PREVALENCE OF PATHOGENIC ORGANISMS AND HYGIENIC PRACTICES AT PUBLIC TOILETS IN SELECTED LOW-INCOME AREAS IN KUMASI

By:

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DECLARATION

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

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DEDICATION

This work is dedicated to my family;

Mr Kwabena Owusu Dwomo

Madam Abena Pokuah Halimah

Rev. Kwame Owusu-Ansah

Kwabena Owusu Dwomo (Jnr)

Osei Tutu Kwaku

Yaa Ansah Takyiwaa

You form a great and united team of which I am proud to be part.

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ABSTRACT

Public toilets in three low income communities within the Kumasi metropolis were sampled to determine the prevalence of pathogenic organisms and the varying hygienic practices by users and caretakers of the facilities. A total of 288 human excreta samples and 72 toilet cubicle wall swab samples were collected and analyzed for *Salmonella, E. coli and Enterococci* using standard methods. Observational studies and key informant interviews were also carried out. Mean *Enterococci* numbers (log_{10} per 100 ml) in human excreta samples were 4.15, 4.18 and 4.14 in Manhyia, Aboabo and Ayigya, respectively. Relative frequency of occurrence of pathogenic microorganisms in the excreta samples were 25, 29 and 22 for *E. coli* and 6.3, 10.4 and 9.4 for *Salmonella* at Manhyia, Aboabo and Ayigya, respectively. Microbial numbers (log_{10} per 100 ml) in the toilet cubicle swab samples were 3.19, 3.29 and 3.24 for *Enterococci.* Swab samples also 16.7%, 25% and 25% for *E. coli* at Manhyia, Aboabo and Ayigya. *Salmonella* isolates showed high resistance to tetracycline (68%) and ampicillin (64%). Standard of hygiene at all public toilets were very low with faecal matter and waste papers on toilet floors. Waste baskets had no cover and were hardly emptied causing overflow of waste papers. Resulting from poor management, most of the toilets had an obnoxious smell causing users to remove their clothes before accessing them. Hand washing materials were not available in Aboabo and Ayigya. KMA must implement proper planning and effective management of public toilets in order to protect public health in Kumasi.

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CHAPTER ONE

1.0 INTRODUCTION

The Millennium Development Goal (MDG) 7 calls on countries to "Halve, by 2015, the proportion of people without sustainable access to safe drinking water and basic sanitation". It is estimated that 2.6 billion of the world's population lack access to improved sanitation and over one billion to clean water (WHO, 2008). Although the majority of these people live in rural areas in developing countries, the problem is also surfacing within the urban areas (Dahlman, 2009) due to urbanization. The regions presenting the lowest coverage of improved sanitation are in sub-Saharan Africa (31%), Southern Asia (33%) and Eastern Asia (65%) (WHO and UNICEF, 2006). If the MDG sanitation target is to be achieved, innovative approaches need to be developed to reduce the time span from policymaking to services delivery.

The provision of improved sanitation in developing countries is an important social process with implications for public health, sanitation policy and planning, and sanitation design and technology development (Jenkins and Scott, 2006). Good sanitation is a foundation for health that affords protection from a wide range of infections including diarrhea, a leading cause of child deaths (WHO, 2004). This can be achieved by the isolation of the user from their own excreta and prevention of nuisance organisms (e.g. flies) from contacting the excreta and subsequently transmitting disease to humans. Most urban poor households in low and mid-income countries depend on public toilets and latrines (Adubofour, 2010). Urbanization has lead to increased pressure on public sanitation facilities leading to further deterioration of the already bad condition, putting users and residents at risk of various diseases.

Poor sanitation gives many infectious diseases the ideal opportunity to spread. Human excreta have been implicated in the transmission of many infectious diseases including cholera, typhoid, infectious hepatitis, polio, cryptosporidiosis, and ascariasis (Huuhtanen and Laukkanen, 2006). WHO (2004) estimates that about 1.8 million people die annually from diarrheal diseases with 90% being children under five, mostly in developing countries. *Salmonella, Cryptosporidium, Vibrio cholerae* and *E. coli* are some of the causative organisms of diarrhea related diseases in humans. Users of sanitary facilities are likely to contact these organisms due to unhygienic practices. Sanitation and hygiene are critical to health, survival and development.

Hygiene refers to conditions and practices that help to maintain health and prevent the spread of diseases. Preventing the spread of diseases means breaking the chain of infection transmission and the simple principle is, if the chain of infection is broken, infection cannot spread (Netherland Water Partnership, 2010). Common hygiene practices which can break infection transmission includes: cooking foods for the appropriate length of time and at the appropriate temperature to kill pathogens, storing food at proper temperatures, the use and maintenance of latrines, hand washing after defecation and keeping drinking water free from fecal contamination (WaterAid, 2009).

In recent times, several reports and studies have emphasized the transmission of diseases by pathogenic organisms due to poor sanitation and hygiene. In the UK, Greed (2006) reported that the lack of maintenance and cleaning of public toilets resulted in proliferation of toilet-related diseases and medical conditions: a major issue underestimated by the media and government. Methicillin-Resistant *Staphylococcus aureus* (MRSA) in hospital toilets had grabbed the headlines as it contributed to the

death of 5,000 patients each year. But, there is a wide range of other bacteria and viruses that are associated with dirty toilets wherever they are located including public toilets.

In Ghana, studies have shown that 44.6% of poor households use public toilets (Boadi, 2004). The high sharing of sanitation facilities among the poor creates unsanitary conditions at facilities with the breeding of pests and disease vectors, thereby exposing the poor to infectious diseases especially among children. Nketia *et al.* (2007) also reported that even though inhabitants were aware of sanitation related diseases, there were no strong primary barriers to prevention of pathogens or bacteria transmission from public toilet facilities to new hosts. There are no hand washing facilities and the immediate surroundings of the facilities are polluted with both faecal and solid waste. Poor maintenance of the facilities and wrong usage of the public sanitation facilities create additional disease transmission routes.

The emergence of resistance to antimicrobial agents in bacterial pathogens is a global public health problem. Antimicrobial resistance results in increased illness, deaths, and healthcare costs (Archibald *et al.*, 1997). The increasing number of immune compromised patients and increased use of indwelling devices, as well as widespread use of antimicrobial agents in both hospital and community settings contributes to resistance among bacterial pathogens causing infections (Chen *et al.*, 2003). Resistance can result from modification of an antibacterial target or from functional bypassing of that target, or it can be contingent on impermeability, efflux, or enzymatic inactivation (Livermore, 2003).

Ajibade *et al.* (2010), in Nigeria reported on the survival and antimicrobial resistant *Salmonella enterica* isolated from toilets and bathrooms following an outbreak of salmonellosis in some homes in Ado-Ekiti. The total percentage isolate from toilets was 44%; the highest being from the bowl water (70%) and 26% from the bathrooms with the highest being from sinks (44%). The antimicrobial drug susceptibility of forty-four (44) of the isolates against ten different antibiotics was tested. The highest resistance was observed in Nalidixic acid (82%) and Ciprofloxacin (70%). Eight isolates from the bathrooms were resistant to Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, Nalidixic acid, Trimethoprin, sulfamethoxazole and Tetracycline. All isolates were susceptible to Cefotaxime and Cefepime; five (5) isolates from the toilets were intermediately susceptible to Cefepime (MIC 16µg/mL).

1.1 PROBLEM STATEMENT

The provision of water and sanitation services in poor urban areas remains a critical challenge for the realization of the Millennium Development Goals but more importantly for poverty reduction (Osumanu *et al*., 2010). In Ghana, 49% of the population resides in urban areas, of which only 17.8% have access to improved sanitation and 90% to improved drinking water sources (WSMP, 2009). This underscores the problem of sanitation in the urban cities especially in low-income areas like the slums characterized by unplanned settlements. There is growing concern about environmental degradation in Kumasi due to poor sanitation and pollution of waterways. It is estimated that more than 900,000 people lack access to safe drinking water and nearly one million people lack access to improved sanitation facilities (WSUP, 2010). Kumasi, with a population of approximately 1.7 million has only 414 public toilets across the city which is used by about 40% of the populace (MCI, 2010; KMA, 2006). This implies that, an average of

1,500 persons make use of the facility per day for defecation, urination and menstrual hygiene purposes. Toilet facilities were invented to deal safely with human waste, but still have risks associated with them, which may become critical at certain times, especially during episodes of diarrhea. A stakeholder analysis of public toilets in Kumasi conducted in June, 2010 concluded that, majority of them represented a clear public health risk (Caplan, 2010**)**.

Poor sanitation and unhygienic practices give many infections the ideal opportunity to spread, plenty of waste and excreta for the flies to breed on, and unsafe water to drink and wash with. Statistics by the Ghana Health Service indicates that about 80% of all OPD cases are sanitation and water related (WSMP, 2008). Human faeces are the primary source of diarrheal pathogens. There are approximately four billion diarrheal cases per year worldwide (WHO, 2004). Access to improved water and sanitation facilities does not, on its own, necessarily lead to improved health. There is a direct link between diseases and unsafe hygiene practices. There is now very clear evidence showing the importance of hygienic behavior, in particular hand washing with soap at critical times: after defecating and before eating or preparing food can significantly reduce the incidence of diarrhea (UNICEF, 2009).

Bacterial infections constitute an important cause of morbidity and mortality among human beings all over the world and for decades, antimicrobial drugs have proven useful for treatment of bacterial infections (Newman *et al*, 2006). Inappropriate antibacterial treatment and overuse of antibiotics have contributed to the emergence of antibacterial-resistant bacteria. In Ghana, antibiotics sold over the counter without prescription has lead to the creation of resistant strains. Widespread usage of

antibacterial drugs in hospitals has also been associated with increases in bacterial strains and species that no longer respond to treatment with the most common antibacterial (Hawkey and Jones, 2009).

Infections due to *Salmonella* species and bacterial indicators remain an important public health problem in many tropical and sub-tropical countries where clean water supply and sanitation are poor. Data on the prevalence of pathogenic organisms and hygiene practices at public toilets have not been well reported. This work is therefore aimed at providing valuable data on the prevalence of pathogenic organisms at public toilets in selected low-income communities (Manhyia, Aboabo and Ayigya) in Kumasi and unhygienic practices of users that are likely to lead to the spread of these bacteria. This will help evaluate the effect of unhygienic sanitation on public health and ensure basic hygiene practices at public toilets.

1.2 OBJECTIVES

1.2.1 General

This study aims at providing data on the prevalence of pathogenic organisms (*Salmonella*, *Enterococci* and *E.coli*) and assessment of hygienic practices at public toilets in selected low-income areas (Manhyia, Aboabo and Ayigya) in the Kumasi metropolis.

1.2.2 Specific

1. Isolate *Salmonella* and determine their sensitivities to commonly used antibiotics.
2. Isolate strains of *Escherichia coli and Enterococci.*
3. Assess hygienic practices at public toilets.

CHAPTER TWO

LITERATURE REVIEW

2.1 Pathogenic Organisms

A pathogen or pathogenic microorganism is usually defined as a biological agent that can cause damage to its host. Damage may be inflicted directly by the microorganism or indirectly through the activity of the host immune responses (Casadevall and Pirofski, 1999). The ability of the pathogen to infect is called its pathogenicity and this is expressed by means of their virulence, a term that refers to the relative capacity of a microbe to cause damage in a host (Casadevall and Pirofski, 1999). Pathogens are differentiated by their virulence from non pathogens, which are considered to be avirulent. Examples of pathogenic organisms include specific strains of bacteria like *Salmonella, E. coli, Clostridium, Campylobacter, Shigella* and *Cryptosporidium.*

Pathogenic microorganisms causing disease have been divided into obligate, facultative and opportunistic pathogens. Obligate pathogens are capable of infecting only within a narrow host range, but can infect healthy, immune competent individuals of susceptible host species. Examples include *Mycobacterium tuberculosis* (tuberculosis) and *Treponema pallidum* (syphilis) (Van Baarlen *et al*., 2007). Facultative pathogens similarly infect within a narrow host range, but are also capable of surviving outside the host in the environment. Examples include *Neisseria meningitides* (bacterial meningitis) and *Bacteroides fragilis* (normal intestinal flora that can cause serious infection if it gets into the bloodstream, usually through intestinal ulceration or trauma) (Van Baarlen *et al*., 2007). Opportunistic pathogens thrive on a wide range of organic substrates, and generally exhibit low virulence towards a broad array of living hosts. However, if

potential host species become injured or become compromised in their immune responses, opportunistic pathogens may be able to attack aggressively, or in a manner that is indolent but progressive. Examples include *Vibrio cholerae* (cholera) and *Pseudomonas aeruginosa* (bacterial pneumonia) (Van Baarlen *et al*., 2007).

There are several substrates including routes where pathogens can invade a host; water, soil, waste or faecal matter. The pathways have different sporadic time frames, but soil contamination has the longest or most persistent potential for harboring a pathogen Pathogens can be spread from person to person in a number of ways. These include airborne, direct or indirect contact, sexual contact, through blood, body fluids and through the fecal-oral route. For example, the influenza virus is transmitted from person to person through the air, typically via sneezing or coughing. Pathogens can also be transmitted to humans through contact with animals, birds, and other living creatures that naturally harbor the microorganism. The agent of anthrax, *Bacillus anthracis* naturally dwells in sheep (Epstein and Price, 2009). Contamination of water by pathogens is another route of disease spread. Water remains crystal clear until there are millions of bacteria present in each milliliter. Viruses, which are much smaller, can be present in even higher numbers without affecting the appearance of the liquid. Thus, water can be easily laced with enough pathogens to cause illness (Ahmed, 2010). Food-borne pathogens cause millions of cases of disease and hundreds of deaths annually. Bacteria, viruses, or protozoa that usually reside in the intestinal tract of humans or other creatures are causative organisms (Atreya, 2004). Examples include *Escherichia coli* O157:H7, *Campylobacter jejuni*, and rotavirus.

2.1.1 Viruses

Viruses are infectious agents consisting of RNA or DNA surrounded by a protein coat (capsid). They do not possess the ability to reproduce by themselves and thus must inhabit living cells and use their hosts' genetic reproduction and protein manufacturing processes in order to multiply. Viruses can cause disease when they inhabit other organisms that in turn become pathogens. Diseases caused by viruses include the common cold, influenza, warts, HIV and smallpox (Boston Cure Project, 2002).

2.1.2 Bacteria

Bacteria are small unicellular organisms that are prokaryotic (lacking a nucleus), unlike other unicellular pathogens which all possess nuclei. Although the vast majority of bacteria are harmless or beneficial, a few pathogenic bacteria can cause infectious diseases. Bacteria affect cells by either breaking down the cells or releasing toxins that affect the entire body. Bacterial diseases are usually named for the bacteria that cause them, like Salmonellosis, caused by the *Salmonella* bacteria. Some serious bacterial diseases include diptheria, anthrax, and various *streptococcus* infections (Boston Cure Project, 2002).

2.1.3 Fungi

Fungi are eukaryotic, non-motile organisms that can be either unicellular or multicellular. They have rigid cell walls composed of chitin, mannans and occasionally cellulose. Their usual role in the environment is to break down dead organic material; however, some species are also capable of parasitizing living creatures. Since healthy people are generally able to resist infection by fungi, most fungal infections are found in immune compromised hosts. Fungal infections are generally cutaneous, subcutaneous, or systemic in nature. Systemic infections result either from inhalation of fungal spores or

particles or the proliferation of commensal fungi in immune compromised hosts. *Tinea*, a type is very good at penetrating our skin and causing infection. This is the fungus that causes athlete's foot. It also causes ringworm (Boston Cure Project, 2002).

2.1.4 Protists

Protists are small single or multi-cellular organisms that can live in a variety of environments. Fungi, amoebas, protozoa and algae are examples of common protists. Malaria, a major cause of death in Africa is caused by *Plasmodium*. Other common causes of illness in humans are *Trypanosoma* (sleeping sickness), and *Entamoeba*, which can cause dysentery. Protozoal diseases are often chronic. Protist caused illnesses can be very bad because they do not respond well to treatments (Hunter, undated).

2.1.5 Worms

Flatworms and roundworms are responsible for a large number of diseases around the world. *Shistosoma* is a parasitic flatworm that is responsible for hundreds of deaths around the world each year. Other parasitic worms that can infect people are tapeworms and hookworms (Hunter, undated).

2.2 The Organisms

2.2.1 Salmonella

Salmonella are enterobacteria that cause typhoid fever, paratyphoid fever and food borne illness. *Salmonella* was named after Daniel Elmer Salmon, an American veterinary pathologist who, together with Theobald Smith, first discovered the *Salmonella* bacterium from pigs (Molbak *et al.*, 2006). *Salmonella* species are generally characterized as Gram negative rod shaped organisms, ranging from 0.7 to 1.5 x 2 to 5 µm in size and non-lactose fermenters. With a few exceptions, they are motile with

peritrichous flagella, facultative anaerobic and produce acid from glucose usually with the production of gas (Health Protection Agency, 2007). *Salmonella* is oxidase negative, catalase positive, indole and Voges Proskauer (VP) negative, methyl red, simmons citrate positive, H_2S producing and urea negative. Some of these characteristics are used for biochemical confirmation of *Salmonella* (WHO, 2003).

Serotypes of *Salmonella* are members of the group *Escherichiae* in the family *Enterobacteriaceae* and regarded to belong to two species: (1) *Salmonella bongori* (formerly subspecies V) and (2) *Salmonella enterica* which is divided into six subspecies (I = *enterica*, II = *salamae*, IIIa = *arizonae*, IIIb = *diarizonae*, IV = *houtenae*, and VI = *indica*). Most (>99.5%) *Salmonella* isolates from humans are serotypes of *Salmonella enterica* (Health Protection Agency, 2007). The Centre for Disease Control (CDC) recommends that *Salmonella* species be referred to only by their genus and serovar: e.g. *Salmonella typhi* instead of the more correct designation, *Salmonella enterica* subspecies *enterica* serovar *typhi*. There are numerous (more than 2500) serovars within both species according to the Kauffman-White classification scheme. The prime division is first by the somatic O antigen, then by flagellar H antigens. H antigens are further divided into phase 1 and phase 2 (WHO, 2003).

Salmonella is perhaps best known as a cause of bacterial food poisoning. *Salmonella* infections are normally associated with raw or undercooked poultry/meat and can also be found on fruits and vegetables that are not cooked or washed properly. A food handler may also spread *salmonella* to foods if they do not properly wash their hands after using the toilet (Molbak *et al*., 2006). The disease caused by *Salmonella* is generally called salmonellosis. Symptoms include diarrhea, abdominal pain, chills, fever, vomiting,

dehydration and headache. In some cases, individuals recovering from salmonellosis may continue to shed *Salmonella* in their feaces for weeks to months after symptoms have disappeared (Molbak *et al*., 2006). A more serious illness may result from *Salmonella* infection especially in infants and the elderly. Diarrhea may become so severe that the person needs to be hospitalized. The bacteria can also get into the bloodstream and cause death unless the person is treated quickly with antibiotics. Drinking plenty of water or an electrolyte solution (Oral Hydrated Salt) is important when you have diarrhea to be sure you do not become dehydrated. Antibiotics are typically not necessary unless the bacteria enter the bloodstream.

2.2.2 Escherichia coli

Escherichia coli, commonly referred to as *E. coli* is a Gram-negative bacterium that is a member of the *Enterobacteriacae* species. Cells are typically rod-shaped, and are about 2.0 μm long and 0.5 μm in diameter, with a cell volume of 0.6 – 0.7 $(\mu m)^3$ .They are facultative anaerobic and non-sporulating (Kubitschek, 1990). *E. coli* is named after a German pediatrician and bacteriologist, Theodor Escherich, who discovered this bacterium in 1885. *Coli* are a reference to bacteria which grows in the colon. A common subdivision system of *E. coli*, but not based on evolutionary relatedness, is by serotype, which is based on major surface antigens (O antigen: part of lipopolysaccharide layer; H: flagellin; K antigen: capsule) (Orskov *et al*., 1977). The combination of letters and numbers in the bacterium name refers to specific surface proteins that distinguish harmful and harmless types of *Escherichia coli*.

Harmless *E. coli* are present in the intestines of people and animals. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K and by preventing the establishment of pathogenic bacteria within the intestine (Eckburg

et al., 2005; Reid *et al*., 2001). However, some strains of the bacteria, 0157:H7, 0121:H19 and 0104:H21 produce potent toxins that can cause severe illness in humans. Fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Symptoms of infection include abdominal cramps, diarrhea, nausea and fever (Olson, 2004). Drinking a lot of water help to flush the bacteria from the system and prevent dehydration. Antibiotics usually don't work against *E. coli* infections because some strains are resistant to them. Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination (Feng *et al*., 2002). Presence of *E. coli* shows the unhygienic conditions practiced by that community.

2.2.3 Enterococci

Enterococci are gram-positive cocci that often occur in pairs (diplococci) or short chains (Gilmore, 2002). *Enterococci* are facultative anaerobic organisms, non spores forming, non motile but are tolerant of a wide range of environmental conditions: extreme temperature (10-45°C), pH (4.5-10.0) and high sodium chloride concentrations (Fisher and Phillips, 2009). Cells predominately occupy human intestines and are one micrometer in diameter. *E. faecalis* and *E. faecium* are the most frequent species found in humans. Other *Enterococcal* species known to cause human infection include *Enterococcus avium, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus durans, Enterococcus raffinosus and Enterococcus mundtii* (De Perio *et al*., 2006). Members of the genus *Enterococcus* were classified as Group D *Streptococcus* until 1984, when genomic DNA analysis indicated a separate genus classification would be appropriate (Schleifer and Kilpper-Balz, 1984). They are differentiated from other *Streptococci* by their ability to grow at high pH (9.6 – 10), high temperature (45°C) and in high salt concentrations (6.5% sodium chloride).

Enterococci are responsible for about 10% of all nosocomial infections with the most common infections being urinary tract infections (UTIs) and bacteremia. Other infections include endocarditis, diverticulitis and meningitis (Fisher and Phillips, 2009). *Enterococci* are generally resistant to many gram positive antibiotics such as the tetracyclines, aminoglycosides, sulfonamides, some penicillins, and lincosamides. In the last two decades, virulent strains of *Enterococcus* have become resistant to vancomycin (vancomycin-resistant *Enterococcus*, or VRE) (Fisher and Phillips, 2009). From a medical standpoint, an important feature of this genus is the high level of intrinsic antibiotic resistance (Ryan and Ray, 2004). *Enterococci* are used as a bacterial indicator for determining the extent of fecal contamination in foods and in recreational surface waters.

2.2.4 Microbiology

Stool samples, animal feaces and environmental samples are the most tested clinical and laboratory materials for *Salmonella*. Large numbers of food ingredients and food products are routinely tested by the food industry, since the presence of *Salmonella* in any ready-to-eat food is not acceptable. Water samples (drinking and recreational) are the most tested laboratory materials for *E. coli* and *Enterococci* since they are commonly used as indicators of faecal contamination.

2.2.4.1 Conventional Methods

Detection of *Salmonella* in samples with low initial cell numbers, or where the cells are stressed due to physical or chemical injury, requires a three-stage procedure involving pre-enrichment in non-selective broth, enrichment in selective broth, and subsequent detection on selective agar media. Clinical samples are typically cultured directly onto

selective agar media, such as Xylose-Lysine-Desoxycholate (XLD) agar, and incubated at 37°C for 18-24 hours (Molbak *et al*., 2006).

The culture media most commonly used for conventional detection are: for pre-enrichment – Buffered Peptone Water (BPW) or Lactose Broth (LB). For selective enrichment – Rappaport–Vassiliadis Broth (RV), Selenite Cystine Broth (SC), or Tetrathionate Broth (TB): These inhibit the growth of other microbes' whiles allowing Salmonella to be enriched in numbers. For plating, Brilliant Green Agar (BGA), Bismuth Sulfite Agar (BSA), MacConkey agar, Xylose Lysine Deoxycholate (XLD) Agar, Desoxycholate-Citrate Agar (DCA), Hektoen Agar and Salmonella Shigella Agar (WHO, 2003).

Preliminary identification based on colony appearance on selective agar media is subsequently confirmed using classical biochemical and serological testing. The ISO-6579 standard recommends using the TSI agar, Urea agar (Christensen), L-lysine decarboxylase, β-galactosidase (ONPG), Voges Proskauer and Indole tests. Serological confirmation tests typically use polyvalent antisera for flagellar (H) and somatic (O) antigens. Isolates with a typical biochemical profile, which agglutinate with both H and O antisera are identified as *Salmonella* spp. (WHO, 2003; www.rapidmicribiology.com/*Salmonella*).

Most-probable-number (MPN) multiple-tube fermentation is a technique that was widely used for measuring coliform and *E. coli* concentrations. The mechanism is based on the lactose fermentation ability of coliforms and *E. coli*, which can be separated by different formulations of the growth medium. The examination of replicates and dilutions gives an

estimated mean density of the microbial indicator; the quantity of microbial indicator in the samples can be estimated by using a probability table (YRIRP Report, 2011)

Membrane filtration (MF) is the alternative traditional method used for enumerating *E. coli* and *Enterococci*. The MF method provides a direct count of bacteria in water, based on the development of colonies on the surface of the membrane filter. Specific media like Slanetz and Bartley Agar are chosen to make the microbial indicator colonies identifiable through unique growth features (YRIRP Report, 2011)

Pathogenic *E. coli* strains that ferment lactose and are not adversely affected by elevated temperatures (e.g. 44°C) can be isolated using standard procedures for *E. coli*. In the Food and Drug Administration Bacteriological Analytical Manual method, the recommended procedure for pathogenic *E. coli* is to pre-enrich the sample in brain heart infusion (BHI) broth at 35°C for 3 hours to facilitate resuscitation of sub-lethally injured cells. The entire pre-enrichment is transferred to tryptone phosphate (TP) broth and incubated at 44°C for 20 hours, after which time an aliquot of enriched broth is plated onto eosin-methylene blue (EMB) agar and MacConkey agar plates. These are incubated at 37°C for 24 hours. Some pathogenic *E. coli* strains may exhibit atypical colony morphology on these media. Therefore typical (green metallic sheen on EMB or red colony on MacConkey agar) and atypical colonies should be selected for further identification (O'Sullivan *et al*., 2007).

Identification and confirmatory steps include biochemical tests, serotyping and examination for key virulence associated genes. Typing of pathogenic *E. coli* may involve the use of a variety of typing techniques, examples; pulsed field gel

electrophoresis, multiplelocus variable-number tandem repeat analysis, amplified fragment length polymorphism and ribotyping (O'Sullivan *et al*., 2007).

2.2.4.2 Rapid Methods

The rapid detection of pathogens and other microbial contaminants in food and water is critical for ensuring the safety of consumers/users. Conventional methods to detect bacteria often rely on time-consuming growth in culture media, followed by isolation, biochemical identification, and sometimes serology. Recent advances in technology make detection and identification faster, more convenient, more sensitive, and more specific than conventional assays in theory. These new methods are often referred to as "rapid methods", a subjective term used loosely to describe a vast array of tests that includes miniaturized biochemical kits, antibody- and DNA-based tests, and assays that are modifications of conventional tests to speed up analysis. Some of these assays have also been automated to reduce hands-on manipulations. With few exceptions, almost all assays used to detect specific pathogens require some growth in an enrichment medium before analysis (Noble and Weisberg, 2005).

The rapid test and screening kits utilise several different technologies, including novel culture techniques, immunomagnetic separation, EIA and ELISA-based assays incorporating fluorescent or colorimetric detection, simple lateral flow assays incorporating immune chromatographic technology, and molecular techniques such as DNA hybridization, PCR-based assays and nucleic acid sequence based amplification (NASBA). Some methods can be automated to screen large numbers of samples (Noble and Weisberg, 2005).

2.2.4.3 Detection of *Salmonella* by enzyme immunoassay (EIA)

The detection of *Salmonella* by EIA offers a sensitive and cost-effective method for mass screening of animal flocks/herds for indications of a past/present *Salmonella* infection. The EIA is a well-established technique for assaying antigens. Antibodies labeled with an enzyme are bound to *Salmonella* antigens, and the level of antigen present is determined by enzymatic conversion of a substrate, usually resulting in a color change which can be read visually or by a spectrophotometer. One of the reagents is usually bound to a solid matrix, such as the surface of a micro titer plate well. Some of the commercially available EIA kits for *Salmonella* antigen detection are ***TECRA*** and the Salmonella-Tek ELISA test system.

The EIAs rely on the standard cultural procedures for pre-enrichment and selective enrichment to provide enough *Salmonella* cells for detection. EIA technology that enables detection at an earlier stage of resuscitation and/or culture can provide even more rapid results. A number of such assays have been commercialized. The Foss *Salmonella* method uses a combination of immune capture to concentrate cells and automated EIA testing. The assay is completed within 18 hours. A dipstick based assay has been developed to detect *Salmonella* in foods, which utilizes an antibody coated dipstick to capture *Salmonella*. The dipstick is transferred to EIA reagents to detect *Salmonella*. This assay is complete within 22 hours (Molbak *et al*., 2006).

2.2.4.4 Detection of *E. coli* by Polymerase Chain Reaction (PCR)

A number of nucleic acid based methods have been reported for the detection and characterization of *E. coli*. The most commonly reported methods are based on the use of the polymerase chain reaction (PCR) to amplify a specific gene in E. coli. The primers used in the PCR may detect a characteristic virulence factor in *E. coli*. PCR relies on

amplification of the target gene in a thermocycler, separation of PCR products by gel electrophoresis, followed by visualization and analysis of the resultant electrophoretic patterns. The development of real-time PCR which uses fluorescence to detect the presence/absence of a particular gene in real time has greatly increased the sensitivity and speed of PCR-based detection methods (O'Sullivan *et al.*, 2007).

2.3 Antimicrobial resistance

Antimicrobial resistance describes the ability of a micro-organism to resist the action of antimicrobial drugs. This is important as it can make the treatment of infections more difficult and increase hospital costs. Undertaking laboratory testing of organisms causing infections help in deciding the most effective treatment options (Donadio *et al*., 2010).

In some instances some micro-organism are naturally resistant to particular antimicrobial agents, but a more common problem is when micro-organisms that are normally susceptible to the action of particular antimicrobial agents become resistant. The resistance often arises as a result of changes in the micro-organisms genes. The genes causing resistance can be transferred between different strains of micro-organism, and when this happens the recipient organisms will also become resistant. Regardless of how they arise, resistant micro-organisms may spread and it seems likely that the extensive use of antimicrobial agents helps this process along by eliminating competing susceptible micro-organisms (Donadio *et al*., 2010).

In Ghana, Newman *et al* (2006) found out that the high percentage of resistance to common antimicrobial agents by a wide range of bacterial isolates especially

enterobacteria from hospitals. High percentage of resistance was observed for tetracycline (82 per cent), cotrimozaxole (73 per cent), ampicillin (76 per cent) and chloramphenicol (75 per cent). Generally, the prevalence of multiple drug resistance (resistance to three or more drugs) was widespread among the various isolates and some multiple resistant strains of *Staphylococcus aureus*, *Salmonella typhi*, and non typhoidal *Salmonella* had high MIC to cefuroxime (>256), gentamicin (>256), and ciprofloxacin (>32).

In another study, Grob *et al* (2011) monitored bacterial distribution and antimicrobial resistance in patients in rural hospitals in Ghana and found *Salmonella Typhi* isolates to be resistant to chloramphenicol.

Ajibade *et al* (2010) conducted a study in Nigeria that showed the antimicrobial resistance in *Salmonella* to Nalidixic acid, Ciprofloxacin, Ampicillin, Chloramphenicol, Gentamicin, Trimethoprin-sulfamethoxazole and Tetracycline.

In Thailand, Hoge *et al* (1998) conducted a fifteen (15) year study and found out that enteric pathogens (*Shigella* species, nontyphoidal *Salmonella* species, enterotoxigenic *Escherichia coli* (ETEC), and *Campylobacter* species isolates from indigenous persons and travelers) in Thailand have developed resistance to virtually all antibiotics routinely used in the treatment of diarrhea, as well as the newer fluoroquinolone and macrolide classes of drugs.

Chen *et al* (2003) conducted a study in Taiwan about antimicrobial susceptibility of common bacterial pathogens isolated from a new Regional Hospital in Southern Taiwan

and concluded that, the high rates of antimicrobial resistance among the major bacterial pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and nontyphoid *Salmonella*) in the new hospital are impressive and alarming. Judicious use of antimicrobial agents can never be overemphasized so continued surveillance of the changes of resistance patterns over time is necessary.

2.4 Antimicrobial susceptibility methodology

Susceptibility testing is indicating for any organism that contributes to an infectious process warranting antimicrobial chemotherapy if its susceptibility cannot be reliably be predicted from existing antibiograms. Susceptibility testing of anaerobes is recommended for surveillance purposes and for specific clinical situations (CLSI, 2006). A wide range of antimicrobial susceptibility testing methods is used but three primary methods have been shown to be accurate and reliable. These are disk diffusion, broth and agar dilution susceptibility tests.

Disk diffusion test is a qualitative assay whereby discs of paper or tablets are impregnated with a single concentration of different antibiotics. The discs or tablets are placed on the surface of an agar plate that has been inoculated with test bacteria. During incubation, the antibiotics diffuse outward from the discs or tablets creating a concentration gradient. After 18-24 hours, the zone diameter (zone of inhibition) is measured and reference tables are used to determine if the bacteria are Sensitive (S), Intermediate (I) or Resistant (R) to the antimicrobial drugs (CLSI, 2006).

The broth dilution method is a liquid culture method whereby a standard amount of bacteria are inoculated into standardized liquid medium that contain different dilutions of antimicrobial drugs. For example in the standard bovine/porcine panel, five tubes contain the antibiotic ceftiofur with dilutions of 8, 4, 2, 1 and 0.5 ug/ml and four tubes contain spectinomycin with dilutions of 64, 32, 16 and 8 ug/ml, respectively. After 18-24 hours, the plates are examined either visually or with an analytical instrument for evidence of bacterial growth. Results are recorded as minimum inhibitory concentrations (MIC). The MIC is the highest dilution (lowest concentration) of antimicrobial drug that completely inhibits bacterial growth. The MIC value is reported with interpretation guidelines (S, I, R) that have been established by the Clinical and Laboratory Standards Institute (CLSI, 2007).

Agar dilution is similar to the broth dilution but in this case standardized suspension is inoculated onto agar containing a varying concentration of antibiotic, when the inoculum has dried the plate is incubated and examined for zones of growth.

A number of guidelines are available for antimicrobial susceptibility testing and subsequent interpretive criteria. These include the Clinical and Laboratory Standards Institute (CLSI, USA), British Society for Antimicrobial Chemotherapy (BSAC, UK), AFFSAPS (France), Deutsches Institut für Normung e.V. (DIN, Germany), ISC/WHO and others (www.rapidmicrobiology.com/ Antibiotic Sensitivity Testing).

2.5 Sanitation

World Health Organization (WHO) describes sanitation as combined techniques for the collection of human excreta, urine and community wastewater in a hygienic way, where

human and community health is not altered. Sanitation includes interventions to reduce people's exposure to diseases by providing a clean environment in which to live; measures to break the cycle of disease. This usually includes disposing of or hygienic management of human and animal excreta, refuse, and wastewater, the control of disease vectors and the provision of washing facilities for personal and domestic hygiene. Sanitation involves both behaviours and facilities which work together to form a hygienic environment (Simpson-Hebert and Wood, 1998). An attempt to set a lowest standard for facilities defined on health criteria resulted in the expression improved sanitation (Dahlman, 2009).

An improved sanitation facility is defined as one that hygienically separates human excreta from human contact. Water closets (WC's), pour-flush latrine, ventilated improved pit (VIP), pit latrine with slab, composting toilet, ecological sanitation are examples. Unimproved sanitation facilities include flush or pour-flush to elsewhere, pit latrine without slab or open pit, bucket, hanging toilet or hanging latrine and no facilities or bush or field defecation (WHO and UNICEF, 2006).

2.6 Sanitation in Ghana

Data reported by the 2010 UNICEF/WHO Joint Monitoring Programme (JMP) for Ghana reports that as at 2008, 13% of the population has improved sanitation, 54% uses shared facilities and 20% practice open defecation. A country with a population of about 22 million of which 49% reside in the urban settlements, improved sanitation coverage for the urban settlements stands at 17.8% and that for the rural settlements stands at 8.2% (WSMP,2009). Ghana will very likely miss the MDG target for sanitation (54%), given the predominant use of shared facilities, which are considered unimproved according to definitions used by the JMP. Further analysis of available data indicates that, for Ghana

to reach the MDG target for use of improved sanitation by 2015, as much as 1.2 million people need to use or have access to improved sanitary facilities every year till 2015 from 2008 (WSMP,2009). By far the greatest challenge is in eliminating open defecation, which is high (CSO, 2010).

2.7 Sanitation in Kumasi

The Waste Management Department (WMD) of the Kumasi Metropolitan Assembly (KMA) is the institution responsible for environmental sanitation services in Kumasi. It supervises the design, construction and management of public sanitation facilities and provides financial and technical assistance for their establishment and maintenance (KMA, 2011). Under the UNDP Water and Sanitation Program, KMA produced a Strategic Sanitation Plan for Kumasi (SSP-Kumasi) in 1999 to help in the attainment of the Target 7C of the Millennium Development Goals, which mandates that the number of people without sustainable access to water and sanitation be reduced by half by 2015(MCI, 2010). In the Strategic Sanitation Plan, it concluded that no one solution is reasonable for the whole city, but rather different sanitation systems for different kind of habitation. Simplified sewerage was recommended for high density areas, KVIP's for medium density areas and WC's with septic systems for low density areas. Stated in the SSPs is also the aim to phase out the unhygienic bucket latrines (Dahlman, 2009).

Sanitation in Kumasi has seen a major improvement from the colonial, when the unhygienic pan latrines were prominent through 90's to the current. Most residents in the metropolis about 38% use public toilets for which they pay a fee. Another 25% use household water closet facilities. The unhygienic bucket latrine system caters for 12% of the population, 8% rely on sewerage (Asafo, 4BN, KATH, KNUST, Ahinsan and

Chirapatre Housing Estates); whilst 10% use pit latrines (KVIP/Traditional) and 6% ease indiscriminately *(*KMA, 2006).

2.7.1 Public toilets in Kumasi

As noted above, 38% of the population makes regular use of 414 public toilets facilities across the city of Kumasi (KMA, 2006: KMA, 2011). Public toilet facilities usually consist of a row of toilet cubicles, with separate blocks for men and women. Different facilities with different service levels could be located on the same site or on adjacent sites. The level of maintenance of facility depicts the service level rendered. Top services may provide a ceramic squat toilet, provision of toilet roll on payment and entry into the facility, fans and lights on the ceiling, doors on the cubicles and may be tiled, nicely painted and cleaned regularly. The other end of the field consists of a hole in the floor with two pads, walls that are rarely cleaned and are soiled with mud or feaces, little or no water for washing and no lighting (Caplan, 2010). The payment of usage of facilities ranges between GHC 0.10 – GHC 0.30. The management of public toilets in Kumasi is under three primary models: facilities run by the Assembly (KMA), by the community (that is Sub-Metropolitan Districts/Unit Committee and by private franchises under the term build-operate-transfer (KMA, 2011).

2.7.2 Sanitation technologies in Kumasi

Five different sanitation technologies are used by Kumasi populace: the water closet (WC) or pour flush; the KVIP; the Enviro-Loo; the aqua privy; and the bucket/pan latrine (MCI, 2010).

The water closets (WCs) are connected to sewer systems or septic tanks (Thrift, 2007). A pour-flush toilet is like a cistern flush toilet except that instead of the water coming from

the cistern above, it is poured in by the user. The KVIP is an improved version of pit latrines with two chambers, allowing the contents of one chamber to decompose while the other is in use. When the second chamber is full, the contents of the first chamber should be sufficiently decomposed as to pose no health hazard and ready for emptying. Since this model was developed in Kumasi, in Ghana this model is called the Kumasi VIP, or KVIP (Thrift, 2007)

An Enviro-Loo is an on-site, dry sanitation toilet system that functions without water. The system separates liquid and solid waste as it enters the container via the custom designed ceramic toilet bowl. The liquid waste drains into the liquid trap below a solid waste drying plate to promote dehydration and evaporation which avoids anaerobic conditions occurring. Both the liquid and solid wastes are subjected to continuous flow of air driven through the unit by the forced aeration ventilation system. The movement of air is assisted by the ventilation extraction unit positioned on top of the outlet vent pipe with air being drawn into the container via the inlet vent pipes and toilet bowl (MCI, 2010). An aqua privy is a pit latrine with an underground watertight vault filled with water. Excreta drop into the vault and wastewater is displaced into a storage chamber, a seepage pit or a sewer line. The excreta then decomposed anaerobically in the tank (MCI, 2010).

Bucket or pan latrines are mostly used by low-income individuals and are unhygienic because they have to be emptied by laborers who collect the buckets several times per week. The contents of bucket latrines are deposited into tanks located at various sanitary sites (MCI, 2010). The practice is now considered a major public health hazard because of the significant health effects suffered by the workers who were responsible for

emptying the buckets (many workers died young), and because the buckets often ended up being emptied within or near the neighbourhood (example in nearby streams) rather than at contained disposal sites (Thrift, 2007).

2.7.3 Treatment

90% of faecal sludge from public toilets and septic tanks are collected and taken to the landfill site at Dompoase by trucks, while the rest is dumped within the city, posing hazard of pollution of ground and surface water (Dahlman, 2009). Improper management of the site has led to ineffective treatment such that effluent poses a danger when released from site.

2.8 The need for improved sanitation and hygiene practices

There is the need for improved sanitation and hygiene in that, on the average, human beings produce 1150 g of urine and 200 g of faeces per day. Thus, globally, about 500 million kg per day of human faeces are generated in urban areas and about 600 million kg in rural areas, producing a total of over one million tons per day (Adubofour, 2010). Sanitation and human health are closely connected to each other. The lack of treatment for these organic materials before disposal pollutes the environment with organisms that are hazardous to human health. Pathogens can be transmitted by direct contact to human excreta, by contaminated water and food, through contact with infected person, contact with animals acting as hosts for parasites and pathogenic bacteria and in the case of some helminthes worm infections, directly through the skin. Ingestion of faecal pathogens can cause diarrheal disease, cholera, intestinal worm infections and typhoid fever with children being the most susceptible. Consequently, it is important to safeguard adequate sanitation and hygiene education to reduce the amounts of infections (Huuhtanen and

Laukkanen, 2006). The most effective way to break the cycle of diseases is by improving sanitation coverage and hygiene practices.

2.9 Effects of poor sanitation

The inadequacy of sanitation has an effect on the economic, social, cultural, gender, health, tourism, environmental, income and to a greater extent hinders the full realization of human development of the affected persons (Adubofour, 2010). The lack of good excreta management is a major environmental threat to the world's water resources, and a fundamental stumbling block in the advancement of human dignity (Simpson-Hebert and Wood, 1998).

2.9.1 Health Effects

Diseases related to poor sanitation, unsafe water and unhygienic practices are some of the most common causes of illness and death among the poor of developing countries. These diseases fill half the hospital beds in developing countries (UNDP, 2006). Human excreta are responsible for the transmission of diarrhea, schistosomiasis, cholera, typhoid, and other infectious diseases affecting thousands of millions (Simpson-Hebert and Wood, 1998).

2.9.1.1 Diarrhea

Diarrhea is the most important excreta related diseases. It is transmitted by ingesting contaminated food or drink, by direct person-to-person contact, or from contaminated hands. Approximately 4 billion people are infected and 2.2 million die annually to diarrhea. Diarrhea is the passage of loose or liquid stools more frequently than is normal for the individual. It is primarily a symptom of gastrointestinal infection which can be caused by a variety of micro organisms including viruses, bacteria and protozoan. It is an

acute malfunction of digestive system which causes watery excrement and continuous need for excretion. It creates rapid weakening of liquid and salt balance and the body starts to dehydrate. Children are remarkably more vulnerable to diarrhea than adults. Diarrhea is the main cause of malnutrition of children.

Main factors in transmitting of diarrhea are inadequate personal and food hygiene, lack of safe drinking water, high residential density and increase of bottle-feeding instead of breast-feeding. Diarrhea cases are preventable through improved sanitation and hygienic practices especially hand washing (Huuhtanen and Laukkanen, 2006: WHO, 2008).

2.9.1.2 Cholera

Cholera is probably the best known and the most feared of the diarrheal diseases which is mostly transmitted by feacal-oral route. Cholera outbreaks can occur intermittently in any part of the world where water supplies, sanitation, food safety and hygiene practices are inadequate. Overcrowded communities with poor sanitation and unsafe drinking-water supplies are most frequently affected. Approximately 140 000 people are infected of which 5000 die of cholera every year. It is caused by Vibrio cholerae – bacteria. Cholera is an acute infection of the intestine, which begins suddenly with painless watery diarrhea, nausea and vomiting. Cholera epidemics spread more widely than diarrhea which usually occurs locally. As high as 90 percent of all cholera cases are symptomless, but the carrier of the disease can still infect others. Similar to diarrheal cases also cholera causes dehydration. Adequate drinking water and food hygiene is the primary measure to prevent cholera. It is also recommended to avoid raw fish and seafood in areas where cholera is met. (Huuhtanen and Laukkanen, 2006: WHO, 2008).

2.9.1.3 Typhoid

Typhoid and paratyphoid fevers are infections caused by bacteria which are transmitted from faeces through ingestion. Salmonella typhi or Salmonella paratyphi are the causative organisms. Estimates of 17 million people are infected yearly. Symptoms include fever, abdominal pains, insomnia, headache, constipation or diarrhea, rose-coloured spots on the chest area and enlarged spleen and liver. Safe water supply, proper sanitation systems and hygienic practices prevent the spread of typhoid and paratyphoid (Huuhtanen and Laukkanen, 2006: WHO, 2008).

2.9.1.4 Hepatitis

Hepatitis, a broad term for inflammation of the liver, is caused by virus. Two of the viruses that cause hepatitis (hepatitis A and E) can be transmitted through water and food in contact with: contaminated water or soil, infected individual, or excreta contaminated water, and directly from one individual to another. Hepatitis A and hepatitis E are associated with inadequate water supplies and poor sanitation and hygiene, leading to infection and inflammation of the liver. Hygiene is therefore important in their control. Though there is no connection between hepatitis A and E viruses, both are transmitted via the faecal-oral route, most often through contaminated water and from person to person. Symptoms include: fever, body weakness, loss of appetite, nausea and abdominal discomfort, followed by jaundice. Symptoms may vary from mild to severe. Majority of the infected are children, who after a recovery from the disease gain immunity (WHO, 2008).

2.9.2 Effects on environment

The failure of provision of satisfactory sanitation for large proportion of the population creates condition such that sewage flows directly into streams, rivers, lakes and wetlands,

affecting coastal and marine ecosystems and fouling the environment (United Nations, 2003).

Improved sanitation reduces environmental burdens, increases sustainability of environmental resources and allows for a healthier, more secure future for children (United Nations, 2003).The perceived impact of human excreta on aesthetics is the fact that waste produces odour and spoils visual appearance of the environment, especially in urban slums. In most urban slums of developing countries, excreta by children are usually disposed off in gutters, on sidewalks, or in some cases on open land. This polluted air quality creates unpleasant atmosphere to not only the households nearby, but also the pedestrians, travelers, and tourists passing by the areas (Adubofour, 2010).

2.10 Interventions to improve sanitation and hygienic practices

Obviously, a new model is required in order to improve sanitation and hygiene practices and to help in the achievement of MDGs and sustainability in the field waste management.

2.10.1 Hygiene Promotion

Hygiene Promotion is the planned, systematic attempt enabling people to take action to prevent or mitigate water and sanitation related diseases (Burnham and Abdallah, 2008). Hygiene promotion encourages people to replace their unhygienic practices with simple and safe alternatives. In many parts of the developing world these practices are not traditionally seen as ways to prevent disease and therefore must be actively promoted within water and sanitation projects (Netherland Water Partnership, 2010). The construction of sanitation facilities and their accessibility does not guarantee they will be used or that they will be used properly. Hygiene promotion tries to ensure that people gain the greatest health benefits possible from these facilities through the proper use and

maintenance of the facilities and by improving hygiene practices. (Burnham and Abdallah, 2008) The first generation of hygiene improvement programmes consisted of top-down communication and educational activities that mainly addressed the link between good hygiene and better health. This is known as hygiene education (Netherland Water Partnership, 2010). The provision of information on health alone is not sufficient to change people's practices. It is important to understand what motivates people to make healthy choices and what motivates them to change their behaviour. The desire for good health is often not the primary motivating factor for change but factors such as convenience, social status, the esteem of others and financial gain might be the driving forces behind change (Burnham and Abdallah, 2008). Hygiene promotion builds upon the knowledge, behavior and beliefs that people already have.

2.10.2 Cleaning of sanitation facilities

Cleaning of toilets is important to prevent odors and make them socially acceptable. Cleaning should be clearly defined and appropriately carried out. Dirty facilities make it more likely that people will continue to use the facilities badly or not at all. Clean facilities set a good example to users (WHO, 2008). Social acceptability is an important part of encouraging people to use toilets.

2.10.3 Handwashing.

It is widely recognized that, to reduce the risk of diarrheal disease transmission, handwashing with soap at critical times, is one of the most important ways to prevent the spread of infections. Washing hands the correct way at the right times can help reduce child morbidity rates from diarrheal diseases by almost 50 per cent (UNICEF, 2009). A latrine without a proper hand washing material will not serve its ultimate objective of

disease prevention. Every latrine or toilet must have proper handwashing materials so that users can wash their hands after visit.

The first ever Global Handwashing Day was launched on 15th October 2008. This multi-partner global awareness raising initiative was celebrated in 85 countries, with large and small events often involving the participation of children. The focus of Global Handwashing Day was on schools and school children. Many countries used it as an opportunity to raise awareness and to launch year-round programmes in schools. Global Handwashing Day has now become an annual event (UNICEF, 2009).

2.10.4 Public education on the importance of sanitation and hygiene practices

It is important to make sure that information about health and hygiene practices are available at public places. Such information should be displayed in an eye-catching, simple and accurate way. Where appropriate, large posters with bright colors and well chosen messages, put up in obvious places, are effective. These messages should include the promotion of: hand washing, use of refuse bins, care of toilet facilities and protection of water supplies (WHO, 2008).

2.10.5 Ecological sanitation

There is a need for a paradigm shift away from the present conventional approach to a holistic approach, taking into account that sanitation is a system where the environment is a key element. To achieve the demand of ecological sanitation, we must have ecological toilets (Simpson-Hebert and Wood, 1998). This method acknowledges human excreta more of a resource than waste and it is based on nutrient cycle approach. Excrement is treated in situ and the formed end product can easily be used as fertilizer in agriculture. Ecological sanitation techniques take into consideration the surrounding environment by decreasing contamination as well as keeping it clean and safe

(Huuhtanen and Laukkanen, 2006). The primary aim of sanitation is to break the life cycle of pathogens and this is achieved with this approach. The use of the end product as fertilizer can help in resource preservation.

2.11 Low income areas (Slums)

It is estimated that at least 1.5 million urban dwellers in Ghana can be classified as poor. They tend to group in identifiable areas of Ghana's major cities like slums (World Bank, 2002). A slum household is a group of individuals living under the same roof in an urban area who lack one or more of the following five conditions: durable housing, sufficient living area, security of tenure, improved access to water and sanitation. Ghana has over the years experienced rapid slum formation as a result of rapid urbanization in connection with natural increase and the insufficiency of the housing sector. Most of the facilities have exceeded their carrying capacities. The number of slum dwellers in Ghana was estimated to be 4,993,000 with a 1.8% growth rate per annum as at 2001. Slums are a physical and spatial manifestation of urban poverty. People living in slums have little or no access to services such as water, sanitation, and solid waste collection. There are about 25 slum settlements in Accra whereas Kumasi can have more than 10 (Dakpallah, 2011).

The lack or inadequacies of basic infrastructures like refuse dumping grounds and toilets, leads to indiscriminate disposal of refuse into drains, gutters and waterways and to open defecation in these areas. There are visible unsightly scenes of heaps of rubbish in containers, which are overflowing. Livestock are often found feeding on some of the rubbish on or along the streets and other open places. Housing structures in slums are sub-standard and do not comply with local building codes. Often, slum dwellers lack

legal ownership of the dwelling in which they reside or any other form of secure tenure. Public authorities do not mostly consider them as an integral part of the city (Dakpallah, 2011). Uncontrolled development is a common feature. The majority of the urban poor lives in these areas and pays rent to other legal householders, often for a room. Compound style living is the most common in many of these areas, sometimes with up to 20 families living in one or two rooms and sharing toilet facilities (World Bank, 2002).

Slum areas have very poor drainage systems. Drains, which are very essential in residential areas, are lacking slums. The well constructed ones along roads are in a deplorable state, dirty and filled with rubbish, and some are running through compounds of houses. Adubofour (2010) found that most residents of slums (Aboabo and Asawase) worked in the informal sector with very few having white collar jobs. The women mostly engage in trading with the men doing works such as welding, security and laborers. There is a linkage between the occupational level of residents and their income level which hardly cater for the needs of their family.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Study Area

Kumasi, the capital of the Ashanti Region is the second-largest city in Ghana and located in the transitional forest zone. It is between latitude 6.35º – 6.40º and longitude 1.30º – 1.35º covering a total land area of 254 km^2, with an elevation which ranges between 250 – 300 metres above sea level (Dakpallah, 2011). With a population of 1,170,270 during the 2000 population census, it now estimated to be 1.7 million (MCI, 2010). There are different kinds of housing in Kumasi, but the most common is the compound and the villa households as well as the one-roomed wooden/ sleet shacks found in shanty and slums areas (Akumiah, 2007). About 38% of residents in the metropolis use public toilets, a facility for which they must pay to access (KMA, 2006).

The study was conducted in three predominantly low income communities in the Metropolis; Manhyia, Aboabo and Ayigya. With a population of 21,636 (Manhyia sub-mtero, 2008), 40,978 (Ghana Statistical Service, 2002) and 23,880 (Oforikrom sub-metro, 2008) respectively, they are considered to be among the most densely populated areas in the Kumasi Metropolis. These settlements were selected because of their slum characteristics; high poverty levels, lack of common basic facilities, poor and overcrowded housing, poor environmental sanitation, lack of improved sanitation, high unemployment levels, lack of access to quality health care and generation of thousands of tons of municipal solid waste (MSW) that must be managed daily but hardly or not managed (Adubofour, 2010). There are no drains and if the drains exist, they have either collapsed or are choked with refuse, with others passing through houses. There is also

indiscriminate garbage disposal, stray livestock, poor toilet facilities and unauthorized building extension.

Barriers to public health delivery services in Kumasi are many; data from 2007 - 2008 for Kumasi show that maternal and infant deaths were on the rise and diseases such as malaria, tuberculosis, diarrhea, malnutrition, hypertension and diabetes continued to be major causes of morbidity. Many of the city's health facilities require refurbishment, and most hospitals need to be expanded to accommodate the increasing numbers seeking care (MCI, 2010).

3.2 Sample

According to KMA (2011), there are 19 public toilets in Manhyia, 11 in Aboabo and 11 in Ayigya. Based on this information, 4 public toilets were selected in each community for this study based on easy accessibility to the feacal sample from their septic tanks. A total of 288 human excreta samples were collected biweekly from November, 2011 – February, 2012. Additionally, 72 swab samples from the toilet cubicle walls were also collected.

3.2.1 Sample collection

Approximately 50 gram human excreta samples were collected in triplicate into air tight sterile plastic bags and transported in an ice box to the laboratory for microbiological analysis. Sterile cotton wool on wooden sticks were also swabbed on toilet cubicle and door handle, immersed in sterile Cary Blair Broth transport media and sent to the laboratory.

3.3 Isolation, Enumeration and Identification of Microorganisms.

Standard microbiological methods were used in the isolation, enumeration and identification of *Salmonella, E. coli* and *Enterococci* (ISO 6579:2002; ISO 7899-2).

3.3.1 Pre-enrichment in non-selective medium

Samples (50 g) were added to 50 ml sterile buffered peptone water and incubated at 37°C for 24 hours.

3.3.2 Selective enrichment

After pre-enrichment, 10 ml each of the pre-enriched sample was transferred into Selenite broth and Rappaport Vassiliadis Soy peptone (RVS) broth respectively and incubated at 44°C for 48 hours.

3.3.3 *Enterococci* count

Enterococci numbers were estimated by inoculating 1 ml of the pre-enrichment media (from the buffered peptone water) onto solidified Slanetz and Bartley (oxoid) agar plates. The plates were incubated at 37°C for 4 hours and at 44°C for 44 hours. Red or maroon colonies after incubation were counted using the Gallenkamp colony counter as *Enterococci*.

3.3.4 Spread on selective agar plates

A loop full from the incubated Selenite broth and RVS broth were separately streaked on Salmonella Shigella Agar (SSA) and on SSI Enteric medium agar plates and incubated at 37°C for 24 hours. A typical *Salmonella* colony has a slightly transparent zone of cream colour with a black centre and a typical *E. coli* colony is pink on SSA and SSI enteric medium.

3.3.5 Confirmation of suspected *E. coli* colonies

Suspected *E. coli* colonies were sub-cultured on Eosin Methylene Blue Agar (EMBA) and incubated at 37ºC for 24 hours. Positive *E. coli* showed green-metallic sheen around colonies on the agar plates.

3.3.6 Sub-cultivation of *Salmonella* suspected colonies and confirmation with Triple Sugar Iron (TSI)

Suspected *Salmonella* colonies were sub-cultured on SSA and incubated at 37ºC for 24 hours to obtain pure cultures. Isolated colonies were inoculated into Triple Sugar Iron (TSI) Agar.

3.3.6.1 Triple sugar iron (TSI) agar

Five different reactions can be observed in a TSI slant but that which is indicative of *Salmonella* are alkaline slant/acid butt with/without gas production and alkaline slant/acid butt, gas, H_2S production.

3.4 Antibiotic susceptibility testing

Susceptibilities to antibiotics were determined using the Kirby-Bauer disk diffusion method. This was used because it is reproducible and reliable. Disk diffusion susceptibilities were interpreted according to guidelines provided by the National Committee for Clinical Laboratory Standards (2002). The antibiotics tested were; Ampicillin (Amp) of 10 µg, Chloramphenicol (Clr) of 30 µg, Gentamicin (Gen) of 10 µg, Trimethoprim (Trm) of 5 µg, Ciprofloxacin (Cpr) of 1 µg, Sulphonamide (Sul) of 240 µg, Amoxycillin clavulanic acid (Amc) of 20 µg and Tetracycline (Tet) of 10 µg.

A standardized suspension of the *Salmonella* isolates was prepared in 0.85% sodium chloride. A densimat was used to ensure the turbidity of the resulting solution was 0.5 McFarland. This was then spread evenly on Mueller-Hinton agar in a Petri dish. The lid was left ajar for 3 to 5 minutes to allow for any excess surface moisture to be absorbed before applying the antimicrobial susceptibility test tablets. Rosco Neo-Sensitab tablets was then placed on the inoculated agar surface and incubated at 37°C for 16 to 18 hours. The antibiotics diffused into the agar, establishing a concentration gradient. Inhibition of microbial growth is indicated by a clear area (zone of inhibition) around the antibiotic disks. The diameter of the zone reflects the concentration gradient established. The zones of inhibition were then compared to a set of standards and the organism was then said to be susceptible (S), intermediately susceptible (I), or resistant (R) to the antibiotics used. The set standards for the antibiotics used were Amc (R<17 S≥17), Amp (R<14 S≥14), Clr (R<17 S≥17), Cpr (R<19 S≥22) Gen (R<14 S≥17), Sul (R≤12 S≥17), Tet (R≤14 S≥19) and Trm (R<15 S≥18) (EUCAST, 2011).

3.5 Field Observational Studies

Observations were made to assess the hygiene conditions of the toilets and the behavioral practices of users. This was done one week per community between 06:00 – 09:00 hours and 17:00 – 19:00 hours, because they are the periods that facilities are mostly used. To assess the standard of hygiene on these public toilets, notes were made on the existence and types of bins, hand washing materials and the state of cleanliness of the toilets. Secondly behavioral practices of users of the toilets were also recorded. Pictures were taken to complement this study.

3.6 Key Informant Interviews

Key informant interviews were conducted with the caretakers of the toilets. Questions centered on the management of toilets, frequency of cleaning, detergents used for cleaning, average number of people that access the toilet daily and water supply to the toilet. These interviews with the caretakers were conducted during the observational studies. It was a face-to-face mode of interview with an average time of about fifteen minutes in the local language (Twi).

3.7 Data Analysis

Statistical Package for Social Science (SPSS) version 17 was used for analysis of variance (ANOVA). *Enterococci* counts were normalized by log transformation before analysis. Tables were done with Microsoft Excel.

CHAPTER FOUR

RESULTS

4.1 State of public toilet facilities in the study areas

Water closets and aqua privy were the most dominant sanitation technology option used in the study areas. Averagely, the number of persons using each toilet cubicle per day varied between 20–50 in Manhyia with mean of 29, 31–50 in Aboabo (mean of 38) and 45–60 (mean of 53) in Ayigya respectively (Table 4.1). In all three communities, 58% of the public toilets were owned by the Assemblies (KMA and sub-metro) whiles 42% were built, owned and managed by Private Franchise under the term Build-Operate-Transfer scheme. However, all the aqua privies were owned by the Assemblies.

Table 4.1: State of sampled public toilets in the study areas

Community	Public Toilet ID	Technology	Capacity (No of cubicles)	Average No of people accessing facility (Daily)	Type of ownership
Manhyia	MA1	Pour flush	40	800	Private Franchise
	MA2	Aqua Privy	20	400	Assembly Owned
	MA3	Enviro-Loo	10	500	Assembly Owned
	MA4	Water Closet	20	500	Assembly Owned
Aboabo	AB1	Aqua Privy	14	500	Assembly Owned
	AB2	Aqua Privy	16	500	Assembly Owned
	AB3	Water Closet	26	900	Private Franchise
	AB4	Pour Flush	20	1000	Private Franchise
Ayigya	AY1	Aqua privy	10	500	Assembly Owned
	AY2	Water Closet	20	900	Private Franchise
	AY3	K.V.I.P	10	600	Assembly Owned
	AY4	Water Closet	12	700	Private Franchise

4.2 Bacterial indicator numbers in faecal samples from public toilets in the study communities

Enterococci

Mean *Enterococci* numbers (log_{10} per 100 ml) in feacal samples from Manhyia, varied between 4.10–4.20 with the highest numbers, 4.19 obtained at MA1 and the lowest, 4.11 at MA2 (Table 4.2). Similarly, *Enterococci* numbers (log_{10} per 100 ml) at Aboabo varied between 4.05–4.30 with the highest counts, 4.27 at AB3 and the lowest 4.09 at AB1. *Enterococccci* numbers (log_{10} per 100 ml) at Ayigya were 4.19 at AY3 for the highest and the lowest of 4.10 at AY4 (Table 4.2). Generally, *Enterococci* numbers (log_{10} per 100 ml) were highest, 4.18 at Aboabo followed by Manhyia, 4.15 and Ayigya 4.14 (Table 4.3). There were no statistically significant differences (p>0.05) between the levels for public toilets in Manhyia (p=0.129) and Ayigya (p=0.167) but Aboabo showed significant differences (p=0.001). However, between the three communities, there were no statistically significant differences (p=0.249).

Table 4.2: Mean *Enterococci* numbers in human excreta samples (n = 24 per public toilet)

Community	PT ID	Mean Value of *Enterococci* (log units per 100 ml)
Manhyia	MA1	4.19 (± 0.12)*
	MA2	4.11 (± 0.14)
	MA3	4.18 (± 0.15)
	MA4	4.12 (± 0.13)
Aboabo	AB1	4.09 (± 0.14)
	AB2	4.21 (± 0.13)
	AB3	4.27 (± 0.11)
	AB4	4.15 (± 0.18)
Ayigya	AY1	4.13 (± 0.15)
	AY2	4.15 (± 0.14)
	AY3	4.19 (± 0.13)
	AY4	4.10 (± 0.16)

**Standard deviations in parenthesis*

Table 4.3: Mean *Enterococci* numbers in human excreta samples (n = 96 per community)

Community	Mean Value of *Enterococci* (log unit per 100 ml)
Manhyia	4.15 (± 0.14)*
Aboabo	4.18 (± 0.16)

Ayigya	4.14 (± 0.15)

**Standard deviations in parenthesis*

4.3 Presence or absence of *E. coli* in human excreta from public toilets in the study communities

Percentage recovery of *E. coli* was highest, 33% at MA1 and lowest 17% at MA3 in the Manhyia area. In the Aboabo community, AB4 recorded the highest 42% *E. coli* whilst it was lowest 13% at AB1. However, in Ayigya, AY3 was 25% with AY1, AY2 and AY4 having the lowest with 21% each. The percentage suspected confirmed ranged between 44% - 78% for Manhyia, 43% - 88% in Aboabo and 50% - 63% in Ayigya (Table 4.4).

Table 4.4: Percentage presence of *E.coli* in human excreta samples (n = 24 per public toilet)

Community	PT ID	% suspected *E. coli*	% confirmed *E. coli*	% of suspected confirmed
Manhyia	MA1	54.2 (13)*	33.3 (8)*	61.5
	MA2	29.2 (7)	20.8 (5)	71.4
	MA3	37.5 (9)	29.2 (7)	77.8
	MA4	37.5 (9)	16.7 (4)	44.4
Aboabo	AB1	29.2 (7)	12.5 (3)	42.9
	AB2	50.0 (12)	33.3 (8)	66.7
	AB3	33.3 (8)	29.2 (7)	87.5
	AB4	54.2 (13)	41.7 (10)	76.9
Ayigya	AY1	33.3 (8)	20.8 (5)	62.5
	AY2	41.7 (10)	20.8 (5)	50.0
	AY3	50.0 (12)	25.0 (6)	50.0
	AY4	37.5 (9)	20.8 (5)	55.6

**Numbers of samples with suspected and confirmed E. coli in parenthesis*

Overall, *E. coli* presence in human excreta from the three communities was highest in Aboabo (29.2%) followed by Manhyia (25%) and Ayigya (21.9%) (Table 4.5).

Table 4.5: Percentage presence of *E. coli* in human excreta samples (n = 96 per community)

Community	% suspected *E. coli*	% confirmed *E.coli*	% of suspected confirmed
Manhyia	39.6 (38)*	25.0 (24)[b]	63.2
Aboabo	41.7 (40)	29.2 (28)	70.0
Ayigya	40.6 (39)	21.9 (21)	53.8

**Number of samples with suspected and confirmed E. coli in parenthesis*

4.4 Percentage presence or absence of *Salmonella* in human excreta from public toilets from the three study communities

From the three study communities, *Salmonella* presence was highest in MA1 and MA2 (17%) at Manhyia AB4 (21%) at Aboabo and AY3 (17%) Ayigya. However, presence of *Salmonella* was low at MA3 and MA4 (4%), AB1 (4%) and AY1 (4%) (Table 4.6). Generally, *Salmonella* presence was highest in human excreta from public toilets in Aboabo (10.4%), followed by Ayigya (9.4%) and Manhyia (6.3%) (Table 4.7).

Table 4.6: Percentage (%) presence of *Salmonella* in human excreta samples (n = 24 per public toilet)

Community	PT ID	% suspected *E. coli*	% confirmed *E. coli*	% of suspected confirmed
Manhyia	MA1	45.8 (11)*	8.3 (2)*	18.2
	MA2	66.7 (16)	8.3 (2)	12.5
	MA3	62.5 (15)	4.2 (1)	6.7
	MA4	45.8 (11)	4.2 (1)	9.1
Aboabo	AB1	58.3 (14)	4.2 (1)	7.1
	AB2	41.7 (10)	8.3 (2)	10.0
	AB3	50.0 (12)	8.3 (2)	16.7
	AB4	66.7 (16)	20.8 (5)	31.3
Ayigya	AY1	50.0 (12)	4.2 (1)	8.3
	AY2	37.5 (9)	8.3 (2)	22.2
	AY3	58.3 (14)	16.7 (4)	28.6
	AY4	58.3 (14)	8.3 (2)	14.3

**Numbers of suspected and confirmed E. coli in parenthesis*

Table 4.7: Percentage (%) presence of *Salmonella* in human excreta samples (n = 96 per community)

Communities	% suspected *Salmonella*	% confirmed *Salmonella*	% of suspected confirmed
Manhyia	55.2 (53)*	6.3 (6)*	11.3
Aboabo	50.0 (48)	10.4 (10)	20.8
Ayigya	51.0 (49)	9.4 (9)	18.4

**Numbers of suspected and confirmed Salmonella in parenthesis*

As observe in Table 4.3, Table 4.5 and Table 4.7, Aboabo recorded the highest *Enterococci* numbers (log_{10} per 100 ml) and *E. coli* presence of 4.18 and 29% respectively. This was coupled with Aboabo also having highest *Salmonella* presence (10.4%). The reverse was true for Manhyia.

4.5 Bacterial indicator numbers in Swab samples made on walls of public toilets in the three study communities

Enterococci

Enteroccoci numbers (log_{10} per 100ml) in swab samples were highest, 3.31 at MA2, 3.35 at AB2 and 3.32 at AY3. However, numbers were low at MA1 (3.04), AB3 (3.24) and AY2 (3.06) (Table 4.8). There were no statistically significant differences ($p>0.05$) in *Enterococci* numbers between the different public toilets in Manhyia ($p=0.221$) and Ayigya ($p=0.221$) but there were differences in public toilets in Aboabo ($p=0.015$). Mean *Enterococci* numbers (log_{10} per 100 ml) was highest at Aboabo (3.29), followed by Ayigya (3.24) and Manhyia (3.19) (Table 4.9). There were no statistically significant differences ($p=0.211$) between the numbers counted in the communities.

Table 4.8: Mean *Enterococci* numbers in swab samples (n = 6 per public toilet)

Communities	PT ID	Mean Value of *Enterococci* (log units per 100 ml)
Manhyia	MA1	3.04 (± 0.25)*
	MA2	3.31 (± 0.13)
	MA3	3.18 (± 0.14)
	MA4	3.22 (± 0.19)
Aboabo	AB1	3.31 (± 0.06)
	AB2	3.35 (± 0.10)
	AB3	3.24 (± 0.09)
	AB4	3.25 (± 0.12)
Ayigya	AY1	3.30 (± 0.05)
	AY2	3.06 (± 0.10)
	AY3	3.32 (± 0.07)
	AY4	3.29 (± 0.09)

**Standard deviations in parenthesis*

Table 4.9: Mean *Enterococci* numbers in swab samples (n = 24 per community).

Communities	Mean Values of *Enterococci* (log units per 100 ml)

Manhyia	3.19 (± 0.20)*
Aboabo	3.29 (± 0.10)
Ayigya	3.24 (± 0.13)

**Standard deviations in parenthesis*

4.6 Percentage presence of *E.coli* in swabs of sampled public toilets

Percentage recovery of *E. coli* from toilet cubicles swab samples were 33% at MA2, 33% at AB2, 33% at AB3, highest 50% at AY3. However there were no *E. coli* in swab samples from MA1 and AY2 (Table 4.10). In all, Aboabo had an *E. coli* recovery of 25%, 25% in Ayigya and 16.7% in Manhyia (Table 4.11).

Table 4.10: Percentage (%) of *E.coli* in swabs samples (n = 6 per public toilet)

Communities	PT ID	% suspected *E. coli*	% confirmed *E. coli*	% of suspected confirmed
Manhyia	MA1	50.0 (3)*	0 (0)*	0
	MA2	66.7 (4)	33.3 (2)	50.0
	MA3	33.3 (2)	16.7 (1)	50.0
	MA4	50.0 (3)	16.7 (1)	33.3
Aboabo	AB1	83.3 (5)	16.7 (1)	20.0
	AB2	50.0 (3)	33.3 (2)	66.7
	AB3	66.7 (4)	33.3 (2)	50.0
	AB4	83.3 (5)	16.7 (1)	20.0
Ayigya	AY1	66.7 (4)	33.3 (2)	50.0
	AY2	66.7 (4)	0 (0)	0
	AY3	83.3 (5)	50.0 (3)	60.0
	AY4	50.0 (3)	16.7 (1)	33.3

**Numbers of suspected and confirmed E. coli in parenthesis*

Table 4.11: Percentage (%) of *E. coli* in swab samples (n = 24 samples per community)

Communities	% suspected *E. coli*	% confirmed *E.coli*	% of suspected confirmed
Manhyia	50.0 (12)*	16.7 (4)*	33.3
Aboabo	70.8 (17)	25.0 (6)	35.3
Ayigya	66.7 (16)	25.0 (6)	37.5

**Numbers of suspected and confirmed E. coli in parenthesis*

4.7 Antimicrobial susceptibility testing using Kirby-Bauer disk diffusion

Isolates from Manhyia were all susceptible to Amoxycillin clavulanic acid, Chloramphenicol and Sulphonamide whilst 50% were resistant to Ampicillin and Tetracycline. Similarly, isolates from Aboabo and Ayigya were 100% susceptible to Chloramphenicol and Gentamicin. In Aboabo, 70% of the isolates were resistant to Ampicillin and Tetracycline whilst 67% and 78% of Ayigya's isolates were resistant to Ampicillin and Tetracycline respectively (Table 4.12).

The highest susceptibility was observed in Chloramphenicol (100%) followed by Gentamicin (96%) and Amoxycillin clavulanic acid and Sulphonamide with 92%. Tetracycline and Ampicillin recorded the highest resistance with 68% and 64% respectively. Sixteen percent (16%) of the isolates were intermediately susceptible to Ciprofloxacin and Tetracycline (Table 4.13).

Table 4.12: Antibiogram of *Salmonella species* isolates for the study communities

	No. of isolates		Amc	Amp	Clr	Cpr	Gen	Sul	Tet	Trm
Manhyia	6	R** (%)		3 (50.0)					3 (50.0)	1(16.7)
		I (%)				2 (33.3)	1 (16.7)		1 (16.7)	
		S (%)	6 (100)*	3 (50.0)	6 (100)	4 (66.7)	5 (83.3)	6 (100)	2 (33.3)	5 (83.3)
Aboabo	10	R (%)	1 (10.0)	7 (70.0)				1 (10)	7 (70.0)	1 (10.0)
		I (%)				1 (10.0)			2 (20.0)	
		S (%)	9 (90)	3 (30)	10 (100)	9 (90)	10 (100)	9 (90)	1 (10)	9 (90)
Ayigya	9	R (%)		6 (66.7)				1 (11.1)	7 (77.8)	1 (11.1)
		I (%)	1 (11.1)			1 (11.1)			1 (11.1)	
		S (%)	8 (88.9)	3 (33.3)	9 (100)	8 (88.9)	9 (100)	8 (88.9)	1 (11.1)	8 (88.9)

** Numbers in parenthesis are percentages*

***Resistance ranges: R – Resistant, I – Intermediary Susceptible, Susceptible*

Table 4.13: Antibiogram of *Salmonella species* isolates.

No of isolates		Amc	Amp	Clr	Cpr	Gen	Sul	Tet	Trm
25	R (%)	1 (4)	16 (64)				2 (8)	17 (68)	3 (12)
	I (%)	1 (4)			4 (16)	1 (4)		4 (16)	
	S (%)	23 (92)	9 (36)	25 (100)	21 (84)	24 (96)	23 (92)	4 (16)	22 (88)

** Numbers in parenthesis are percentages*

***Resistance ranges: R – Resistant, I – Intermediary Susceptible, Susceptible*

4.8 Hygiene practices

Table 4.14: Observed Hygienic Practices in the communities

Communities	Number of times cleaning is done daily	Provision of Bins	Covering for Bins	Handwashing materials
Manhyia	3	2	0	3
Aboabo	2	2	0	0
Ayigya	2	4	0	0

4.8.1 Cleaning of public toilets

It was observed that in all the public toilets in the three study communities, standard of hygiene was very low. Often times cleaning was done early mornings and late evenings. However, although most of the caretakers claimed through questionnaire interviews that detergents were not used in cleaning, it was mainly water that was used in cleaning. In cases where detergents such as liquid and powder soaps were used, they were scantly applied. In between cleaning times and especially around noon, feacal matter and waste paper (old newsprint) could be seen on the toilet floors outside the squat holes,

On the average, public toilets in Manhyia were cleaned three times in a day. MA1 and AY2 public toilets were always well cleaned during the study period.

4.8.2 Existence and types of bins

From the three study communities, waste bins were available in two-thirds (2/3) of the public toilets sampled. All the 4 public toilets in Ayigya had bins, 2 toilets in Manhyia and 2 in Aboabo (Table 4.14). The types of bins used at the various public toilets included cane baskets, plastic baskets and plastic buckets. However, none of these waste bins had a cover. The bins were hardly emptied and were often seen overflowing with waste papers onto the floors. In the public toilets without bins, waste papers were dropped on the floor and swept onto the corridors of the cubicles.

4.8.3 Hand washing materials

Hand washing materials were available at one-fourth (1/4) of the public toilets sampled with all of them in Manhyia. Even at these toilets it was only a bucket of water with little or no soap placed outside the toilets. Unfortunately, there were no running taps at any of the public toilets for proper recommended hand washing after visiting the toilet. One had a tap but provided no soap for users except on occasions when users made the request.

In some of the public toilets there were hand washing basins but these had broken down with no water running through its taps. Public toilet caretakers did not see the need to have them repaired because they thought they could be damaged again by users.

4.8.4 Nearness to residence

An interesting observation was the nearness of some public toilets to places of residence especially in Aboabo and Ayigya. Some houses were as close as 2 – 3 metres from public toilets and this posed a risk to inhabitants considering the breeding of flies and transfer of infections. The unsightly scenes and the unpleasant smell from the public toilets also create a nuisance for persons living nearby.

4.9 Behavioral practices

4.9.1 Queue

Waiting in queues to access the public toilets was a normal feature at most of the toilets. This was particularly evident during the mornings between the hours of 06:00 – 8:00 GMT and evenings between 18:00 – 19:00 hours GMT. During these periods, there was a lot of pressure on the public toilets making them untidy and putting users at risk of infections.

4.9.2 Removal of clothes

Due to poor management and high users per squat hole, some of the facilities, especially the aqua privy toilets, had the septic tanks/pit almost full and were hardly emptied. Such condition makes the smell of the public toilets obnoxious and as such users often took off their clothes before entering the toilets in order to prevent them smelling after visiting the toilet.

4.9.3 Usage of hand washing materials

The provision of substandard hand washing materials was hardly used by users of the public toilets. Users were often seen walking off from the toilet premises after using the facilities. A head count of users who made use of the hand washing materials recorded on the average, 3 out of ten for the three (3) public toilets in which hand washing materials were available at Manhyia.

Pitchers were common feature at the public toilets in Aboabo because of the high percentage of Muslims in that community. As a tradition, they wash their anus with water instead of using the toilet paper whenever they visit the toilet. This was however done without soap since it was not available.

4.9.4 Use of waste paper

A fee ranging between GHC 0.10 – GHC 0.30 was paid by users before they could access the public toilets. Each user was provided with an old newsprint paper or toilet roll. Almost all the caretakers did not charge children for using the toilet and as such they were not provided with a paper or toilet roll. If the children were unable to bring along their own paper or toilet roll, they often were observed using waste papers that had earlier been used and lying close to the squat holes.

CHAPTER FIVE

DISCUSSION

The sanitation technologies observed during the study include aqua privy, enviro-loo, KVIP, pour flush and water closet, with aqua privy and water closet being the foremost. This agrees with Thrift (2007) that flush toilets (including public toilets) are used by a large portion of the population. MCI (2010) in their social sector working paper series, "Water and Sanitation needs assessment for Kumasi", also reported that aqua privy toilets were generally found at public facilities. According to the Kumasi Metropolitan Assembly, the maximum capacity for one seat public toilet is 25 persons per day. The interviews with the caretakers of the public toilets revealed an average of 29, 38 and 53 users per squat hole per day for Manhyia, Aboabo and Ayigya respectively. This means that the facilities are overstretched accounting for the overflow of most septic tanks observed. Most of them are desludged biweekly at huge cost to management. Kumar *et al* (2002) in their field survey on water supply, sanitation and associated health impacts in urban poor communities in Mumbai City, India, reported an average of 129, 93, and 101 users per toilet seat in Rajiv Gandhi Nagar, Mukund Nagar and pavement dwellers respectively.

According to Van der Geest and Obirih-Opareh (2002), in Accra, there are two types of public toilet ownership, namely (i) those built by the local authority, and (ii) those built by private firms and individuals for commercial purposes as observed in Kumasi. Franchising of environmental sanitation services in Ghana is a major strategic objective outlined in the Environmental Sanitation Policy. Long queues in accessing public toilets facilities during early morning and evening rush hours has also been reported by Van der Geest and Obirih-Opareh (2002).

Generally, Aboabo is a densely populated area and hygiene practices in this community were the poorest of all the study sites and this explains why *enterococci* numbers (log_{10} per 100ml) in faeces and swabs ranged between 4.10 – 4.20 and 3.00 – 3.40 respectively. Varying enterococci numbers in faecal samples from public toilets have been reported by Zubrzycki and Spaulding (1962) ranging from 10^4 to 10^9 per gram of stool, Noble (1978) 10^2 to 10^8 per gram of faeces and Srinivasan *et al*. (2011) 6.36 and 4.07 (log_{10} per 100 ml) for raw sewage and its effluent at a sewage treatment plant.

As a result of the cubicles sizes and the general hygiene of these public toilets, *E. coli* was present in 25%, 29% and 22% of the faecal samples and 16.7%, 25% and 25% in the swab samples from Manhyia, Aboabo and Ayigya, respectively. The overall isolation rate of 25% *E. coli* in feaces was higher than the 10% reported by Addy *et al.* (2004) in infants with diarrhea in Kumasi. The reason for the higher isolation rate may be due to the fact that, interest was not in specific strains of *E. coli* as was in the study by Addy *et al*. (2004). A study in Nigeria by Obi *et al*. (1997) on 1200 patients with diarrhea and 1200 without diarrhea gave an isolation rate of 20% and 3% respectively which are all lower than the isolation rate recorded for this study.

Persistence of *Salmonella* in the environment is an important characteristic in its prevalence. *Salmonella* can survive for long periods of time in water and in dry materials such as dust, faeces and animal feed (Akhtar *et al.*, 2010). Low numbers of *Salmonella* surviving in the environment in a dormant state can multiply rapidly if suitable conditions are present. This study has shown that the isolation rate for the *Salmonella* was low in all three communities. Aboabo recorded the highest presence (10%), followed by Ayigya (9%) and Manhyia (6%). The difference in the prevalence rates

reported could be due to differences in the population densities and hygienic standards in the study communities. The overall Isolation frequency was 9%. A similar study in Pakistan on prevalence and antibiogram studies of *salmonella enteritidis* isolated from human and poultry sources showed a higher isolation rate of 46% for human (Akhtar *et al*., 2010). Molla *et al* (2003) also reported a 6% isolation frequency from human in a study in Ethiopia.

Antibiotic resistance in Salmonella has assumed alarming proportions worldwide. Monitoring drug resistance pattern among the isolates give vital clues to the clinician regarding therapeutic regime to be adopted. It is also an important tool in devising a comprehensive chemotherapeutic drug for a population within a geographical area. In the present study, the highest susceptibility was observed in Chloramphenicol (25; 100%) followed by Gentamicin (24; 96%) and Amoxycillin and Sulphonamide (23; 92%). High resistance was against Tetracycline (17; 68%) and Ampicillin (16; 64%). All the isolates were susceptible to at least one of the 8 antibiotics tested. Murugkar et al (2005) in India analyzed the resistance profile for 15 antimicrobial agents of 23 *Salmonella* strains from humans and reported that 13 (57%) were resistant to Ampicillin and Amoxicillin, 4 (17%) were resistant to Gentamicin and 2 (9%) were resistant to Tetracycline, Trimethoprim and Chloramphenicol. Ajibade *et al*. (2010) in Nigeria reported of resistance to Chloramphenicol (75%), Ampicillin (78%), Tetracycline (82%), Gentamicin (78%), Teimethoprim (82%) and Ciproflaxacin (74) by 44 *Salmonella enterica* isolated from the bathroom and toilets in some homes.

Although the cleaners engaged in cleaning public toilets clean them at least two or three times daily, the sanitary condition and physical outlook of most of them were dirty and

covered with filth. Lack of hygiene on the part of the cleaners, misconduct of users and the high user per squat hole seem to be the major causes of bad sanitary and physical condition of public toilets. MA1 and AY2 were found to be good from the sanitary point of view whiles the others were bad. A survey by Nketia *et al.* (2007) in Appiadu, a suburb of the Kumasi metropolis had 52% of respondents describing the cleanliness of the public toilet as either bad or poor. A sanitation survey of Aboabo and Asawase by Adubofour (2010) also had 31.2% and 23.5% of the respondents indicating presence of faecal matter on the floor of public toilets in Aboabo and Asawase respectively and 100% indicating poor maintenance of facilities in both communities. These findings validate the statement by Ayee and Crook (2003) that,- public toilets are not at a satisfactory level, both from the point of public health standards and in the eyes of the public users of these facilities.

An inspection of two public toilets in Nima, a suburb of Accra by Van der Geest and Obirih-Opareh (2002) reported that,- used toilet papers were often seen lying on the floor or in large overflowing baskets that blocked the passage to toilet cubicles and corridors. These also produced stench. It is often a miracle that public toilet users manage to ease themselves in such conditions and reappear from the toilet totally spotless. The non availability of hand washing materials at public toilets has also been reported by Nketia *et al.* (2007) in Appiadu. Proper hand washing is one of the most effective ways of preventing spread of diseases especially after using the toilet.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Public toilets have become a basic necessity in the life of urban poor dwellers because of the lack of private toilets in most houses. The public toilets in the three communities (Manhyia, Aboabo and Ayigya) pose a great challenge to public health. The isolation of pathogenic organisms (*Enterococci, E. coli* and *Salmonella*) from the public toilets validates the risk associated with their usage. The high-risk part of users of the public toilets, that is infants, elderly, immune compromised and malnourished persons are highly susceptible and the presence of these pathogenic organisms even in low numbers constitutes a major public health concern. The risk is further accentuated by the bad hygienic practices observed with users of the facilities. Proper location, good management and sanitary facilities for public toilets will not only promote general public health and the city's beauty but will also be economically beneficial.

Additionally, the isolation of pathogenic organisms from faecal matter and the walls of the public toilets are of significance to public health in Kumasi as it underlines the necessity for a coordinated surveillance and monitoring program of public toilets in the metropolis.

6.2 Recommendations

- Cleaning of the public toilets especially those managed by the Assemblies should be supervised thoroughly by KMA as most of them were the bad in terms of sanitary conditions.

- There is the need for proper education to help in eradication of bad habits at public toilets. Local NGOs can help in this regard.
- The provision of sinks/taps and soap should be enforced at public toilet facilities because hand washing is a life-saver and a cost-effective intervention. Additionally posters which encourage users to wash their hands after usage should be placed at vantage point in the public toilet.
- A study should be undertaken to know the levels of pathogenic organisms carried by users who refuse to make use of hand-washing materials. Clearly, ethical clearance and cooperation of user would be needed.

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APPENDICE

APPENDIX A

MEDIA USED, COMPOSITION AND MODE OF PREPARATION

Buffered Peptone Water

It is used as a non-selective pre-enrichment medium for the detection of *Salmonella* in food products and environmental specimens and also a diluent for the enumeration of micro-organisms.

Typical formula (g/l):	Peptone mix	10.0
	Sodium Chloride	5.0
	Disodium Hydrogen Phosphate	3.5
	Monopotassium Phosphate	1.5
	pH	7.2 ± 0.2

Mode of preparation – Dissolve 20 grams in 1 litre of distilled water and mix thoroughly. Sterilize by autoclaving at 121°C for 15 minutes.

Rappaport-Vassiliadis Soy Peptone Broth

It is a selective media used for the enrichment of *Salmonella* species from food and environmental samples. It is primarily used following a pre-enrichment of the specimen in a suitable medium, such as BPW.

Typical formula (g/l):	Pancreatic Digest of Casein	4.54
	Sodium Chloride	7.2
	Monopotassium Phosphate	1.45
	Magnesium Chloride (anhydrous)	13.4

Malachite Green Oxalate	0.036
pH	5.2 ± 0.2

Mode of preparation – Dissolve 26.6 grams in 1 litre of distilled water and mix thoroughly. Sterilize by autoclaving at 121°C for 15 minutes.

Selenite Broth

It's a base for an enrichment medium for the isolation of Salmonella.

Typical formula (g/l):

Pancreatic Digest of Casein	5.0
Lactose	4.0
Sodium Selenite	4.0
Sodium Phosphate	10.0
pH	7.0 ± 0.2

Mode of preparation – Dissolve 23 grams in 1 litre of distilled water and heated to boiling.

Slanetz and Bartley Agar

It is a selective medium used for the enumeration of Enterococci.

Typical formula (g/l):

Tryptose	20.0
Agar	12.0
Yeast Extract	5.0
Glucose	2.0
TTC	0.1
Potassium Phosphate	4.0
Sodium Azide	0.4
pH	7.0 ± 0.2

Mode of preparation – Suspend 43.5 g in 1 litre of distilled water. Slowly bring to boiling, stirring with constant agitation until complete dissolution.

Salmonella-Shigella Agar (SSA)

It is a selective and differential medium widely used to isolate Salmonella and Shigella

Typical formula (g/l):		
	Meat Extract	5.0
	Yeast Extract	5.0
	Peptone	5.5
	Lactose	10.0
	Sodium citrate	1.0
	Sodium Thiosulphate	8.5
	Ferric Ammonium Citrate	1.5
	Bile Salt	1.5
	Brilliant Green	0.00033
	Neutral Red	0.025
	Agar	14.0
	pH	7.0 ± 0.2

Mode of preparation – Suspend 52 g in 1 litre of distilled water. Take to boiling until complete dissolution. Do not autoclave.

Statens Serum Institut (SSI) Enteric Medium

It is a suitable single plate agar for the isolation of enteric pathogens except Campylobacter spp and anaerobic bacteria

Typical composition: Pancreatic digest, yeast extract, trisodium citrate, $CaCl_2$, $MgCl_2$, L-tryptophane, L- phenylalanine, ferric citrate, sodium dodecylbenzenesulfonate, glucose,

lactose, sodium glycerophosphate, sodium pyruvate, sodium thiosulphate, sodium deoxychocolate, neutral red and agar.

Modes of preparation – Suspend 41 g in 1L of distilled water, mix thoroughly, add 2.75 ml of NaOH and boil at 110 °C for 10 minutes.

Eosin Methylene Blue Agar (EMBA)

EMB agar is selective for gram-negative bacteria against gram-positive bacteria.

Typical formula (g/l): Peptone 10.0

Lactose	10.0
Dipotassium Hydrogen Sulphate	2.0
Yellow Eosin	0.4
Methylene Blue	2.0
Agar	5.0
pH	7.0 ± 0.2

Mode of preparation – Suspend 37.5g to 1 litre of distilled water. Sterilise at 121℃ for 15minutes. Mix well and pour in sterile plates.

TSI Agar (Oxoid, England)

This is a medium for the differentiation of gram negative enteric bacteria on the basis of carbohydrate fermentation and the production of hydrogen sulphide.

Typical formula (g/l): Powder 3.0

Yeast extract	3.0
Peptone	20.0
Glucose	1.0
Lactose	10.0

Sucrose	10.0
Sodium chloride	5.0
Sodium thiosulphate	0.3
Ferric citrate	0.3
Phenol red	0.0024
Agar	12.0
pH	7.4 ± 0.2

Mode of preparation – Suspend 65 g in 1 litre of distilled water and boil to dissolve the medium completely. Dispense into test tubes and sterilize by autoclaving at 121ºC for 15 minutes. Allow to set as slopes with 2.5 cm butts.

Mueller-Hinton Agar

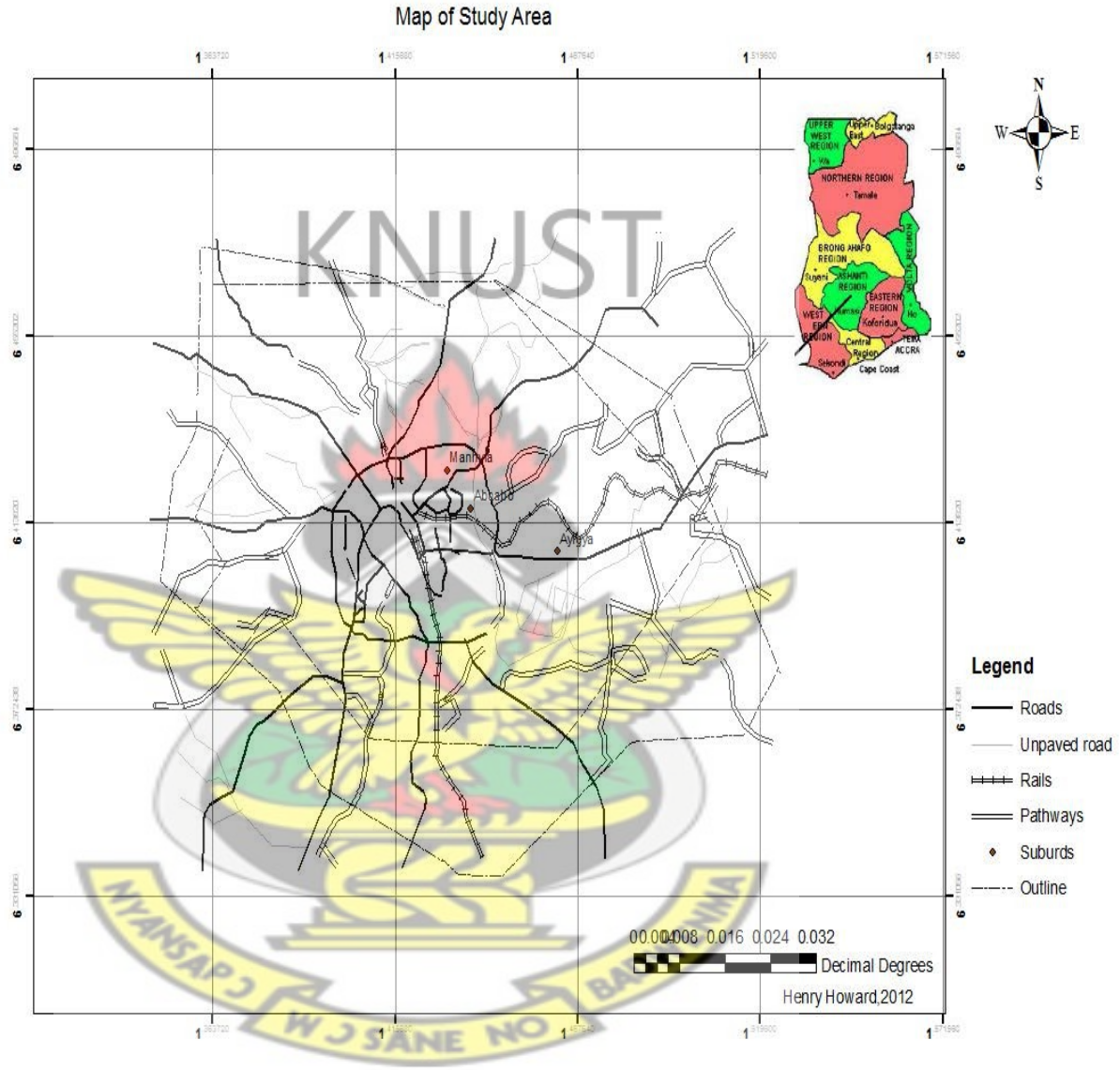
Typical formula (g/l):

Beef dehydrated infusion	300
Casein hydrolysate	17.5
Starch	1.5
Agar	17.0

Mode of preparation – Suspend 38 g in 1 litre of distilled water. Bring to boil to dissolve the medium completely. Sterilize by autoclaving at 121ºC for 15 minutes.

APPENDIX B

MAP OF KUMASI SHOWING THE STUDY AREAS



APPENDIX C

PLATES

Plate 1: Dirty corridor: Section 4.8.1

Plate 2: Dirty cubicle with no bin: Section 4.8.2

Plate 3:Overflow of waste paper: Section 4.8.2

Plate 4: Dirty cubicle and bin: Section: 4.8.1

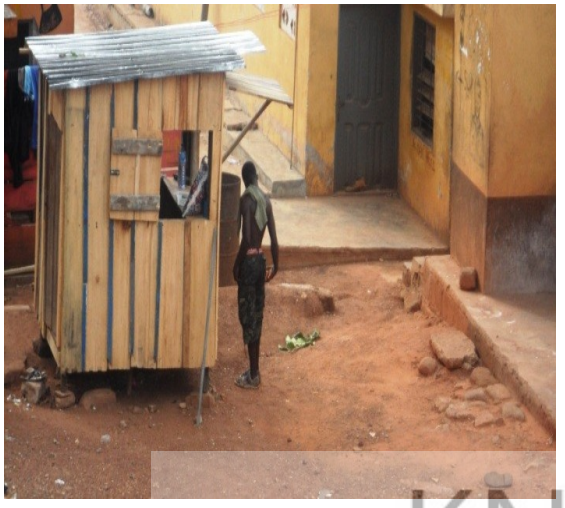


Plate 5: Removal of clothes: Section 4.9.2

Plate 6:Use of waste paper by a child

Section: 4.9.4

Plate 7: Available hand washing material

Section: 4.9.3

Plate 8: Hand washing material is hardly used

Section: 4.9.3

Plate 9: Woman blowing her nose with her hand just after using the public toilet

Plate 10: Waiting in queue to use public toilet: Section: 4.9.1

Plate 11: Closeness of residence to public toilet Section: 4.8.4

Plate 12: Waste paper swept and kept in the corridor: Section: 4.8.2