευση / Ursprung / Oprindelse / Pochodzenie :

ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX	NO2	N2	MOB	McC	OF-O	OF-F
+	+	-	+	+	-	-	-	+	-	-	+	+	-	+	-	+	+	+	+		+					
1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4

Code: 3 3 + 4 5 6 3 1

Autres tests / Other tests / Andere Tests / Otras pruebas / Altri test / Outros testes / Άλλες εξετάσεις / Andra tester / Andre tests / Inne testy : Catalase

Ident. / Ταυτοποίηση : Citrobacter diversus

Cet Red

api® 20 E — CE — BIOMÉRIEUX

REF :

Origine / Source / Herkunft / Origen / Origem / Προέλευση / Ursprung / Oprindelse / Pochodzenie :

ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX	NO2	N2	MOB	McC	OF-O	OF-F
+	-	+	+	+	-	-	-	-	+	-	+	+	+	+	-	+	-	+	-		+	-				
1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4

Code: 5 3 0 7 7 2 1 1

Autres tests / Other tests / Andere Tests / Otras pruebas / Altri test / Outros testes / Άλλες εξετάσεις / Andra tester / Andre tests / Inne testy :

Ident. / Ταυτοποίηση : Serratia Marcescens

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