ANTIBIOTIC PROFILES OF ENTEROBACTERIACEAE ISOLATED FROM DIARRHOEIC STOOL OF CHILDREN BELOW FIVE YEARS AT MUKURU SLUMS, NAIROBI- KENYA

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DECLARATION

DECLARATION BY THE CANDIDATE

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DEDICATION

This study is dedicated to my wife Joyce, my sons Frampton K. Rotich (University of Eldoret, School of Natural Resources Management, Department of Wildlife); Ryan K. Rotich (University of Eldoret, School of Science, Department of Actuarial Sciences); Joshua Kiprop, my daughters Naomi Chemutai and Abigael Chelang'at, Little Lambs School, Eldoret.

ABSTRACT

Diarrhoea in young children continues to be a major public health concern in developing countries, including Kenya. Poor sanitation among other factors can predispose a child to diarrhoea. Faecal carriage of bacteria with antibiotic resistant traits greatly affects treatment outcome. The main objective of this study was to determine the aetiological causes of diarrhoea and response of bacterial isolates to selected antibiotics from children presenting with diarrhoea in the government health facility at Mukuru Slums, Nairobi- Kenya. It employed a cross-sectional design targeting children below 5 years of age. Stool specimens were obtained asceptically and cultured on MacConkey agar and Salmonella Shigella agar. Biochemical tests were used to identify the isolated bacteria to genus and species using biochemical characterization scheme and the Analytic Profile Index 20E. Drugs sensitivity tests were done using standard techniques. *Escherichia coli* ATCC 25922 was included as a control strain. The study showed that there was no statistical significant association ($p>0.05$) between the gender and areas of residence in relation to diarrheoea. Age of the participants showed significant association with the prevalence of diarrhoea ($p=0.00$).The *E.coli* isolates from Mukuru Kwa Njenga showed a higher percentage of pathogens (35.2%) from female children,while 29.4% were from male children. *Salmonella spp* isolates (4.9%) were isolated from female children and the least was *Shigella sonnei* (3.2%) from female children from Mukuru Kwa Reuben and Sinai respectively. More than 33.3% of all the isolates from both male and female in all the slums were resistant to Amoxicillin/ Clavulanic Acid. Above 93.4% of *E.coli*, 100% of *Salmonella spp, Salmonella typhi*, *Shigella sonnei* and *Shigella dysenteriae* were sensitive to Ceftazidime. 52.4% of *E. coli*, 100% of *S. typhi*, 66.7% of *S. dysenteriae* were sensitive while 33.3% of *S. dysenteriae* and 37.5% of *S. sonnei* were resistant to Sulphamethoxazole/ trimethoprim. Also 63.9% of *E. coli*, 80.0% of *S. typhi*, 66.7% of *S. dysenteriae* and 25.0% of *S. sonnei* were sensitive to Tetracycline. 59.6% of *E. coli*, 100% of *Salmonella sp*, 20% of *S. typhi*, 33.3% of *S. dysenteriae* and 87.5% of *S. sonnei* were resistant to Ampicillin. 89.8% of *E. coli*, 100% of *Salmonella sp* and *S. dysenteriae*, 80% of *S. typhi* and 87.5% of *S. sonnei* were sensitive to Ciprofloxacin. 86.1% of *E. coli*, 100% of *Salmonella sp* and *S. dysenteriae*, 80.0% of *S. typhi* and 87.5% of *S. sonnei* were sensitive to Nalidixic acid. Likewise 91.0% of *E.coli*, 87.5% of *Salmonella sp*, 100% of *S. typhi* and *S. dysenteriae* and 87.5% of *S. sonnei* were sensitive to Chloramphenicol. All the isolates were above 90.0% sensitive to gentamicin and ceftriaxone. There were isolates which showed multidrug resistance in this study. The *E. coli* isolate was resistant to Amoxicillin/ Clavulanic Acid and Ampicillin, *Salmonella sp* was resistant to Amoxicillin/ Clavulanic Acid (100%), Ampicillin (100%) and Tetracycline (87.5%). The *S. dysenteriae* isolate was resistant to Amoxicillin/ Clavulanic and streptomycin (66.7%), Ampicillin, tetracycline and kanamycin (33.3%). *Shigella sonnei* isolate was resistant to Amoxicillin/ Clavulanic Acid (50.0%), Ampicillin (87.5%), tetracycline (75.0%), Sulphamethoxazole/ trimethoprim (37.5%). The findings showed that, although there are a number of causative agents of diarrheal diseases, bacteria still remain one of the major causes with *Shigella, Salmonella* and *Escherichia coli* being the most prevalent and are usually waterborne. Emphasis should therefore be placed on primary preventive measures such as ensuring good sewerage management and safe supply of drinking water in the study area.

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LIST OF ABBREVIATIONS/ACRONYMNS

AD	Acute Diarrhoea
API	Analytic Profile Index
CMR	Centre for Microbiology Research
EAgg	Enteroaggregative
ECD	Early Childhood Diarrhoea
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ESBL	Extended Spectrum Beta Lactamases
ERIC	Enterobacterial Repetetive Intergenic Consensus
ETEC	Enterotoxigenic *E. coli*
HIV	Human Immuno-deficiency virus
KEMRI	Kenya Medical Research Institute
KDHS	Kenya Demographic and Health Survey
MUAC	Mid Upper Arm Circumference
MSF	Medicines' San Frontiers
OriR	Origin of replication
OriT	Origin of Transfer
ORS	Oral Rehydration Solutions
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PD	Persistent Diarrhoea
ProD	Prolonged Episodes of Acute Diarrhoea
RDA	Required Daily Amounts
UNICEF	United Nations International Children's Educational Fund
WHO	World Health Organization
CLSI	Clinical Labortory Standards Institute

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Major strides have been made to reduce diarrhoea-related mortality, although diarrhoeal diseases are still a leading cause of morbidity and mortality (WHO, 2005). Diarrhoea accounts for 1.7 billion illnesses and between 760,000 to 1.5 million deaths annually in children below five years of age globally (Rohde, 1984; UNICEF/WHO, 2009; WHO, 2013). In sub-Sahara Africa, an estimated 16% of deaths in children below 5 years of age are diarrhoea related (Bryce *et al.*, 2005). Human Immunodeficiency Virus (HIV) is also prevalent in Sub-Sahara Africa and diarrhoea can exacerbate HIV related symptoms (Obimbo *et al.*, 2004).

Studies have shown that prolonged episodes of diarrhoea in early childhood leads to stunting (Mcauliffe & Souza 1992; FAO, 2008; WHO, 2009). Poverty, poor sanitation and lack of balanced diet are also risk factors in diarrhoeal diseases (United Nations, 2010; MOH, 2010). In Kenya, under five year's mortality rate is seventy four (74) deaths per 1000(KDHS, 2010). Sixteen per cent (16%) of children under five are underweight using weight for age index (KDHS, 2010). In Nairobi county, stunting in children increased by 4% in 2010 from an earlier survey done in 2003 (KDHS, 2010). Diarrhoea episodes increase with age peaking at six to eleven months at 30% experiencing diarrhoea because during this age bracket most of the children will have started crawling while others are already walking (KDHS, 2010).

The family of enterobacteriaceae includes a group of bacteria that inhabits the intestinal tract of human and other animals. They are either motile or non-motile.

Among the most important genera include *Escherichia coli*, *Salmonella spp*, and *Shigella speci*es among others (Lima *et al.,* 2000). In a study by Tadesse *et al.,* (2012) diarrhoeagenic *E.coli* isolates were 63% (n-76) resistant to tetracycline. The traditional antibiotic Tetracyline (TC) showed low activity against these *E. coli* strains among other resistant enteric bacteria (Sang *et al.,* 2012). Although antibiotics resistance patterns are region-specific, major etiologic causes of diarrhoea are resistant to commonly used antibiotics such as ampicillin and trimethoprim-sulfamethoxazole (Mandomando *et al*., 2007; Irena *et al*., 2011; Mazel 2004; Mondal *et al*., 2009). The current study determined aetiologic causes of diarrhoea and response of bacterial isolates to selected antibiotics from children presenting with diarrhoea in a slum set up in Kenya.

1.2 Statement of the problem

Children living in the slums are vulnerable to diarrhoeal diseases mainly due to poor sanitation. Malnutrition can also predispose a child to diarrhoea or be a consequence of poor feeding during a diarrhoeal episode. Antibiotics are used in management of diarrhoea to prevent further intestinal damage. Faecal bacteria with antibiotic resistant traits is of importance whether pathogenic or commensals. The ability of bacteria to evolve mechanisms to resist attack by antibiotics was recognized soon after the widespread deployment of the first antibiotics. Antibiotic resistance is recognized as a growing problem that poses a major threat to the continued effectiveness of antibiotics used in the treatment of human illnesses. Further exacerbating the problem, pharmaceutical companies are developing fewer new antibiotics to replace those that are no longer effective. Disease outbreaks due to antibiotic resistant bacteria are not an emerging problem, but an established phenomenon that needs more routine

scrutiny and prevention by public health officials. Distribution of bacterial etiologic agents of diarrhoea by age, gender and other slum conditions are not well known hence the need to carry out this study.

1.3 Justification

Mukuru slums are situated along the highly polluted Nairobi River which flows through the industrial area. It is a home to the urban-poor population. In a large informal settlement like Mukuru, sanitation is compromised and hence a higher prevalence of diarrhoea may be observed compared to other non- slum regions of Nairobi County. Majority of the inhabitants are labourers who survive on minimal wages. The slum is heavily contaminated with animal and human waste with inadequate drainage and limited access to clean water supply hence predisposing children to illnesses. Determination and the distribution of antibiotic sensitivity profiles greatly assist clinicians in the choice of drugs resulting in better patient's response. This study yielded useful data that could guide policy formulation on managing antibiotic resistance and public health intervention.

1.4 Objectives

1.4.1 General objective

To determine aetiologic causes of diarrhoea and response of bacterial isolates to selected antibiotics.

1.4.2 Specific objectives

1. To isolate, identify and determine distribution of enterobacteriaceae from stool specimens of children below five years presenting with diarrhoea in Mukuru Slums, Nairobi- Kenya and analyse them by age, gender and residential distribution

2. To determine and separate pathogenic from non-pathogenic isolates and compare their antibiotic sensitivity profiles by gender, age and residence of the children in Mukuru Slums, Nairobi- Kenya

1.5 Hypotheses

1.5.1 Null hypothesis:

1. Enterobacteriaceae from stool specimens of children below 5 years presenting with diarrhoea in Mukuru Slums, Nairobi are not diverse
2. The isolated enterobacteriaceae from children below five years presenting with diarrhoea in Mukuru Slums, Nairobi- Kenya are not resistant to antibiotics and are not associated with gender, age and residence

CHAPTER TWO

LITERATURE REVIEW

2.1 Enterobacteriaceae

The enterobacteriaceae are a large heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of humans and animals (Lakshmi *et al.,* 2014). The family includes many genera such as *Escherichiae, Shigellae, Salmonellae, Enterobacter, Klebsiella, Serratia, Proteus* among others. Some enteric organisms, for example, *Escherichia coli* are part of the normal flora and incidentally cause disease while others such as salmonellae and shigellae, are regularly pathogenic to humans (Abbott, 2003; Kariuki *et al.*, 2013). The enterobacteraceae are facultative anaerobes or aerobes, ferment a wide range of carbohydrates, posses a complete antigenic structure, and produce a variety of toxins and other virulence factors (Sang *et al.,* 2013). Enterobacteraceae, enteric gram-negative rods and enteric bacteria may also be called coli forms (Farmer, 2003).

2.1.1 Classification

The Enterobacteriaceae are the most common group of gram-negative rods cultured in the clinical laboratory and along with *staphylococci* and *streptococci* are among the most common bacteria that cause disease (Cheesbrogh, 2005). The taxomony of Enterobacteriaceae is complex and is rapidly changing since the introduction of techniques that measure evolutionary distance, such as nucleic acid hybridization and sequencing (Eugene, 2012). More than 25 genera and 110 species or groups have been defined; Moreover, the clinically significant Enterobacteriaceae comprise about 20-25 species (Farmer, 2003).

2.1.2 General characteristics

The family of Enterobactericeae are gram-negative rods; they are motile with peritrichous flagella or non- motile. They grow on peptone or meat extract media without the addition of sodium chloride or other supplements. They can be cultured on MacConkeys' agar. Enterobactericeae can grow either aerobically or anaerobically (are facultative anaerobes), can ferment rather than oxidise glucose, often with gas production, are catalase-positive,oxidase-negative, and reduce nitrate to nitrite and have a 39-59% G+C DNA content (Farmer, 2003; Cheesbrogh, 2005).

2.1.3 Antigenic structure

Enterobacteriaceae have a complex antigenic structure. They are classified by more than 150 different heat stable somatic O (Lipopolysaccharide) antigens, more than 100 heat-labile K (capsular) antigens, and more than 50H (flagella) antigens. In *Salmonella typhi* the capsular antigens are refered to as VI antigens (Bopp, 2003). O Antigens are the most external part of the cell wall lipopolysaccharide and consist of repeating units of polysaccharide. Some O specific polysaccharides contain unique sugars (Benna, 2002). O antigens are resistant to heat and alcohol and usually are detected by bacterial agglutination. Antibodies to O antigens are predominantly I_gM. While each genus of Enterobacteriaceae is associated with specific O groups, a single organism may carry several O antigens (Brooks *et al.*, 2006). Thus most *Shigellae* share one or more O antigens with *E. coli.* *E. coli* may cross-react with some *Providencia, Klebsiella* and *Salmonella* species. Occasionally, O antigens may be associated with specific human diseases, e.g. specific O types of *E. coli* are found in diarrhoea and in urinary tract infectinus (Eisenstein *et al.*, 2003).

K antigens are external to O antigens on some but not all Enterobacteriaceae. Some are polysaccharides including the K antigens of *E. coli*. Others are proteins. K antigens may interfere with agglutination by O antisera, and they may be associated with virulence (e.g. *E. coli* strains producing KI antigens are prominent in neonatal meningitis, and K antigens of *E. Coli* cause attachment of the bacteria to epithelial cells prior to gastro intestinal invasion (Bopp , 2003).

H antigens are located on the flagella and are denatured or removed by heat or alcohol. H antigens agglutinate with anti-H antibodies, primarily 1gG. The determinants is H antigens are a function of the amino acid sequence in flagella protein (flagellin). H antigens on the bacterial surface may interfere with agglutination by anti-O antibody (Eisenstein *et al.,* 2003). There are many examples of overlapping antigenic structures between Enterobacteriaceae and other bacteria. Most Enterobacteraceae share the O14 antigen of *E. coli*. The type 2 capsular polysaccharide of *klebsiellae* is only similar to the polysaccharide of type 2 *pneumococci*. Some K antigens cross react with capsular polysaccharides of *Haemophilus influenza* and O *Neisseria meningitidis*. Thus *E. coli* 075:K100:H5 can induce antibodies that react with *H. Influenza* type b (Mandell *et al.,* 2000). The antigenic classification of Enterobacteriaceae often indicates the presence of each specific antigen. Thus the antigenic formula of an *E. coli* may be 055:KS:H21; that of *Salmonella schottmülleri* is 01, 4,5,12:H6:1,2 (Murray *et al.,* 2003).

2.2 Disease caused by Enterobacteriaceae

2.2.1 Diarrhoea

Acute diarrhoea is defined as the abrupt onset of abnormally high fluid content in the stool: more than the normal value of approximately 10 ml/kg/bd in the infant and

young child and more than 200 kg/bd in the teenager and adult. This situation typically implies an increased frequency of bowel movements, which can range from 4-5 to more than 20 times per day. The augmented water content in the stools is due to an imbalance in the physiology of the small and large intestinal processes involved in the absorption of ions, organic substrates, and thus water (Molbak *et al.,* 2002). Diarrhoeal episodes are classically distinguished into acute, chronic (or persistent) based on their duration. Acute diarrhoea is thus defined as an episode that has an acute onset and lasts no longer than 14 days: chronic or persistent diarrhoea is defined as an episode that lasts longer than 14 days. The distinction, supported by the World Health Organization (WHO), has implications not only for classification and epidemiological studies but also from a practical standpoint because protracted diarrhoea often has a different set of causes, poses different problems of management, and has a different prognosis (Snyder *et al.,* 1982).

Diarrhoea is the reversal of the normal net absorptive status of water and electrolyte absorption to secretion. Such a derangement can be the result of either an osmotic force that acts in the lumen to drive water into the gut or the result of an active secretory state induced in the enterocytes. In the former case, diarrhoea is osmolar in nature, as is observed after the ingestion of non- absorbable sugars such as lactose or lactose in lactose malabsorption. Instead, in the typical active secretory state, enhanced anion secretion (mostly by the crypt cell compartment) is best exemplified by enterotoxin induced diarrhoea (Guerrant *et al.,* 1990).

2.3 Entero pathogens causing diarrhoea

2.3.1 *Escherichia coli*

Escherichia coli are members of the normal intestinal flora. Other enteric bacteria *(proteus, enterobacter, klebsiella, morganella, providencia, citrobacter* and *serratia* species) are also found as members of the normal intestinal flora but are considerably less common than *E. coli.* The enteric bacteria generally do not cause disease in the intestine but they contribute to normal function and nutrition (Bopp *et al.,* 2003). *Escherichia coli* that cause diarrhoea are extremely common worldwide, they are classified by characteristics of their virulence properties and each group causes disease by different mechanisms. The small or large bowel epithelial cell adherence properties are encoded by genes on plasmids. Similarly, the toxins often are plasmid or phage mediated (Geo, 2004).

Enteropathogenic *E. coli* (EPEC) is an important cause of diarrhoea in infants, especially in developing countries. EPEC previously was associated with outbreaks of diarrhoea in nurseries in developed countries. EPEC adhere to the mucosal cells of small bowel. Chromosomally mediated factors promote tight adherence. There is loss of microvilli (effacement), formation of filamentous action pedestals or cup-like structures and occasionally entry of the EPEC into the mucosal cells. The result of EPEC infections is watery diarrhoea, which is usually self- limiting but can be chronic. EPEC diarrhoea has been associated with multiple specific serotypes of *E. coli*. Strains are usually identified by O antigen and occasionally by H antigen typing. The duration of the EPEC diarrhoea can be shortened and the chronic diarrhoea cured by antibiotic treatment (Bopp *et al.,* 2003).

Enterotoxigenic *E. coli* (ETEC) is a common cause of "traveller's diarrhoea" and a very important cause of diarrhoea in infants in developing countries. ETEC colonization factors specific for humans promote adherence of ETEC to epithelial cells of the small bowel. Some strains of ETEC produce a heat-labile exotoxin (LT) (MW 80,000) that is under the genetic control of a plasmid (Chart, 1998). Some strains of ETEC produce the heat-stable enterotoxin STa (MW 1500 – 4000), which is under the genetic control of a hereterogenous groups of plasmids; the enzyme STa activates guanyl cyclase in enteric epithelial cells and stimulate fluid secretion. Many STa-positive strains also produce LT. The strains with both toxins produce more severe diarrhoea (Murray *et al.*, 2003). The plasmids carrying the genes for enterotoxins (LT, ST) also may carry genes for the colonization factors that facilitate the attachment of *E. coli* strains to intestinal epithelium. Recognised colonization factors occur with particular frequency in some serotypes. Certain serotypes of ETEC occur worldwide. However, others have a limited recognised distribution. It is possible that virtually any *E. coli* may require a plasmid encoding for enterotoxins. There is no definite association of ETEC with the EPEC strains causing diarrhoea in children. Likewise, there is no association between enterotoxigenic strains and those able to invade intestinal epithelial cells (Bopp, 2003).

Enterohemorrhagic *E. coli* (EHEC) produces verotoxin named for its cytoxic effect on vero cells, a line of Africa green monkey kidney cells. There are at least two antigenic forms of the toxin. The EHEC has been associated with hemorrhagic colitis, a severe form of diarrhoea, with haemolytic syndrome, a disease resulting in acute renal failure, microangiopathic haemolytic anaemia and thrombocytopenia. Verotoxin has many properties that are similar to the shiga toxin produced by some

strains of *Shigella dysenteriae* type 1; however, the two toxins are antigenically and genetically distinct. Of the *E. coli* serotypes that produce verotoxin, 015:H7 is the most common and is the one that can be identified in clinical specimen (Chart *et al.*, 1999; Bopp, 2003).

Enteroinvasive *E. coli* (EIEC) produces a disease very similar to shigellosis. The disease occurs most commonly in children in developing countries. It also infect travellers to developing countries hence refered to as travellers diarrhoea. Like *shigella*, EIEC strains are non-lactose or late lactose fermenters and are non- motile. The EIEC produce disease by invading intestinal mucosal epithelial cells (Bopp, 2003). Enteroaggregative *E. Coli* (EAEC) causes acute and chronic diarrhoea (>14 days in duration) in persons in developing countries. These organisms also cause food borne illnesses in industrialized countries. They are distinguished by their characteristic pattern of adherence to human cells, EAEC produce ST- like toxin and a haemolysin (Law *et al.*, 1998; Bopp, 2003).

2.3.2 *Shigellae*

The natural habitat of *Shigellae* is limited to the intestinal tracts of human and other primates, where they produce bacillary dysentery. *Shigellae* are mostly slender gram-negative rods while cocobacilliary forms occur in young cultures. *Shigellae* are facultative anaerobes but grow best aerobically. They are convex, circular and transparent colonies with intact edges reaching a diameter of about 2 mm in 24 hrs. All *shigella*e with the exception of *shigella sonnei* ferment glucose, they do not ferment lactose. The inability to ferment lactose distinguishes *Shigellae* on different media, *shigellae* form acid from carbohydrates but rarely produce gas (Dupont, 2000). *Shigellae* have a complex antigenic pattern. There is great overlapping in the

serologic behaviour of different species, and most of them share O antigens with other enteric bacilli (Sang, 2007). The somatic O antigens of *shigellae* are lipopolysaccharides. Their specificity depends on the polysaccharide. There are more than 40 serotypes. The classification of *shigellae* relies on biochemical and antigenic characteristics (Dupont, 2000).

Shigellae infections are almost limited to the gastrointestine fact; bloodstream invasion is quite rare. *Shigellae* are highly communicable; the infective dose is on the order of 10^3 organisms (whereas it usually is $10^5 – 10^3$ for *salmonellae and vibrios*). The essential pathologic process is invasion of the mucosal epithelial cells (e.g. M cells) by induced plasmylosis, escape from the phagocytic vacuole, multiplication and spread within the epithelial cell cytoplasm, and passage to adjacent cells, microabsceses in the wall of the large intestine and terminal ileum lead to necrosis of the mucous membrane, superficial ulceration, bleeding, and formation of a 'pseudomembrane' on the ulcerated area. This consists of fibrin, leukocytes, and cell debris, a necrotic mucous membrane and bacteria (Dupont, 2000; Kariuki *et al.*, 2013).

2.3.3 *Salmonellae*

Salmonellae are often pathogenic in humans or animals when acquired through the oral route. They are transmitted from animals and animal products to humans where they cause enteritis, systemic infection and enteric fever, (Miller *et al*., 2003; Saidi *et al.,* 2005). *Salmonellae* vary in length and most isolates are motile with peritrichous flagella. *Salmonellae* grow readily on simple media, but they almost never ferment lactose or sucrose. They form acid and sometimes gas from glucose. They usually produce hydrogen sulphide (Hs) gas. They survive freezing in water for long periods.

Salmonellae are resistant to certain chemicals that inhibit other enteric bacteria. Such compounds are therefore useful for inclusion in media to isolate *salmonellae* from faeces (Miller *et al.,* 2003). In common with many pathogenic enteric bacteria, strains of *S. enterica* require a range of pathogenic mechanisms to colonize a host and cause disease. Infection is initiated by ingestion of sufficient number of organisms to colonize the gut, to overcome the host's defences and express the mechanisms resulting in overt disease. For infection of man, the number of bacteria that must be swallowed in order to cause infection is uncertain and varies with the serotype (Mandel *et al.,* 2000).

Once *salmonellae* enter the lumen of the intestine they are able to tolerate the action of digestive bile, but need to be able to compete with the prevailing gut flora, adhere to the gut mucosa and multiply (Mandel *et al.,* 2000). Attachment to the host mucosa is followed by degeneration of the microvilli to form breaches in the cell membrane through which the *salmonellae* enter the intestinal epithelial cells. For certain strains, further multiplication in these cells and in macrophages of the peyer's patches follows. Some bacteria penetrate into the submucosa and pass to the local mesenteric lymph modes. All the clinical manifestation of infection with *salmonellae*, including diarrhoea, begins after intestinal (ileum) penetration (Kariuki *et al.,* 2006).

2.4 Historical perspective of diarrhoea

Acute bacterial diarrhoea constitute a major health problem on a global scale since time immemorial, but this is much more so in developing countries of the world, especially among children where they are a major cause of mortality and morbidity in all other age groups. From early 1980's, aetiologies of diarrhoea have been studied

with enterotoxigenic *E. coli,* enteropathogenic *Escherichia coli, Salmonella spp. Shigella spp. Camphylobacter jejuni* and *Vibrio cholera* 01 being identified as causes of diarrhoea according to a survey in five developing countries (Huilan, 1991). In *Enterobacteriaceae,* plasmid mediated resistance was recognized in Japan in 1959 in *Shigella dysentriae* resistant to ampicillin, chloramphenicol, aminoglycoside, tetracycline and trimethoprim/sulfamethoxazole (Gerding *et al.,* 2003). *Salmonella typhi and Vibrio cholerae also* acquired resistance genes to the commonly used antibiotics at that time (Gerding *et al.*, 2003). *Campylobacter jejuni* was also shown to have acquired tetracycline resistance gene tetM and was also intrinsically resistant to trimethoprim due to carriage of dihydrofolate reductase genes, *dfrAs* (Neu, 1993).

Extended spectrum beta lactamases are enzymes that confer resistance to most beta lactam antibiotics. The first extended β-lactamase (ESBL) was TEM-1 reported in 1965 (Bradford *et al.,* 2001) from *E. coli* and named Temoneira after the girl it was isolated from. The first report of plasmid-mediated β-lactamases capable of hydrolyzing the extended-spectrum cephalosporins was published by Knothe (1983). About 500 million episodes of acute diarrhoea occur annually among children below the age of 5 years in the developing countries of Africa, Asia and Latin America (Rhode *et al.,* 2001). Assuming that a quarter of these episodes of diarrhoea are of bacterial origin and 1.0 to 3.5% mortality, between 1.0 and 3.5 million in those countries would die annually from attacks of acute diarrhoea. It has been estimated that also the great majority of acute diarrhoeal illness in adults and children over the age of two years is attributable to bacterial pathogens or germs (Carpenter, 1980).

2.5 Effects of diarrhoea

Of the estimated total of 1.7 billion cases of dirrhoeal disease every year, an estimated 760,000 children below five years are reported to die (WHO, 2013). Diarrhoea

disease is a leading cause of child mortality and morbidity in the world, and mostly results from contaminated food and water sources.Worldwide, 780 million individuals lack access to improved drinking water and 2.5 billion lack improved sanitation.Although mortality rates among the under fives have been declining globally,the situation in Africa is different (Tiruneh, 2009).The diarrhoea specific deaths in children below five years of age in Africa has been estimated at about 10.6 per thousand (WHO, 2013). As compared with other regions of the world, the Africa region shows the smallest reductions in mortality rates and the most marked slowing down trend (MOH, 2010). The under-five mortality rate in the African region is seven times higher than that in the European region.

2.6 Mechanisms of bacterial diarrhoea

A thorough understanding of the mechanisms is crucial not only in promoting new rational and better treatment measures but it will also go a long way to putting in place strategies aimed at prevention and control of these diseases. In osmotic diarrhea, stool output is proportional to intake of the unabsorbable substrate and is usually not massive: diarrhoeal stools promptly regress with discontinuation of the offending nutrient, and the stool ion gap is high. In fact, the fecal osmolality in this circumstance is accounted for not only by the electrolytes but also by the unabsorbed nutrient(s) and their degradation products (Murray *et al.*, 2003).

In secretory diarrhoea, the epithelial cells' ion transport processes are turned into a state of active secretion (Rhode & Northrup, 2001). The most common cause of acute-onset secretory diarrhoea is bacterial infection of the gut. Several mechanisms may be at work (Naik, 2006). After colonization, enteric pathogens may adhere to or invade the epithelium; they may produce enterotoxins (exotoxins that elicit secretion

by increasing an intracellular second messenger) or cytotoxins. They may also trigger release of cytokines attracting inflammatory cells, which, in turn, contribute to the activated secretion by inducing the release of agents such as prostaglandins or platelet-activating factor (Lima *et al.,* 2000). Features of secretory diarrhoea include a high purging rate, a lack or response to fasting, and a normal stool ion gap, indicating that nutrient absorption is intact (Bryce *et al.,* 2005).

2.7 Role of Enterotoxins

Enterotoxins are poisonous substances called exotoxins which are produced by some pathogenic bacteria such as *Vibrio cholerae* the causative organism of cholera and enterotoxigenic strains of *Escherichia coli*. Enterotoxins are of two types, the traditional enterotoxins and cytotoxins (Sang *et al.,* 2012). The traditional enterotoxins are either heat labile or heat stable and are represented by those produced by *Vibrio cholerae* and Enterotoxigenic *Escherichia coli (ETEC).* They act on enterocytes without causing any change in mucosal histology (Banwell *et al.,* 1973). The prototype of the traditional enterotoxins is cholera toxin called choleragen – which is functionally similar to heat labile toxin (LT) of ETEC. It acts only on the entrerocytes. Choleragen and LT stimulate adenylate cyclase located on the inner surface of the enterocyte membrane causing an increase in the intra-enterocyte cyclic adenosine mono phosphate (cAMP). Heat stable enterotoxin (ST) on the other hand, stimulates guanylate cyclase and causes in the same way an increase in intra-enterocyte cyclic guanosine monophosphate (cGMP) (Gianella *et al.,* 2009).

The increase in intra-enterocyte cAMP and cGMP triggers off a cascade of reactions involving calcium and a calcium-dependent regulator protein called calmodulin

culminating in the leakage of chloride ions thus providing the osmotic force for the reverse fluid movement into, rather than away from, the bowel lumen from the intestinal mucosa (Naffalines, 2001). The volume of fluid thus secreted into the lumen of the small bowel exceeds the colonic re-absorption capacity thereby overwhelming the colonic salvage mechanism and diarrhoea ensues. Cytotoxic enterotoxins are typified by those produced by *Staphylococcus aureus, Bacillus cereus, Clostridium perfringens, Vibrio parahaemolyticus* and *Aeromonus hydrophilia* which cause some damage to the brush border of enterocytes, and bring about diarrhoea by mechanisms which are yet to be clearly elucidated (Gianella *et al.,* 2009).

2.7.1 Bacterial diarrhoea mediated by enterocyte invasion

The second main pathogenic mechanism involves invasion of enterocytes and colonocytes by entero-invasive bacteria, thereby causing diarrhoea. When colonocytes are predominantly affected in the invasion process, the diarrhoea may be associated with the passage of blood, mucus and pus cells in the stool (Sang *et al.,* 2012). Among the bacteria which utilize this mechanism are *Salmonella typhi*, the causative organism of typhoid, *Shigella dysenteriae* which along with its close allies causes bacilliary dysentery*; Campylobacter fetus, Vibrio parahaemolyticus,* Entero-*invasive Escherichia Coli (ETEC),* and *Yerssinia enterocolitica* (Kariuki *et al.,* 2006). The mechanism of enterocyte invasion by these bacteria has been elucidated by several investigators who have carried out studies on Human epithelial cells in culture as well as on animal and human intestinal tissues *in-vitrio* using cinemicrographic technique (Ogawa *et al.,* 2009) and transmission electron microscopy (Takeuchi, 2008).

2.8 Treatment and management strategies for diarrhoea cases

2.8.1 Fluid and electrolyte replacements

Managing diarrhea involves a combination of various strategies which reduce dehydration. The WHO (2005) recommends use of oral rehydration solutions (ORS), zinc supplementation and constant feeding, breast feeding and use of antibiotics only in special circumstances (WHO, 2005). The aim of any anti-diarrheal treatment is to replace or minimize fluid and electrolyte loss, reduce stool frequency and any other symptoms such as abdominal pains, reduce faecal losses and ultimately reduce duration and severity of illness (Casburn-Jones *et al.,* 2007). Therefore, the administration of ORS to replace fluid and electrolyte loss in diarrheal patients is the rule to effective treatment (Casburn-Jones *et al.,* 2007).

Different formulations of these solutions exist but the basic ingredients are water, electrolyte (e.g. sodium) and glucose. Their mechanism of action lies in the fact that sodium/glucose co-transport proteins on the brush border cells of the intestinal lumen pulling sodium and glucose from the gut into the cells (Forceberg *et al.,* 2007). As the cellular osmotic pressure increases, water is reabsorbed out of the gut into the body. This action reverses electrolytes imbalances and rehydrates the patient. Rice starch and other concentrated carbohydrates are also being used in the formulations of ORS with the advantage that an increased amount of cellular substances will also drive active solid absorption bringing about relief of symptoms (Afia *et al.,* 2009).

Combined administration of ORS and zinc has also been reported to alleviate diarrheal symptoms and expedite recovery of many patients in different parts of the world (WHO, 2013). This treatment is being encouraged because it may be a way to avoid unnecessary use of antibiotics, especially in children. Furthermore 10 – 14 day

course of zinc during and after diarrhoea has been reported to decrease the recurrence of the disease in the next 2-3 months. Despite the relief obtained with ORS, lack of parental knowledge concerning their applications is among the major factors that limit their usage in rural and semi-urban areas of developing world. It is also difficult to administer the therapy successfully to patients whose purging episodes are accompanied by vomiting (Casburn-Jones *et al.*, 2007). In these cases, intravenous fluid replacement by a professional medical staff may be required. Such staffs are difficult to find in rural areas of African countries.

2.8.2 Use of antibiotics as a management strategy

The feasibility of using antibiotics in-*vivo* as chemotherapeutic agents is dependant primarily upon the specificity of action of such substances. The purpose is to kill microorganisms without significant harmful effects on the host. It was the basis of Ehrlich's search, which begun in 1904, for a 'magic bullet', a compound strongly germicidal for a given microorganism yet sufficiently nontoxic that it could be injected in a suitable amount to give effective concentrations in the tissues (Ehrlich, 1904). His work, originally directed toward the therapy of African sleeping sickness of trypanosome aetiology, attempted to retain the antimicrobial activity of arsenic compounds and at the same time reduce toxicity for the host. It culminated in the synthesis of salvarsan (Amyes, 2001). Penicillin discovered by Sir Alexander Fleming in 1929, was the first microbial product to be used as a significant chemotherapeutic agent. Although the trial and error search for therapeutic synthetic chemicals continues, it has been largely over shadowed by the trial and error search for antibiotics (Hare, 1970).

The WHO discourages the use of antibiotics and only approves its use in laboratory confirmed cases of dysentery and cholera. In these scenarios, ciprofloxacin of 15mg/kg body weight for three days in children presenting with dysentery should be incorporated in treatment. The WHO also recommends use of ampicillin or gentamicin as broad spectrum antibiotics in hospitalised severe malnourished children (WHO 2005). According to guidelines by the Infectious Diarrhoea Society of America (IDSA), diarrhoea caused by *E. Coli* is generally best managed by sulfamethoxazole -trimetioprim (SMZ -TMP), 800 and 160 mg, respectively, *bis in die* (b.d) that is twice daily for 3 days, or fluoroquinolone (e.g., 300 mg ofloxacin, 400mg norfloxacin, or 500 mg ciprofloxacin b.d for 3 days (Guerrant *et al*. 2001).

2.9 Antibiotic modes of action

Effective antibiotic agents work because they inhibit essential metabolic reactions of the target microorganism and are relatively harmless to the host (Kelly *et al.,* 2000). This selective toxicity can be due to distinct differences between the metabolism of the host and that of the target organism. For example, penicillin inhibits bacterial cell wall synthesis, an anabolic process peculiar to bacteria, and except for allergic reactions is harmless as far as animals are concerned (Cheesbrogh 2005). Selective toxicity can also be due to selective binding to grossly similar metabolic structures. Finally, selective toxicity could be due to differences in permeability of cells to chemotherapeutic agents (figure2.1) Eugene *et al.,* (2012).

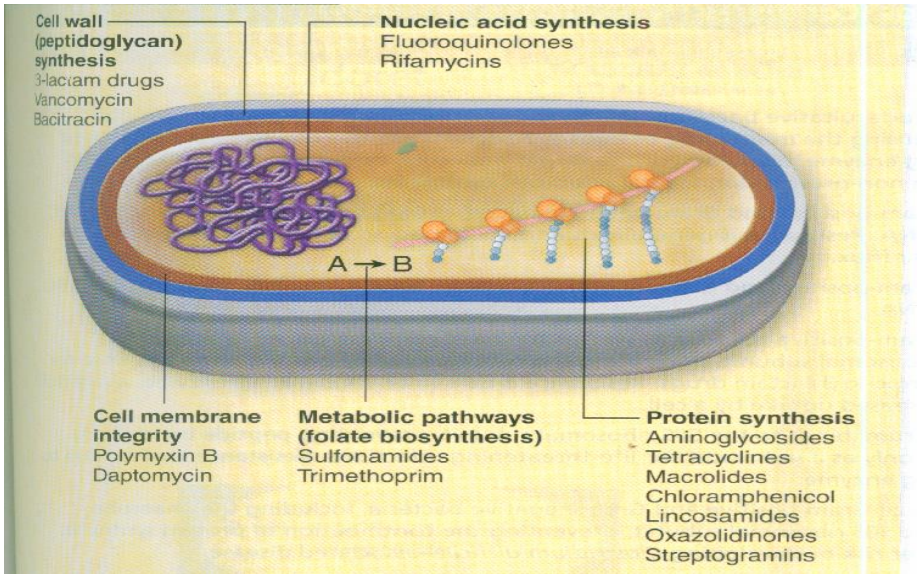

Figure 2.1: Targets of Antibiotic medications Eugene *et al., (*2012).

2.9.1 Inhibition of folic acid synthesis

Sulfanomides do not inhibit organisms that cannot synthesize folic acid, including man and those bacteria that require preformed folic acid for growth. *Escherichia coli* synthesize folic acid from para-aminobenzoic acid (PABA) (figure 2.2b) but appear to be impermeable to exogenous folic acid therefore, are particularly susceptible to inhibition by sulfanomides (Schmid, 1998).Sulfanomides are structural analogs and competitive antagonists of PABA(figure2.2a). They inhibit normal bacterial utilization of para-aminobenzoic acid molecule for the synthesis of folic acid, an important metabolite in DNA synthesis (Figure 2.2 is a diagrammatic presentation of pathway).

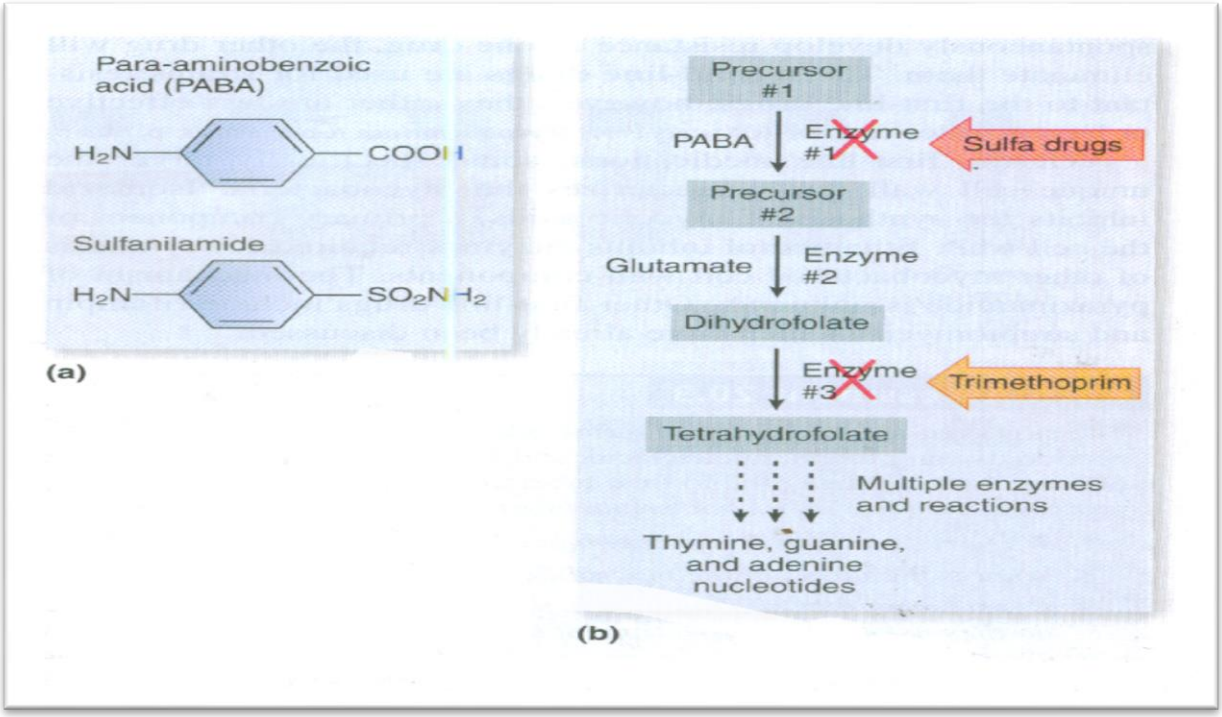

Figure 2.2: Inhibition of the folate pathway Eugene *et al.*, *(*2012).

a) The chemical structure of PABA and sulfa drug sulphanilamide.

b) The sulfanilamides and trimethoprim interfere with different steps of the pathway that leads initially to the synthesis of folate and ultimately to the synthesis of a co-enyme required for nucleotide biosynthesi

2.9.2 Inhibition of peptidoglycan synthesis

Several antibiotics are inhibitors of bacterial peptidoglycan synthesis. All beta-lactum antibiotics are bactericidal and their bactericidal action is on growing cells. After attachment to penicillin-binding proteins on bacteria, they inhibit the transpeptidation enzyme that cross-links the peptide chains attached to the backborne of the peptidoglycan.The continued growth of the cell without synthesis of the rigid structural layer of peptidoglycan leads to lysis of the bacterium through the inactivation of an inhibitor of autolytic enzymes in the cell wall (Green, 2002).

2.9.3 Inhibition of protein synthesis

A variety of antibiotics such as Streptomycin, Gentamycin, Kanamycin and Amikacin (Aminoglycosides); Tetracyclines and Chloramphenicol have been found to be inhibitors of protein synthesis (Chopra *et al.*, 1998). Aminoglycosides are bactericidal and all bind with the 30S subunits of ribosomes and inhibit protein synthesis. Tetracyclines are bacteriostatic and their main site of inhibition of protein synthesis is inhibition of the binding of AA-tRNA to the 30S subunit. Chloramphenicol is another bacteriostatic, broad spectrum antibiotic, but it affects the 50S rather than the 30S subunit and inhibits the transpeptidation stage of protein synthesis (Figure 2.3).

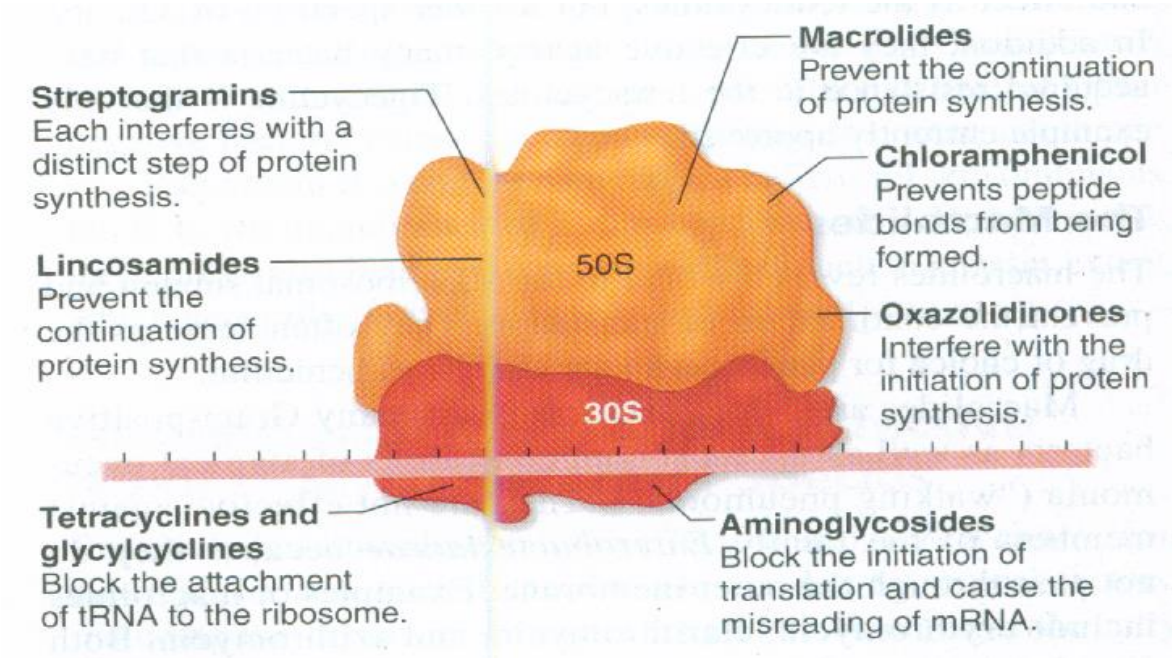

Figure 2.3: Antibiotic medications that inhibit prokaryotic protein synthesis Eugene *et al.*, (2012).

2.9.4 Inhibition of nucleic acid function

Antibiotics (fluoroquinolones) are known to inhibit nucleic acid functions in one of the following ways ; Interaction with DNA and sometimes RNA templates, causing interference with transcription or replication, interaction with polymerases involved in

transcription or replication and some nucleoside antibiotics are analogues of nucleic acid components and interfere with nucleic acid synthesis or are incorporated into a nucleic acid with subsequent alteration of structure and function (Kumar & Schweizer, 2005).

2.9.5 Antibiotics that cause membrane damage

Certain polymixin antibiotics such as Polymixin B and Bacitracin produced by members of the *Bacillus* genus kill bacteria primarily by damaging the cell membrane and destroying the cell's permeability barrier, they have cationic detergent properties and exert their antibacterial action by disrupting the cell membrane phospholipids.They have a selective, rapidly bacteriacidal action on Gram-negative bacilli, especially pseudomonads and coliform organisms (Falagas *et al*., 2008).

2.10 Diarrhoea and antibiotic resistance scenarios in Kenya

Malnutrition and diarrhoea overlap between ''severe'' clinical syndromes among acute paediatric admissions. In a study done in Kilifi District Hospital, 44% of children with malnutrition also had diarrhoea on admission (English *et al*., 2003). *Escherichia coli* O44 causing persistent diarrhoea in Kenyan patients showed resistance to tetracycline, ampicillin, erythromycin, trimethoprim-sulphamethoxazole and amoxicillin/clavulanic acid and susceptibility to chloramphenicol, nalidixic acid, azithromycin and cefuroxime (Sang, 2007). The relatedness of *E.Coli* strains from stool samples of children and rectal swabs of chicken living within close human contact revealed that *E.Coli* from children and those in chicken were contracted from different sources (Kariuki *et al*., 2013). Typical prevalence of inappropriate use of antibiotics exists in Kenya. A study that was done in eight district hospitals showed that children with abdominal pains had increased probability of receiving unnecessary antibiotics. In this study, 1160 children who had non-bloody

diarrhoea and no co-morbidities requiring antibiotics, were the focus of the analysis. A total of 750 (64.7%) of them received antibiotics inappropriately (Opondo *et al*., 2009).

In another study in rural western Kenya, recall of antibiotic use is lower than anti-malarial use probably because anti-malarials are more recognizable by name than common antibiotics recommending shorter recall periods of three to four days (Feikin *et al*., 2010). Drug resistance has therefore been an end-point in antibiotic misuse. To confirm this, a study on non-typhoidal salmonella isolated from both blood and stool of children presenting with bacteraemia and/or with gastroenteritis showed a high level of resistance to commonly used antibiotics including ampicillin, cefuroxime and cotrimoxazole (Kariuki *et al*., 2006). *Escherichia coli* isolates from Kenya were more resistant to antibiotics than those from Japan, in a 2005 study with more resistance (more than 60% of isolates in the study) being mediated against tetracycline, ampicillin (AMP) and Sulfamethoxazole/trimethoprim (SXT/ TMP).

In a study done by Bii *et al*., (2005) a total of 82% of the *E. coli* also harboured virulence genes. Salmonella and Shigella strains from Kenya showed low activity (MIC_{90} = 1,024 to 1,024mg/mL) to the commonly used antibiotics, including AMP, TMP/SXT (Gomi *et al*., 2001). A report on plasmid mediated CTM-12 resistance in *K. pneumoniae* has been documented in clinical samples from Kenyatta National Hospital, Kenya (Kariuki *et al*., 2001). Also plasmid-mediated CTX-M-15 type ESBL, CMY-2 AmpC enzymes and ciprofloxacin acetylating enzyme *aac*(6')-*1b-cr*, were reported in a study on community-acquired urinary tract infections (Kariuki *et al*., 2006). There is limited information about causative agents of diarrhoea and their antibiotic susceptibility profiles in Mukuru slums in Kenya and therefore this study

seeks to document antibiotics susceptibility profiles of enteric bacteria from the study area.

Non-typhoidal Salmonella isolated from diarrhoeagenic stools in children were resistant to mainly ampicillin, chloramphenicol and tetracycline (Kariuki *et al.*, 2006). Antibiotics are clearly one of the greatest triumphs of medical science, although they are not without their problems and disadvantages. One of the greatest concerns with the misuse of antibiotics is the likelihood of emergence of resistant strains of pathogens (Sanders, 1987). Bacteria become resistant to antibiotics in a number of different ways. Some resistance is due to the ability of a particular mutant to destroy a given antibiotic (Sanders *et al.,* 1987). For example, some *staphylococci* produce the enzyme penicillinase which destroys natural penicilins. Resistance to trimethoprim can arise when the microbe begins synthesizing very large amounts of the enzymes against the drugs hence leading to drug inactivation. Of particular concern is the possibility that such resistant mutants will increasingly replace the susceptible normal population (Guerrant *et al*., 2001). Hereditary drug resistance is often carried by extra chromosomal genetic elements called plasmids. Some plasmids, including those called resistance (R) factors, can be transferred between bacterial cells in a population and between different but closely related bacterial populations. R factors often contain genes for resistance to several antibiotics (Cundliffe, 1989).

2.11 Resistance to Antibiotics

Since the 1940s the development of effective and safe drugs to deal with bacterial and other infections has revolutionalised medical treatment and the morbidity and mortality associated with these diseases has been dramatically reduced. Unfortunately

the development of effective antibiotics has been accompanied by the emergence of drug-resistant organisms. The short generation time of many bacterial species affords ample opportunities for evolutionary adaptation. The phenomenon of resistance imposes serious constraints in the options available for the medical treatment for many bacterial infections (WHO, 2014). The emergence of antibiotic resistant bacterial pathogens has become a major public health concern. The use of antibiotics in any avenue including disease treatment and growth promotion in domestic livestock can potentially lead to widespread dissemination of antibiotic-resistant bacteria (Kariuki *et al.,* 2013).

Resistance to antibiotic agents in bacteria is mediated by several mechanisms including: Changes in bacterial cell wall permeability, energy-dependent removal of antibitotics via membranes bound efflux pumps, modification of the site of drug action and destruction or inactivation of antibiotics (MSF, 2008). Acquired antibiotics resistance phenotypes most often develop via conjugative transfer of plasmids (Gebreyes *et al*., 2002). Another method of drug resistance is by mutation,where bacteria multiply at such a rapid rate that there is always a chance that a mutation will render a bacterial cell resistant to a particular agent.These mutations occur naturally and randomly and do not require the presence of the drug. Indeed, it is likely that a drug-resistant cell is present in a bacterial population even before the drug is encountered. This was demonstrated with the identification of streptomycin-resistant cells from old cultures of *Escherichia coli* which had been freeze dried to prevent multiplication before the introduction of streptomycin into medicine (Graham, 2005).

The most disturbing development of resistance has been in *staphylococci*; one of the convenient causes of hospital bloodstream infections, many strains of which are now resistant to almost all currently available antibiotics. In addition to resistance to some

β-lactams through production of β-lactamase and the production of an additional β-lactam-building protein that also renders them resistant to methicillin, *staphylococci aureus* may also manifest resistance to other antibiotics as follows:streptomycin (because of chromosumally determined alteration of target site), aminoslycosides (because of altered target site and plasmid -determined inactivating enzymes), chloramplenicol and the macrolides (because of plasmid-determined enzymes), trimethoprim(because of transposon-encoded drug-resistant dihydrofolate reductase), Sulfonamides (because of chromosomally determined increased production of PABA) and quinolones, for example, ciprofloxacin and norfloxacillin (because of chromosomally determined reduced uptake) (Bax *et al.,* 2000).

In the past few years, enterococci have been rapidly developing resistance to many chemotherapeutic agents and have emerged as the second most common nosocomial pathogen. Non-pathogenic enterococci are ubiquitous in the intestine, have intrinsic resistance to many antibiotics and can readily become resistant to other agents by taking up plasmids and transposons carrying the relevant resistance genes. Such resistance is easily transferred to invading pathogenic enterococci (Tan *et al.,* 2000). *Enterococci*, already multiresistant, have recently developed resistance to vancomycin. This is apparently achieved by substitution of D-Ala-D-Ala with D-Ala-D-lactate in the peptide chain attached to N-acetylglucosamine-N-acetylmuramic acid (G-M) during the first steps of peptidoglycan synthesis (Bax *et al.*, 2000).

Prescribers and consumers must also bear a responsibility for the burgeoning problem of resistance. Indiscriminate use of antibiotics in human medicine has undoubtedly encouraged the growth of resistant strains. The issue around declining antibiotic efficiency is, however, not soley to do with bacterial countermeasures. The fact is that there has been a declining interest in the pharmaceutical industry in researching novel

antibiotics. Historically the era has been one of the mainstays of the industry, but most of the drugs available today are the result of incremental changes in the structures of a relatively small number of basic molecular structures, such as when it was possible to discover new and effective drugs in this way are long gone (Barrett & Barret, 2003). Ineffectiveness of antibiotics is as a result of an unavoidable negative selective pressure brought about by increased use and misuse of antibiotics (Spratt, 1994). Gram negative bacteria have shown resistance to aminoglycosides, tetracycline, sulfonamides, β-lactams and quinolones (Pfeifer, 2010). Resistance to β-lactams and fluoroquinolones receive most attention because β-lactam antibiotics are used to treat most systemic infections accounting for a larger percentage of the number of days one is put under antibiotics followed by quinolones (Banja, 2010). Clinical breakpoint is a minimum inhibitory value that links to a possible clinical outcome. It separates those isolates that are sensitive with a high likelihood of treatment success from those that are considered clinically resistant.

2.12 Mechanisms of antibiotic resistance in bacteria

Antibiotic resistance in bacteria spreads in three ways: by transfer of bacteria between people, by transfer of resistance genes between bacteria (usually on plasmids) and transfer of resistance genes between genetic transposable elements within bacteria on transposons (Higgins, 2007). Understanding the mechanisms involved in antibiotic resistance is crucial for the sensible chemical use of existing medicines and in the design of new antibiotics. One by product of the studies of resistance in bacteria was the development of plasmid-based techniques for DNA cloning, leading to the use of bacteria to produce recombinant proteins for therapeutic use (Walsh, 2000).

The spontaneous mutations rate in bacterial populations for any particular gene is very low and the probability is that approximately only one cell in ten million will on division, give rise to a daughter cell containing a mutation in that gene (Martinez & Baquero 2000). However, as there are likely to be very many more cells than this over the course of an infection, the probability of a mutation causing a change from drug sensititvity to drug resistance can be quite high with some species of bacteria and with some drugs (Cirz *et al.,* 2005). Fortunately, in most cases a few mutants are not sufficient to produce resistance as; despite the selective advantage that the resistant mutants possess the drastic reductions of the populations by the antibiotic usually enables the host's natural defences to prevail. However, this may not occur if the primary infection is caused by a drug resistant strain (Martinez & Baquero 2000).

In addition, many species of the bacteria contain extrachromosomal genetic elements called plasmids that exist free in the cytoplasm. There are also genetic elements that can replicate independently. Structurally, they are closed loops of DNA that may comprise a single gene or as many as 500 or even more. Only a few plasmids copies may exist in the cell but often multiple copies are present and there may also be more than one for resistance to antibiotics *(r-genes)* are refered to as R plasmids (Robicseck *et al.,* 2006).

2.12.1 Transfer of resistance genes between genetic elements within the bacterium

Some stretches of DNA are readily transferred from one plasmid to another and also from plasmid to chromosome or vice versa. This is because intergration of these segments of DNA, which are called transposons into the receptor DNA can occur

independently of the normal mechanism of homologous genetic recombination.Unlike plasmids, transposons are not able to replicate independently, although some may replicate during the process of intergtation, resulting in a copy in both the donor and the receptor DNA molecules (Hall & Stokes, 2004).

Transposons may carry one or more resistance genes and can ride on a plasmid to a new species of bacterium. Even if the plasmid is unable to replicate in the new host, the transposon may intergrate into the new host's chromosome or into its indigenous plasmid (Mazel, 2006). This probably accounts for the widespread distribution of certain resistance genes on different R plasmids and among unrelated bacteria. Gene cassettes and integrons which are other mobile elements can spread multidrug resistant. Several cassettes may be packaged together in a multicassette array, which can be intergrated into a larger mobile DNA unit termed as integron. This system transposon/ integron/ multiresistance cassette array-allows particulary rapid and efficient transfer of multidrug resistance between genetic elements both within and between bacteria (Mazel, 2006; Hall & Stokes, 2004).

The transfer of resistance genes between bacteria of the same and indeed of different species is of fundamental importance in the spread of antibiotic resistance. The most important mechanisms include: conjugation, transduction and transformation (Rusi *et al*., 2008). Conjugation involves cell to cell contact during which chromosomal or extra chromosomal DNA is transferred from one bacterium to another and is the main mechanism for the spread of resistance. The ability to conjugate is encoded in conjugative plasmids; these are plasmids that contain transfer genes, which in *coliform* bacteria, code for the production by the host bacteria of proteinaceus surface tubules, termed sex pili, which connect the two cells. The conjugative plasmid then passes across from one bacterial cell to another (generally of the same species).

Many gram-negative and some gram-positive bacteria can conjugate (Skippington & Ragan, 2011). Some promiscuous plasmids can cross the species barrier accepting one host as readily as another. Many R plasmids are conjugative. Non-conjugative plasmids, if they co-exist in a 'donor' cell with conjugative plasmids, ride from one bacterium to the other with the conjugative plasmids. The transfer of resistance by conjugation is significant in populations of bacteria that are normally found at high densities, as in the gut (Skippington & Ragan, 2011; Rusi *et al*., 2008). Transduction is a process by which plasmid DNA is enclosed in a phage and transferred to another bacterium of the same species. It is a relatively ineffective means of transfer of genetic material but is clinically important in the transmission of resistance genes between strains of *staphylococci* and of *streptococci*. A few species of bacteria can under natural conditions undergo transformation by taking up DNA from the environment and incorporating it into the genome by normal homologous recombination (Johnsborg *et al*., 2007).

2.12.2 Biochemical mechanisms of resistance to antibiotics

There are several forms of the transpeptidase enzymes present within any bacterial cell and these vary in their affinity for the different β-lactams. Differences in the relative proportions of these enzymes across bacterial species account in part for the variable susceptibility of these bacteria to different penicillins (MSF, 2008). For example, early strains of *Staphylococcus aureus* contained transpeptidase enzymes which had a high affinity for penicillin and were effectively inhibited (Lakshmi *et al.*, 2014). Penicillin resistant strains of *Staphylococcus aureus* acquired a transpeptidase enzyme called penicillin binding protein (PBP) 2a, which has a much lower affinity to penicillins(MSF, 2008). The presence of lower affinity transpeptidases is also a problem with *enterococci* and *pneumococci*.

Effectiveness of β-lactam antibiotics relies upon accessibility to its targets, extent of enzymatic inactivation and inhibition of target penicillin binding proteins by β-lactams (Gniadkowski, 2008). Enzymatic inactivation of antibiotics causes β-lactamases to preferentially catalyse irreparable hydrolysis of the amide bond in the β-lactam ring leading to inactive end products. The problem of β-lactamase become critical in 1960,when the widespread use of penicillin G led to an alarming increase of penicillin -resistant *Staphylococcal aureus* infections. At one stage 80% of all *Staphylococcal aureus* infections in hospitals were due to virulent, penicillin-resistant strains. Alarmingly, these strains were also resistant to all other available antibiotics (Chambers, 2001). The most important example of resistance caused by inactivation is that of the β-lactam antibiotics. The enzymes concerned are β-lactamases, which cleave the β-lactam ring of pencillins and cephalosporins. Cross-resistance between the two classes of antibiotics is not complete, because some β-lactamases have a preference for pencillins and some for cephalosporins (Lakshmi *et al*., 2014). The β-lactamases are enzymes which have mutated from transpeptidases and so they are quite similar in nature. For example, they have a serine residue in the active site and can open up the β-lactam ring of penicillin to form an ester link to the structure.

Unlike the transpeptidase enzyme, β-lactamases are able to hydrolze the ester link and shed the ring-opened penicillin. It is estimated that about 1000 penicillin molecules are hydrolysed per second. Most if not all Gram-negative bacteria produce β-lactamases, which makes them more resistant to penicillins, moreover, the β-lactamases released is trapped in theperiplasmic space between the cell-membrane and the outer membrane because it cannot pass through the latter (MSF, 2008; Lakshmi *et al.*, 2014). As a result, any penicillin managing to penetrate the outer membrane encounters a higher concentration of β-lactamase than it would with Gram-

positive bacteria (Queenan and Bush, 2007). There are various types of β-lactamase enzymes produced by Gram-negative and Gram-positive bacteria which vary in their substrate selectivity. Some are selective for penicillins (penicillinases), some for cephalosporins (cephalosporinases) and some for both penicillins and cephalosporins. The differing levels of enzyme and their differing affinities for different β-lactams account for the varying susceptibilities of Gram-negative bacteria of different β-lactams (Gniadkowski, 2008; Tharian *et al.*, 2013).

Gram-negative organisms can also produce β-lactamases, and this is a significant factor in their resistance to the semi-synthetic broad-spectrum β-lactam antibiotics. In these organisms, the enzymes may be coded by either chromosomal or plasmid genes. In the former case, the enzymes may be inducible, but in the latter they are produced constitutively (Tharian *et al.*, 2013). When this occurs, the enzyme does not inactivate the drug in the surrounding medium but instead remains attached to the cell wall, preventing access of the drug to membrane-associated target sites. Many of these β-lactamases are encoded by transposons, some of which may also carry resistance determinats to several other antibiotics (Gniadkowski, 2008) as shown in Figure 2.4.

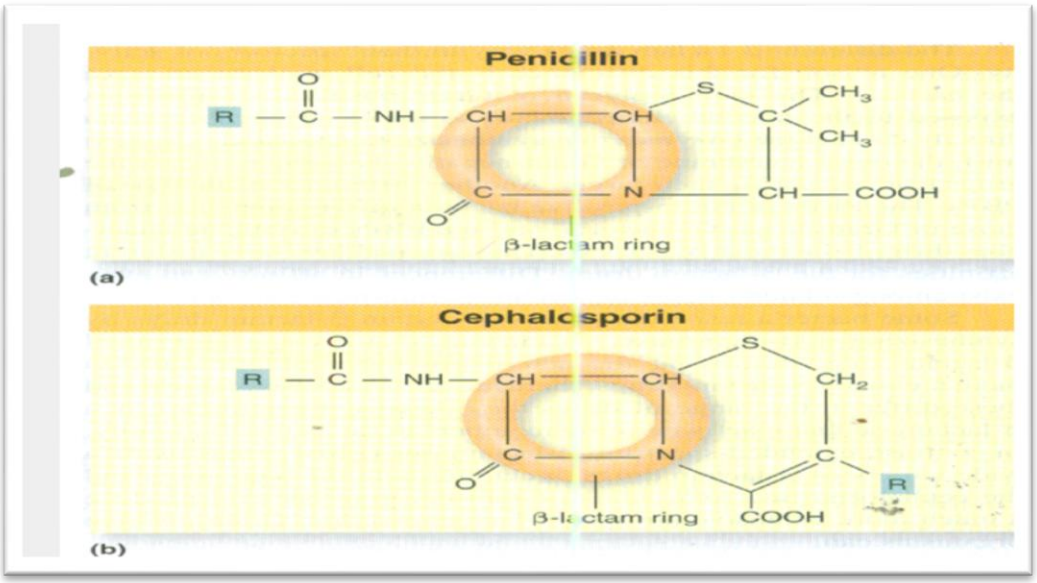

Figure 2.4: The β-lactam ring of penicillins and cephalosporins (Eugene *et al.*, 2012)

The core chemical structure of (a) penicillin (figure 2.4a) (b) cephalosporin (figure 2.4b): The β-lactam rings are marked by an orange circle. The groups vary among different penicillins and cephalosporins (Eugene *et al.,* 2012).

Chloramphenicol is usually inactivated by chloramphenicol acetyl transferase (CAT), an enzyme produce by resistant strains of both gram-positive and gram-negative organisms, the resistance gene being plasmid-borne. Chloramphenicol acetyl transferase covalently attaches an acetyl CoA to chloramphenicol which prevents chloramphenicol from binding to ribosomes (Li & Rufus, 2000). The biochemical mechanism of bacterial resistance to chloramphenicol is the inactivation by O-acetylation of the antibiotic, a reaction catalysed by chloramphenicol acetyl transferase with acetyl-CoA as acyl donor. Bacterial resistance to the antibiotic chloramphenicol, an inhibitor of the peptidyltransferase activity of prokaryotic ribosomes, is commonly conferred by the enzyme chloramphenicol acetyl transferase (Tharian *et al*., 2013). In gram-negative bacteria the enzyme is produced constitutively, resulting in levels of resistance that is fivefold higher than in gram-positive bacteria, in which the enzyme is inducible.

Aminoglycosides are inactivated by phosphorylation, adenylation or acetylation, and the requisite enzymes are found in both Gram-negative and Gram-positive organisms. The resistance genes are carried on plasmids, and also on transposons (Jana & Deb, 2005). The aminoglycoside-binding site on the 30S subunit of the ribosome may be altered by chromosomal mutation. A plasmid-mediated alteration of the binding site protein on the 50S subunit also underlies resistance to erythromycin, and decreased binding of flouroquinolones because of a point mutation in DNA gyrase A. An altered DNA-dependant RNA polymerase determined by a chromosomal mutation is reported to be the basis for rifamycin resistance (Lambert, 2005). In addition to acquiring

resistance to β-lactams susceptible to β-lactamase some strains of *staphylococcus aureus* have even become resistant to some antibiotics that are not significantly inactivated by β-lactamase (eg methicillin), because they express an additional β-lactam-binding protein coded for by a mutated chromosomal gene (Chan *et al*., 2011).

An important example of decreased drug accumulation is the plasmid-mediated resistance to tetracyclines which is encountered in both Gram-positive and Gram-negative bacteria. In this case, resistance genes in the plasmid code for inducible proteins in the bacterial membrane which promote energy-dependent efflux of the teracyclines, by use of proton motive force and hence resistance (Pfeifer, 2010). This type of resistance is common and has greatly reduced the therapeutic value of the tetracyclines in human and veterinary medicine. Resistance of *Staphylococci aureus* to erythmycin and other microlides, and to fluoroquinolones, is also brought about by energy-dependant efflux. There is also evidence of plasmid-determined inhibition of porin synthesis, which could affect these hydrophilic antibiotics from entering the bacterium through these water-filled channels in the outer membrane. Altered permeability as a result of chromosomal mutation involving the polysaccharide components of the outer membrane of Gram-negative organisms may confer enhanced resistance to ampicilin. Mutations affecting envelope components have been reported to affect the accumulation of aminoglycosides, β-lactams, chloramphericol and tetracycline (Pfeifer, 2010). Ciprofloxacin acetylating enzyme aac *(6)-1b-cr*, encodes an acetylase that modifies the amino-group of the piperazine ring of the flouroquinolones (Frederique *et al*., 2008).

Development of a pathway that by-passes the reaction inhibited by the antibiotic-Resistance to trimethoprim is the result of plasmid-directed synthesis of a *dihydrofolate reductase* with low or zero affinity for trimethoprim. It is transferred by transduction and may be spread by transposons. Sulfonamides resistance in many bacteria is plasmid-mediated and results from the production of a form of *dihydropteroate synthetase* with a low affinity for sulpfonamides but no change in affinity for para-aminobenzoic acid. Bacteria causing serious infections have been found to carry plasmids with resistance genes to both sulfonamides and trimethoprim (Skold, 2000). Sulfonamides are structural analogs and competitive antagonists of PABA, they inhibit normal bacterial utilization of PABA for the synthesis of folic acid,an important metabolite in DNA synthesis.Sulfonamides are bacteriostatic and inhibit growth and multiplication of bacteria, but do not kill them. Humans, in contrast to bacteria acquire folate through the diet (Madigan *et al*., 2012). In bacteria, sulfonamides act as competitive inhibitors of the enzyme *dihydropteroate synthetase* (DHPS), an enzyme involved in folate synthesis with a low affinity for sulfonamides but no change in affinity for PABA (Barbosa *et al.*, 2000; Gebreyes *et al.*, 2002; Graham, 2005; Poirel, 2010).

2.12.3 Presence of β-Lactamase enzymes

The problem of β-lactamase became critical in 1960, when the widespread use of penicillin G led to an alarming increase of penicillin-resistant *Staphylococcal aureus* infections. At one point 80% of all *Staphylococcal aureus* infections in hospitals were due to virulent, penicillin – resistant strains. Moreover, these strains were also resistant to all other available antibiotics (Chambers, 2001). The presence of β-lactamases is the most important mechanism by which bacteria gain resistance to penicillin. The β-lactamases are enzymes which have mutated from transpeptidases

and so they are quite similar in nature. For example, they have a serine residue in the active site and can open up the β-lactam ring of penicillin to form an ester link to the structure. Unlike the transpeptidase enzyme, β-lactamases are able to hydrolyse the ester link and shed the ring - opened penicillin. They do this so effectively that 1000 penicillin molecules are hydrolyzed per second (Higgins, 2007).

Most if not all Gram-negative bacteria produce β-lactamases, which makes them more resistant to penicillins, moreover, the β-lactamases released is trapped in the periplasmic space between the cell-membrane and the outer membrane because it cannot pass through the latter. As a result, any penicillin managing to penetrate the outer membrane encounters a higher concentration of β-lactamase than it would with Gram-positive bacteria.

There are various types of β-lactamase enzymes produced by Gram-positive and Gram-negative bacteria which vary in their substrate selectivity. Some are selective for penicillin (penicillinases), some for cephalosporins (Cephalosporinases) and some for both penicillins and cephalosporins. The differing levels of enzyme and their differing affinities for different β-lactams account for the varying susceptibilities of Gram-negative bacteria of different β– lactams (Cirz *et al.*, 2005).

2.12.4 Affinity of the transpeptidase enzyme to penicillin

There are several forms of the transpeptidase enzymes present within any bacterial cell and these vary in their affinity for the different β-lactams. Differences in the relative proportions of these enzymes across bacterial species account in part for the variable susceptibility of these bacteria to different penicillins (Chambers, 2001). For example, early strains of *staphylococcus aureus* contained transpeptidase enzymes which had a high affinity for penicillin and were effectively inhibited (Agafitei *et al*., 2010). Penicillin resistant strains of *staphylococcus aureus* acquired a transpeptidase

enzyme called penicillin binding protein (PBP) 2a, which has a much lower affinity to penicillins. The presence of lower affinity transpeptidases is also a problem with *enterococci* and *pneumococci.* Effectiveness of β-lactam antibiotics relies upon accessibility to its targets, extent of enzymatic inactivation and inhibition of target PBP's by β-lactams (MSF, 2008). Enzymatic inactivation of antibiotics causes β-lactamases to preferentially catalyse irreparable hydrolysis of the amide bond in the β-lactam ring leading to inactive end products.

2.12.5 Transport back across the outer membrane of Gram-negative bacteria

There are proteins in the outer membrane of some Gram-negative bacteria which are capable of pumping penicillin out of the periplasmic space thus lowering its concentration and effectiveness. The extent to which this happens varies from species to species and also depends on the structure of penicillin. This is known as an efflux process. *Efflux* pumps that comprise of cytoplasmic membrane proteins extrude antibiotics including β-lactams from the cytoplasm to the periplasmic space by use of proton motive force (Pfeifer, 2010). Porin loss or modification and alteration of lipopolysaccharide lead to decreased permeability of antimicrobials rendering the target site inaccessible (Agafitei *et al*., 2010). In *Streptococcus* and *Staphylococcus aureus,* alteration of target PBP reduces affinity for β-lactams hence conferring resistance to these antimicrobials (Li *et al*., 2009; Pfeifer, 2010). *aac(6')-1b-cr,* a ciprofloxacin acetylating enzyme gene encodes an acetylase that modifies the amino-group of the piperazine ring of the fluoroquinolones (Fre´de´rique *et al*., 2008; Agafitei *et al*., 2010).

2.13 Treatment options in β-lactamase mediated resistance

The emergence of plasmid-mediated and thus transferable quinolone resistance determinants have been shown to involve the pentapeptide repeat proteins (Martinez *et al*., 1998). Quinolone resistance protein (Qnr) interacts with DNA gyrase and topoisomerase IV to prevent quinolone inhibition (Sang, 2007). The DNA gyrase is responsible for introducing negative supercoils into DNA and for relieving topical stress arising from the translocation of transcription and replication complexes along the DNA. Qnr proteins protect DNA from quinolone binding and compromise the efficacy of quinolones such as nalidixic acid. They have been identified worldwide in a variety of enterobacterial species and were often associated with expanded-spectrum beta lactamases (ESBLs) (Robicsek *et al.,* 2006). Alternative options for the treatment of infections caused by carbapenem-resistant strains of enterobacteriaceae are limited. Current strategies include colistin, fosfomycin, tigecycline and tenocillin. Although *in-vitro* testing suggests strong activity for each of those drugs against a large proportion of carbapenem-resistant strains of *nitrobacteria*, clinical evaluations do not provide strong evidence of equivalent or improved outcome (Schultsz, *et al.,* 2012).

The ESBLs are plasmid mediated and they confer resistance to oxyimino-cephalosporins (Cefotaxime, ceftriaxone, ceftazidime) and to monobactam (aztreonam) but are not active against cephamycins and carbapenems. Carbapenems therefore have been recommended as the drugs of choice for serious infections with ESBL producers. Studies done elsewhere have shown that ESBL producers are susceptible to imipenem (Subha *et al.,* 2002). Treatment with non-β-lactam containing regimens compels the use of quinolones especially in bacteremia, intra-abdominal infections and hospital acquired pneumonia (Patterson, 2005). Aminoglycosides can

also be used as alternatives, preferably in serious infections as injectables such as gentamicin although both quinolones and aminoglycosides show co-resistance with β-lactam antibiotics (Nathisuwan *et al.*, 2001).

Carbapenems (meopenem, imipenem and ertapenem) are relied upon against ESBL-associated infections because they demonstrate in-activation by ESBL *in-vitro*. A clinical study in adults with uncomplicated urinary tract infection caused by ESBL producing *E. coli* showed good treatment outcomes with the use of oral fosfomycin but there is limited use in paediatric patients (Falagas *et al*., 2010). Tigecycline is a good treatment option in ESBL mediated resistance but should be used in older children (above eight years old), adolescents and adults. This is because a study showed that 99% of ESBL and carbapenemase producing *E.coli* were susceptible to tigecycline (Kelesidis *et al*., 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study site was the government hospital located at Mukuru Kwa Njenga slum in Nairobi County. The Hospital serves the residents of Kwa Reuben, Kwa Njenga, Kayaba and Sinai slums along Nairobi River. It is situated within the Industrial area of Nairobi city lying at co-ordinates 1°18'33"S 36°48'12"E (KBS, 2009). Mukuru Kwa Njenga is a slum in the East of Nairobi, the capital of Kenya. It belongs to Embakasi Constituency. It is one of the largest slums in Nairobi. Among other major slums in Nairobi are Korogocho, Kibera and Mathare. The population of the slum exceeds 100,000. There have been cholera deaths in 2009 (WHO, 2010).

(Figure 2).

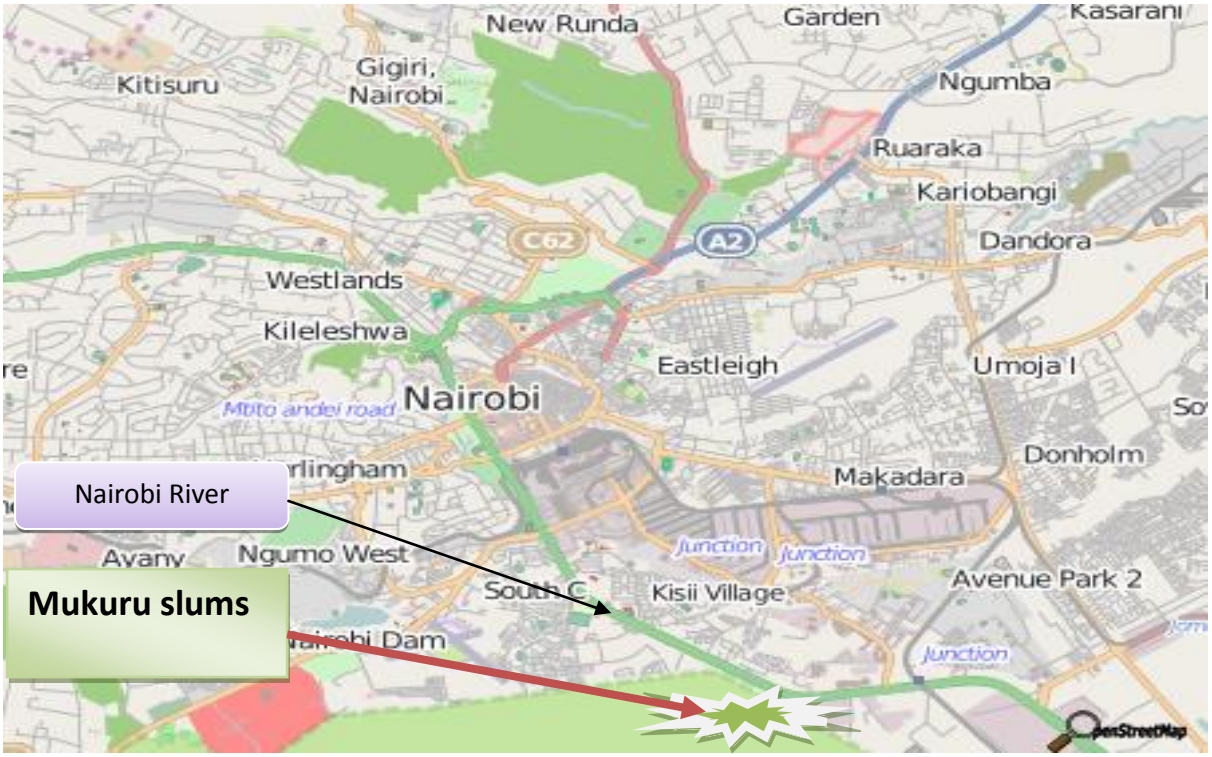

Figure 3.1: A Google map of Nairobi showing Mukuru slums

3.2 Study design and population

The study employed a cross-sectional laboratory based design (Fischer *et al.,* 1986). The study population comprised of children who were five years and below, attended to at the government health facility in Mukuru Kwa Njenga.

3.3 Participants' selection

The guardian/parent was requested to fill and sign the consent form after explaining the purpose of the study to them. Those who accepted their children to participate in the study indeed signed the forms.

3.3.1 Inclusion criteria

- Children under five years verified by child welfare clinic records.
- Children who had diarrhoea or history of diarrhoea i.e. passage of loose or watery stool more than three times a day (WHO, 1988).
- HIV negative.
- Children whose parents/guardians accepted to sign informed consent form.

3.4 Sample size determination

The sample size was determined using the formula below according to Fischer *et al.* (1986).

$$n = \frac{Z^2 \times P(1-p)}{d^2} \quad \text{..... [1]}$$

Where n is the sample size, z is the confidence interval at 95% and p is the prevalence got from Kenya Demographic health survey (KDHS), 2010. d is the margin of error at 5%

$$n = \frac{1.96^2 \times 0.17\ (0.83)}{0.05^2} \quad \text{.....}$$

[2]

Final sample size was 178 stool samples/ anal swabs

N = 178; 190 participants were included in this study. The study sampled 190 children to take care of specimen that might get spoilt on the way before reaching the laboratory for processing.

3.5 Sample collection

Stool samples were collected into sterile, wide-mouthed, screw cap containers and preserved in cool boxes. Anal swabs were collected from participants who were unable to produce stool samples and the specimens were labelled and assigned unique code numbers during the time of sample collection. Specimens once collected were taken to the centre for microbiology research laboratory (CMR)-KEMRI within the shortest time possible for processing.

3.6 Specimen processing

3.6.1 Culturing

The specimens were enriched in selenite F media overnight at 37^{o}C. After enrichment, inoculations were done both on MacConkey Agar and Shigella Salmonella Agar (Oxoid, Basingstoke, United Kingdom). Lactose fermenters and non-lactose fermenters that had grown colonies were inoculated onto biochemically impregnated API 20E strips (BioMerieux, Basingstoke, United Kingdom) for identification.

3.6.2 Biochemical tests

3.6.2.1 Triple sugar iron agar (TSI)

Colonies were selected on plate using a sterile straight wire loop. The centre of the colony was lightly touched and prepared TSI medium were inoculated by stabbing the butt and streaking the slants. These were then incubated at 37^{o}C for 24 hours (Cowan and Steel, 2002).

3.6.2.2 Indole test

The bacteria isolated were sub-cultured in nutrient broth and incubated for 24 hours. About 3 drops of Kovac's indole reagent was added and mixed gently (Cheesbrough, 2005).

3.6.2.3 Urease test

Urea agar was inoculated heavily over the entire surface of the slants in bijou bottles, incubated at 37^{o}C for 24 hours.

3.6.2.4 Citrate utilization test

Simmons citrate slopes were prepared in bijou bottles. The slopes were then stabbed and incubated at 37^{o}C for 48 hours.

3.6.2.5 Motility test

A sterile straight wire loop was used to inoculate motility indole urea media with bacterial isolate and incubated overnight at 37^{o}C. Motility was shown by diffused turbidity in the medium (Cheesbrough, 2005).

N/B: All these tests mentioned above were used for the purpose of identification of Enterobacteriaceae. The results were either positive or negative for a particular entero pathogen.

3.7 Antibiotic sensitivity testing

All the enterobacteriaceae isolated and identified in section 3.6 were tested for various antibiotic sensitivities following Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2011). Antibiotic sensitivity tests were performed using antibiotic discs a method referred to as disk diffusion method (Cypress diagnostics, Langdorp, Belgium) on Mueller Hinton agar (Oxoid, Basingstoke, United Kingdom). The *E. coli* ATCC 25922 was included as a control strain on each test occasion. Sensitivity tests were interpreted using the CLSI guidelines. Twelve (12) different types of antibiotics presented in Table 3.1 were tested. The indicated concentrations were based on the minimum inhibitory concentrations (MIC) as per CLSI standards. The antibiotics are commercially prepared and supplied in different concentrations (10 µg and 30 µg). The results were interpreted according to CLSI (6.0mm-9.5mm is resistant, 10-14.5mm is intermediate while 15mm and above inhibition zones were interpreted as sensitive) (CLSI, 2011). Percentage sensitivity of isolates to the drugs were calculated

by taking the total number of isolates sensitive to a certain drug divided by the total number of strains isolated per organism multiplied by hundred.

B-lactam/β-lactamase inhibitor combinations included were; amoxicillin/clavulanic acid (AMC, comprising amoxicillin 20 µg and clavulanic acid 10 µg). Break points were calculated taking into account different criteria and were mainly influenced by pharmacokinetic/pharmacodynamic (PK/PD) parameters as recommended by the CLSI guidelines (CLSI, 2011).

Table 3.1: Antibiotic panels used in the study

Drugs	**Abbreviations**	**Dosage in micrograms (µg)**
*Amoxicillin/ Clavulanic Acid,	AMC	10
Sulphamethoxazole/Trimethoprim	SXT	30
Ceftazidime	CAZ	30
Tetracycline	TE	30
Ampicillin	AMP	30
Ciprofloxaxin	CIP	30
Nalidixic Acid	NA	30
Chloramphenical	C	30
Gentamicin	CN	30
Ceftriaxone	CRO	30
Kanamycin	K	30
Streptomycin	S	30

*Amoxicillin/ Clavulanic Acid – 10 µg commercially prepared

3.8 Data management and analysis

Data was recorded in Microsoft excel and protected using a password. It was entered into SPSS version 20 (SPSS, Inc. Chigago, IL) and was kept confidential before, during and after analysis. Data on age, gender and residence as well as antibiotic sensitivity test were subjected to Student T test and χ^2 analysis at 95% confidence (0.05), level of significance.

3.9 Permission to carry out the study

The study was nested within a bigger study which was funded by The Centre for Disease Control and Prevention in collaboration with the Kenya Medical Research Institute, Opportunistic infection laboratories and the Ministry of Health Central Microbiology Laboratories. Permission to carry out the study was granted by the investigators of the main study (Appendix 2). The Board of Post Graduate Studies, University of Eldoret also gave permission for the study to be carried out.

CHAPTER FOUR

RESULTS

4.1 Participants characteristics

A total number of 190 children below the age of five years presenting with diarrhoea in the Government health facility in Mukuru Kwa Njenga slum participated in this study. The mean age of the participants was 24.21 months with the youngest child being 3 months and the oldest child being 72 months. More children who participated in the study were less than 40 months in age. The childrens' ages were skewed to the right of the normal curve (Figure 4.1). The mean age of the children was twice more than the median age with a standard deviation of 17.62. (Mean 24.21 ± 17.619, and N 190). In this study there was significant association ($p<0.05$) between the age groups and diarrhoea among the participants.

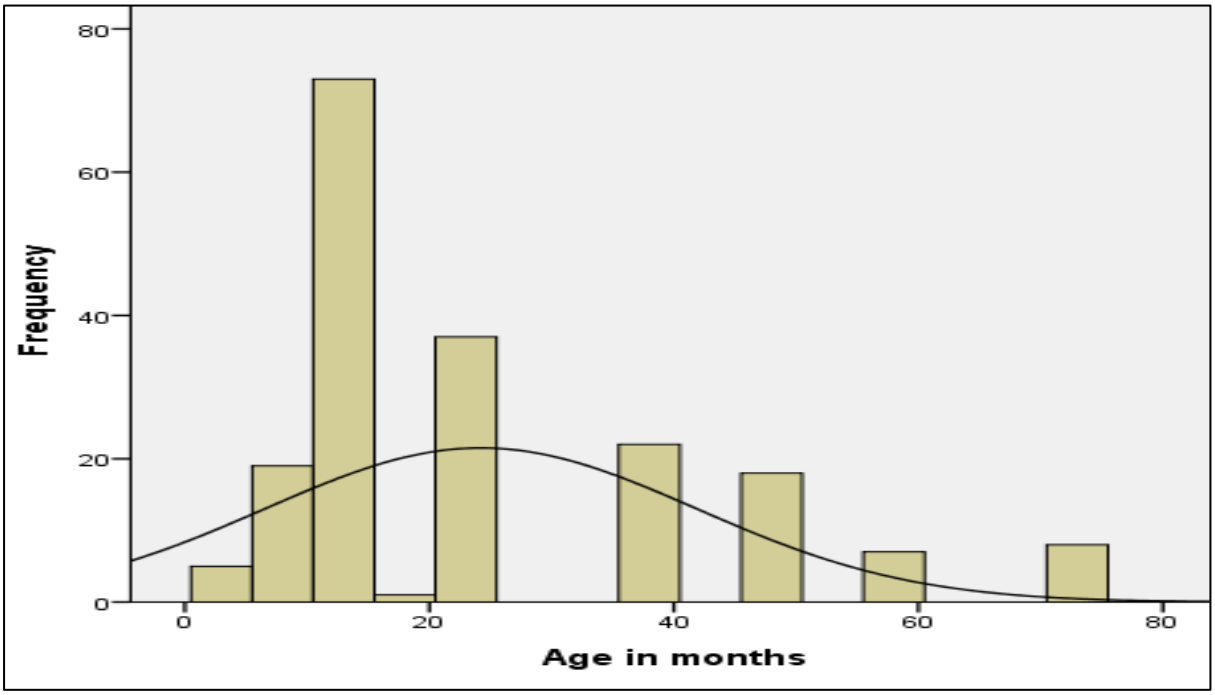

Figure 4.1: Distribution curve of participants ages

In this study the female children were 105(55.26%) and the males were 85(44.74%). Female children were 3.7 months older than the male children with a standard difference error mean of 0.02 months as shown in Table 4.1.

Table 4.1: Analysis of age of the participants

Gender	N	Percentage	Mean age	Age Std. Deviation	Age Std. Error Mean	Std. difference	P-value
Male	85	44.74%	16.414	1.780	16.414	0.02	0.00
Female	105	55.26%	18.449	1.800	18.449		

The analysis of the participants' ages verses gender revealed that there was no significant difference. The $t_{(186)}$ value was 1.458 with probability, $p = 0.146 > 0.05$, the p-value was more than 0.05 therefore there was no association between the gender in relation to diarrhoea in this study. The male participants were 85(45%) while the female were 105(55%) as shown in Figure 4.2. There was significant association between age and diarrhoea in this study (p=0.01).

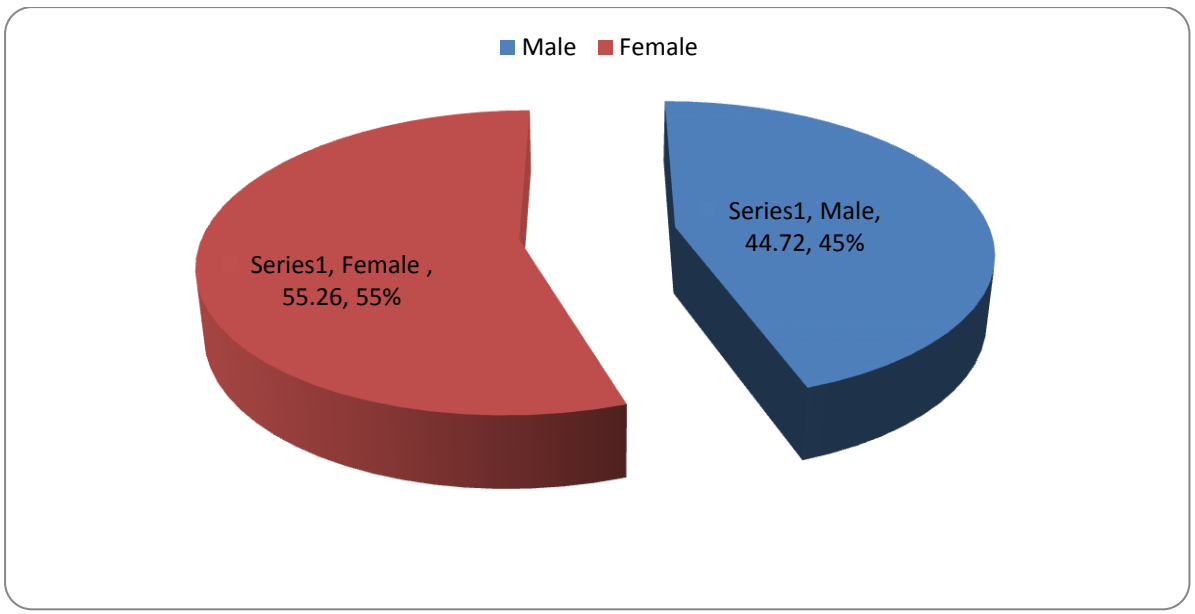

Figure 4.2: Gender of the participants

The participants attended to at Mukuru Kwa Njenga government health facility were noted to be residents of four neighbouring slums namely; Mukuru Kwa Njenga, Mukuru Kwa Reuben, Mukuru Kayaba and Sinai. The majority of the participants were from Mukuru Kwa Njenga 61(32.6%) followed by Mukuru Kwa Reuben 57(30.5%) then Sinai 35(18.9%) and the least were from Mukuru Kayaba 33(17.9%). Mukuru Kwa Njenga had the highest number of female children (35.2%) while Mukuru Kwa Reuben had the highest number of male children (32.9%). The p-values were greater than 0.05 hence there was no significance difference between the participants from different areas of residence (χ^2 =5.41, p= 0.144) as shown in Figure 4.3.

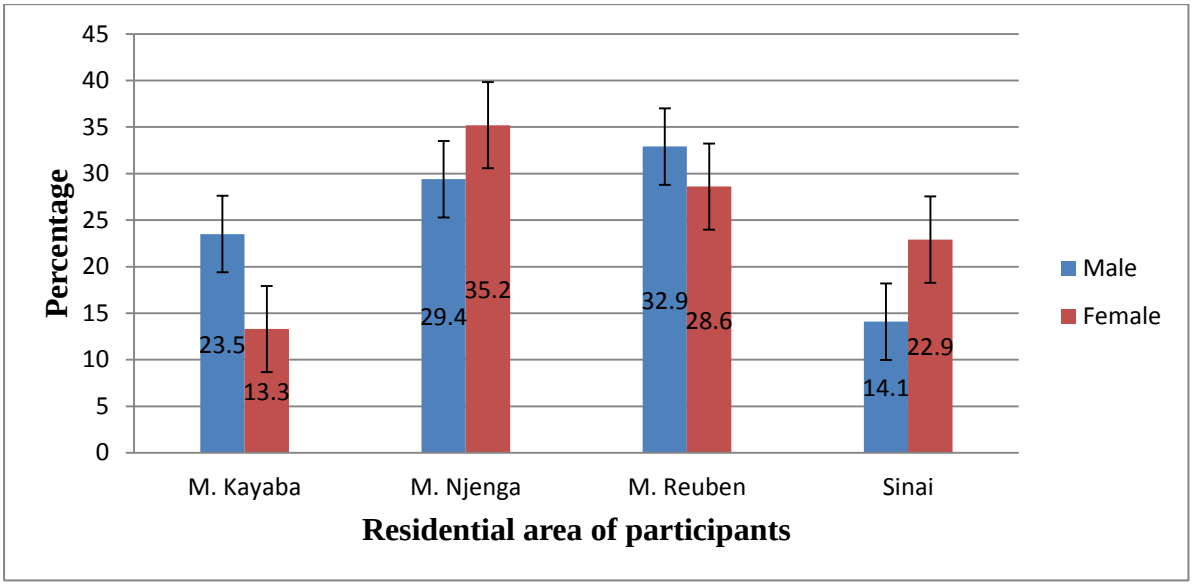

Figure 4.3: Residence of study participants

4.2 Isolation and identification of the bacteria

The prevalence of bacteria isolated from the study were as follows: *Escherichia coli* (87.4%), *Salmonella spp* (4.2%), *Shigella sonnei* (4.2%), *Salmonella typhi* (2.6%),*and Shigella dysenteriae* (1.6%) as shown in Figure 4.4.

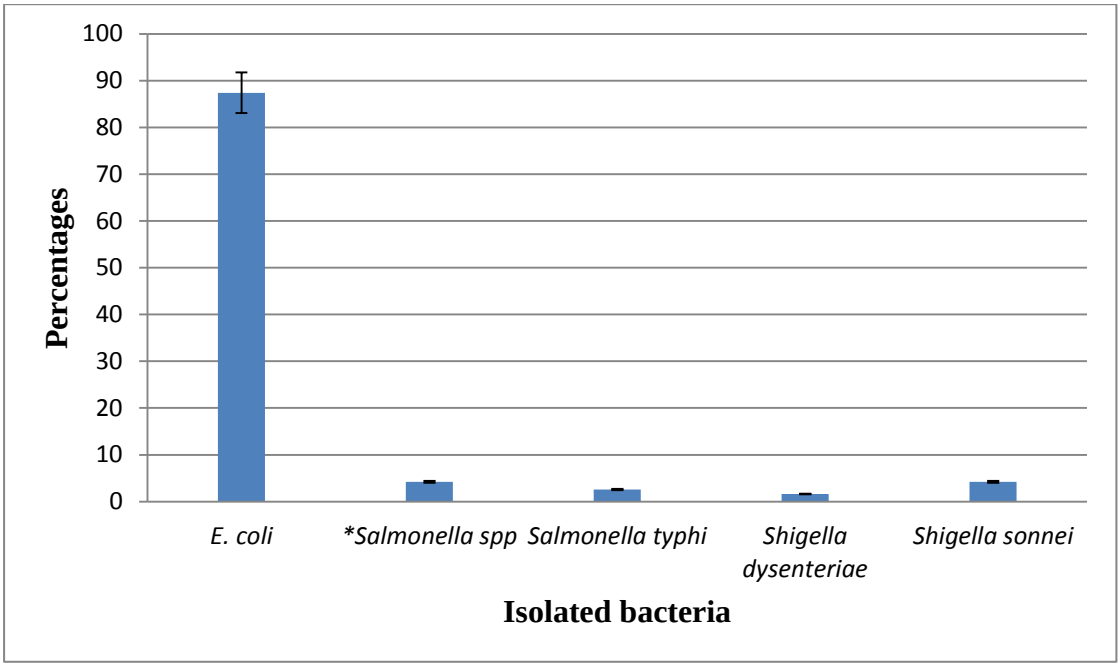

Figure 4.4: Bacteria species isolated from the stool samples

At Mukuru Kwa Njenga *E. coli* was isolated from female children (35.2%) than male children (29.4%) the isolates were uniform in both genders. At Mukuru Kwa Reuben the trend was similar in that more *E. coli* was also isolated from female children (17.5%) than from male children (13.0%). *Salmonella spp* were 4.9% from female children and 0.0% from male children while *S. typhi* were more from male children (3.0%) compared to female children (0.5%). At Sinai the percentage isolates from both male and female children were almost equal (9.0% and 9.9%, respectively). *Shigella sonnei* were more from (3.2) female than male children (1.0%). At Kayaba *E. coli* isolates were more from female (10.7%) than from male children (7.2%). *S typhi* were 1.6% in females and 0.0% in males while the rest were 0.0%. There was no

significant association between the gender and percentage isolates (p>0.05). There was also no significant association between the prevalence of the isolates and the area of residence of the children (χ^2 =2.23, p=0.693). The results are as shown in Table 4.2.

Table 4.2: Prevalence of bacteria isolated by gender and residence of participants

Residence	**Isolated *spp***	**Male (% isolates)**	**Female (% isolates)**	**χ^2 (p-value)**
M. Njenga	*E. coli*	29.4	35.2	2.23 (0.693)
	**Salmonella spp*	1.0	1.1	
	S. typhi	0.0	0.0	
	S. dysenteriae	1.5	1.5	
	Shigella sonnei	1.1	1.0	
M. Reuben	*E. coli*	13.0	17.5	
	**Salmonella spp*	0.0	4.9	
	S. typhi	3.0	0.5	
	S. dysenteriae	1.0	1.1	
	Shigella sonnei	2.9	2.0	
Sinai	*E. coli*	9.0	9.9	
	**Salmonella spp*	2.0	2.2	
	S. typhi	1.6	1.0	
	S. dysenteriae	0.0	1.6	
	Shigella sonnei	1	3.2	
M. Kayaba	*E. coli*	7.2	10.7	
	**Salmonella spp*	0.0	0.0	
	S. typhi	0.0	1.6	
	S. dysenteriae	0.0	0.0	
	Shigella sonnei	0.0	0.0	

**Salmonella spp*- other *Salmonella* isolates which were not identified to species level, *Spp* - species, χ^2 – Chi square test, p-value- level of significance (0.05)

4.3 Antibiotic sensitivity testing

4.3.1 Response of isolates to Amoxicillin/ clavulanic acid

The isolated *E. coli* had varied response to Amoxicillin/ Clavulanic acid.Over thirty six percent (36.7%) of the isolates were sensitive, 15.7% intermediary and 47.6% were resistant. One hundred percent (100%) of the *Salmonella spp* were resistant. Sixty percent (60%) of *Salmonella typhi* were sensitive while 20% each were both intermediary and resistant (Table 4.3). Among the *Shigella dysenteriae* isolates, none were sensitive, 66% were intermediary and 33.3% were resistant. For *Shigella sonnei* 12.5% were sensitive, 37.5% were intermediate sensitive while 50% were resistance. In total, 34.2% were susceptible, 16.3% were intermediate while 49.5% were resistant to Amoxicillin/clavulanic acid. Isolates suscephtibility to Amoxicillin/ Clavulanic Acid chi square test had no significant association in this study because all the p-values ($p>0.05$) are more than 0.05 (Table 4.3).

Table 4.3: Amoxicillin/ Clavulanic Acid sensitivity test

Isolate	Amoxicillin/ Clavulanic Acid	Percentage (%)	χ^2 (p-value)
E. coli	Sensitive	36.7	0.668
	Intermediate	15.7	
	Resistant	47.6	
Salmonella spp	Resistant	100.0	
Salmonella typhi	Sensitive	60.0	0.659
	Intermediate	20.0	
	Resistant	20.0	
Shigella dysenteriae	Intermediate	33.3	0.386
	Resistant	66.7	
Shigella sonnei	Sensitive	12.5	0.641
	Intermediate	37.5	
	Resistant	50.0	
Total	Sensitive	34.2	0.700
	Intermediate	16.3	
	Resistant	49.5	

*** *Salmonella sp*- were only resistant, *Shigella dysenteriae*- were intermediate sensitive and resistant**

When compared in terms of residence and gender the reaction of Amoxicillin/ Clavulanic Acid to the isolates were as follows; the isolated *E. coli* showed varied sensitivity. 35.3% of the *E. coli* isolates from male children were sensitive, 17.6% were intermediate while 47.1% were resistant. One hundred percent of *Salmonella sp, Salmonella typhi, Shigella dysenteria* and *Shigella sonnei* were resistant from male children.

For the isolates from female children; *E. coli* isolates were 30.8% sensitive, 23.1% intermediate and 46.2% resistant. *Shigella sonnei* isolated from female children from this area were 100% resistant. From Mukuru Kwa Njenga; *E. coli* isolates from male children were 20.8% sensitive, 16.7% intermediate and 62.5% resistant while *Shigella sonnei* were 100% resistant. *E. coli* isolates from female children were 33.3% sensitive, 12.1% intermediate and 54.5% resistant. *Salmonella sp* and Salmonella *typhi* isolates were 100% resistant. From Mukuru Kwa Reuben; *E. coli* isolates from male children were 38.5% sensitive, 26.9% intermediate and 34.6% resistant. *Salmonella* sp were 100% resistant while *Shigella dysenteriae were* 100% intermediate.

The *E. coli* isolates from female children were 44.8% sensitive, 13.8% intermediate and 41.4% resistant while *Shigella sonnei* were 100% sensitive. From Sinai; *E. coli* isolates were 66.7 sensitive and 33.3% were resistant. *Salmonella* sp were 100% resistant *S. typhi* were 100% sensitive while *S. sonnei* were 100% intermediate. From female children, *E. coli* isolates were 40.0% sensitive, 6.7% intermediate and 53.3% resistant. *Salmonella* sp were 100% resistant. *Salmonella typhi* were 66.7% sensitive and 33.3% intermediate. *Shigella dysenteria* were 100% resistant while *S. sonnei* were 66.7% intermediate and 33.3% resistant to Amoxicillin/ Clavulanic acid. The p-values were 0.82, 0.77, 0.37, 0.02, 0.58, 0.55, 0.55 and 0.12 hence there was no significant

association between the variables (age, gender and area of residence) verses the drug reaction against the isolates except Sinai which had a p-value less than 0.05 as shown in Table 4.4.

4.3.2 Response of isolates to Sulphamethoxazole/trimethoprim

The isolated *E. coli* had varied response to Sulphamethoxazole/ trimethoprim. 52.4% of the *E. coli* isolates were sensitive, 6.6% were intermediate while 41.0 % were resistant. 12.5% of the *Salmonella spp* isolates were sensitive, 62.5% were intermediate and 25.0% were resistant to the drug.

Table 4.4: Response of isolates to Amoxicillin/ Clavulanic acid by gender and residence

Gender	Residence	Isolate	Sensitive (%)	Intermediate (%)	Resistant (%)	p-value
Male	Mukuru Kayaba	*E.coli*	35.3	17.6	47.1	0.82
		S. spp			100.0	
		S. dysenteriae			100.0	
		S. sonnei			100.0	
	Mukuru Kwa Njenga	*E.coli*	20.8	16.7	62.5	0.77
		S. sonnei			100.0	
	Mukuru Kwa Reuben	*E.coli*	38.5	26.9	34.6	0.37
		S. spp			100.0	
		S. dysenteriae		100.0		
	Sinai	*E.coli*	66.7		33.3	0.02
		S. spp			100.0	
		S. typhi	100.0			
		S. sonnei		100.0		
Female	Mukuru Kayaba	*E.coli*	30.8	23.1	46.2	0.58
		S. sonnei			100.0	
	Mukuru Kwa Njenga	*E.coli*	33.3	12.1	54.5	0.55
		S. spp			100.0	
		S. typhi			100.0	
	Mukuru Kwa Reuben	*E.coli*	44.8	13.8	41.4	0.55
		S.sonnei	100.0			
	Sinai	*E.coli*	40.0	6.7	53.3	0.12
		S. spp			100.0	
		S. typhi	66.7	33.3		
		S. dysenteriae			100.0	
		S. sonnei		66.7	33.3	

Key: *S. Spp- Salmonella species* (Salmonella which were not identified to species level), *S. typhi – Salmonella typhi, E.coli- Escherichia coli, S.sonnei- Shigella sonnei, S. dysenteriae- Shigella dysenteriae*

Hundred percent (100%) of the *Salmonella typhi* isolates were sensitive to this drug (SXT). 66.7% of *Shigella dysenteriae* isolates sensitive, 0.0% were intermediate and 33.3% were resistant. Of *Shigella sonnei*, 25% were sensitive while both intermediates and resistant were 37.5%. In total, all the isolates were 51.1% sensitive, 10% intermediate and 38.9% resistant (Table 4.5). Data analysis showed that there

was no significant association between the drug response and the isolated bacteria (p=0.784, 0.237, 0.083, 0.315 and 0.749).

Table 4.5: Response of isolates to Sulphamethoxazole/trimethoprim

Isolate	Sulphamethoxazole/ trimethoprim	Percentage (%)	p-value
E. coli	Sensitive	52.4	0.784
	Intermediate	41.0	
	Resistant	12.5	
Salmonella spp	Sensistive	6.6	0.237
	Intermediate	62.5	
	Resistant	25.0	
Salmonella typhi	Sensitive	100.0	
Shigella dysenteriae	Sensitive	66.7	0.083
	Resistant	33.3	
Shigella sonnei	Sensitive	25.0	0.315
	Intermediate	37.5	
	Resistant	37.5	
Total	Sensitive	51.1	0.749
	Intermediate	10.0	
	Resistant	38.9	

* ***Salmonella typhi* – were 100% sensitive, *Shigella dysenteriae*- had no intermediate sensitivity**

When compared by gender and residence, all the isolates had varied response to Sulphamethoxazole/ trimethoprim. The *E. coli* isolates from male children at Mukuru Kayaba were 64.7% sensitive and 35.3% intermediate. Hundred percent of *Salmonella sp* and *S. sonnei* were intermediate while *S. dysenteriae* were sensitive from male children. *E. coli* isolates from female children at Mukuru kayaba were 46.2% sensitive, 23.1% were intermediate and 30.8% of the *E. coli* isolates were resistant while 100% of *S. sonnei* isolates were intermediate. From Mukuru Kwa Njenga; *E. coli* isolates from male children were 45.8% sensitive, 4.2% were intermediate and 50.0% resistant while 100% of *S. sonnei* isolates were resistant. From the female children, the results showed that 51.5% of the *E. coli* isolates were sensitive and 48.5% were resistant. Of *Salmonella sp* from female children, 33.3% were intermediate and 66.7% were resistant while 100% of *S. typhi* were sensitive.

From Mukuru Kwa Reuben, isolates from male children showed that 50.0% of *E. coli* was sensitive, 15.4% were intermediates and 34.6% were resistant. Hundred percent of *Salmonella sp* and *S. dysenteria* were sensitive. 51.7% of *E. coli* isolates from female children were sensitive, 6.9% were intermediate and 41.4% were resistant while 100% of *S. sonnei* isolates were sensitive. From Sinai; 66.7% of the *E. coli* isolates were sensitive and 22.2% resistant. Hundred percent of the *Salmonella sp* and *S. typhi* were intermediate and sensitive, respectively while 100% of *S. sonnei* isolates were resistant. From the female children, 46.7% of *E. coli* isolate were sensitive, 6.7% were intermediate and 46.7% were resistant. Hundred percent of *Salmonella sp* and *S. typhi* were intermediate and sensitive, respectively. Hundred percent of *S. dysenteria* isolates from female children were resistant. Of the *S. sonnei*, 33.3% were sensitive, intermediate and resistant. The p-values were 0.00, 0.62, 0.76, 0.02, 0.01, 0.26, 0.64 and 0.03 respectively. Four variables had significant association with the drug tested ($p \leq 0.05$) while four had no significant association between them and the drug Sulphamethoxazole/ trimethoprim ($p > 0.05$) as shown in Table 4.6.

4.3.3 Response of isolates to Ceftazidime

All the isolated bacteria had varied response to Ceftazidime in this study. The results revealed that 93.4% of the E. coli isolates were sensitive, 3.0% were intermediate sensitive and 3.6% were resistant. Hundred percent of *Salmonella spp, Salmonella typhi, Shigella dysenteriae* and *Shigella sonnei* isolates sensitive to the drug. Susceptibility to Ceftazidime was 94.2% sensitive, 2.6% intermediate sensitive while 23.2% were resistant (Table 4.7).

There was no significant association between the isolated bacteria and their response to ceftazidime (p=0.494. 0.516).

Table 4.6: Response of isolates to Sulphamethoxazole/ trimethoprim by gender and residence

Gender	Residence	Isolate	Sensitive (%)	Intermediate (%)	Resistant (%)	p-value
Male	Mukuru Kayaba	*E.coli*	64.7		35.3	0.00
		S. spp		100.0		
		S.dysenteriae	100.0			
		S. sonnei		100.0		
	Mukuru Kwa Njenga	*E.coli*	45.8	4.2	50.0	0.62
		S. sonnei			100.0	
	Mukuru Kwa Reuben	*E.coli*	50.0	15.4	34.6	0.76
		S. spp	100.0			
		S.dysenteriae	100.0			
	Sinai	*E.coli*	77.8		22.2	0.02
		S. spp		100.0		
		S. typhi	100.0			
		S. sonnei			100.0	
Female	Mukuru Kayaba	*E.coli*	46.2	23.1	30.8	0.01
		S. sonnei		100.0		
	Mukuru Kwa Njenga	*E.coli*	51.5		48.5	0.26
		S. spp		33.3	66.7	
		S. typhi	100.0			
	Mukuru Kwa Reuben	*E.coli*	51.7	6.9	41.4	0.64
		S. sonnei	100.0			
	Sinai	*E.coli*	46.7	6.7	46.7	0.03
		S. spp		100.0		
		S.typhi	100.0			
		S.dysenteriae			100.0	
		S. sonnei	33.3	33.3	33.3	

Key: *S. Spp- Salmonella species* (Salmonella which were not identified to species level), *S. typhi – Salmonella typhi, E.coli- Escherichia coli, S.sonnei- Shigella sonnei, S. dysenteriae- Shigella dysenteria*

Table 4.7: Response of isolates to Ceftazidime

Isolate	Ceftazidime	Percentage (%)	p-value
E. coli	Sensitive	93.4	0.494
	Intermediate	3.0	
	Resistant	3.6	
Salmonella spp	Sensitive	100.0	
Salmonella typhi	Sensitive	100.0	
Shigella dysenteriae	Sensitive	100.0	
Shigella sonnei	Sensitive	100.0	
Total	Sensitive	94.2	0.516
	Intermediate	2.6	
	Resistant	3.2	

* ***Salmonella, Salmonella typhi, Shigella dysenteriae*** **and** ***Shigella sonnei*** **– were only sensitve**

When compared in terms of gender and residence, the results were as follows: In Mukuru Kayaba, 94.1% of E. coli isolates were sensitive and 5.9% resistant. Hundred percent of *Salmonella sp*, *Shigella dysenteria* and *Shigella sonnei* were sensitive. Isolates from female children from Mukuru kayaba showed that 92.3% of *E. coli* were sensitive and 7.7% were intermediate. Hundred percent of *S. sonnei* isolates were sensitive. From Mukuru Kwa Njenga 91.7% of *E. coli* isolates from male children were sensitive while 8.3% were resistant. Hundred percent of *S. sonnei* isolates from male children were sensitive. From female isolates 97.0% of *E. coli* isolates were sensitive and 3.3% were intermediate. Hundred percent of *Salmonnela* sp. and *S. typhi* isolates from female children were sensitive. All the isolates from the male children from Mukuru Kwa Reuben were sensitive. 86.2% of *E. coli* from female children at Mukuru Kwa Reuben were sensitive, 3.4% were intermediate and 10.3% were resistant while 100% of *S. sonnei* isolates were sensitive. From Sinai, 88.9% of *E. coli* isolates from male children were sensitive, 11.1% were intermediate while the rest were 100% sensitive. From female children, 93.3% of *E. coli* isolates from Sinai were sensitive and 6.7% were intermediate while all the rest were 100% sensitive to the antibiotic Ceftazidime. There was no significant association beween either the gender or area of residence where the bacteria was isolated from and the response to Ceftazidime (Table 4.8).

Table 4.8: Response of isolates to Ceftazidime by gender and residence

Gender	Residence	Isolate	Sensitive (%)	Intermediate (%)	Resistant (%)	p-value
Male	Mukuru	*E.coli*	94.1		5.9	0.98
	Kayaba	*S. spp*	100.0			
		S.dysenteriae	100.0			
		S. sonnei	100.0			
	Mukuru	*E.coli*	91.7		8.3	0.76
	Kwa	*S. sonnei*	100.0			
	Njenga					
	Mukuru	*E.coli*	100.0			
	Kwa	*S. spp*	100.0			
	Reuben	*S.dysenteriae*	100.0			
	Sinai	*E.coli*	88.9	11.1		0.95
		S. spp	100.0			
		S. typhi	100.0			
		S. sonnei	100.0			
Female	Mukuru	*E.coli*	92.3	7.7		0.77
	Kayaba	*S. sonnei*	100.0			
	Mukuru	*E.coli*	97.0	3.0		0.94
	Kwa	*S. spp*	100.0			
	Njenga	*S. typhi*	100.0			
	Mukuru	*E.coli*	86.2	3.4	10.3	0.92
	Kwa	*S. sonnei*	100.0			
	Reuben					
	Sinai	*E.coli*	93.3	6.7		0.96
		S. spp	100.0			
		S.typhi	100.0			
		S.dysenteriae	100.0			
		S. sonnei	100.0			

Key: *S. Spp- Salmonella species, S. typhi – Salmonella typhi, E.coli- Escherichia coli, S.sonnei- Shigella sonnei, S. dysenteriae- Shigella dysenteriae*

4.3.4 Response of isolates to Tetracycline

All the bacteria isolated had varied response to the drug Tetracycline in this study. The results showed that 63.9% of *E. coli* isolates were 63.9% sensitive, 0.6% intermediate and 35.5% were resistant to the drug. Exactly 12.5% of the *Salmonella spp* isolates were sensitive and 87.5% were resistant to the drug. Eighty percent of *Salmonella typhi* sensitive while 20% were resistant. Of *Shigella dysenteriae* isolates, 66.7% were sensitive while 33.3% were resistant. About 25.0% of *Shigella sonnei* were sensitive and 75.0% were resistant. Therefore all the isolates tested were

60.5% sensitive, 0.5% intermediate and 38.9% were resistant. The p-values show that there was no significant association between the isolated bacteria and the response to Tetracycline in this study (Table 4.9).

Table 4.9: Response of isolates to Tetracycline

Isolate	Tetracycline	Percentage (%)	p-value
E. coli	Sensitive	63.9	0.157
	Intermediate	6	
	Resistant	. 35.5	
Salmonella spp	Sensitive	12.5	0.168
	Resistant	87.5	
Salmonella typhi	Sensitive	80.0	0.576
	Resistant	20.0	
Shigella dysenteriae	Sensitive	66.7	0.083
	Resistant	33.3	
Shigella sonnei	Sensitive	25.0	0.206
	Resistant	75.0	
Total	Sensitive	60.5	0.113
	Intermediate	0.5	
	Resistant	38.9	

***Salmonella spp, Salmonella typhi, Shigella dysenteriae, Shigella sonnei* –no intermediate**

The response of isolates to Tetracycline when compared in terms of gender and area of residence were varied. In Mukuru Kayaba, 70.6% of *E. coli* isolates from the male children were sensitive and 29.4% were resistant. Hundred percent of *Salmonella sp* and *S. sonnei* from male children were resistant while *S. dysenteriae* were sensitive. Results of female children from Mukuru kayaba showed that 61.5% of *E. coli* isolates were sensitive and 38.5% were resistant while 100% *S. sonnei* were resistant. From Mukuru Kwa Njenga, 66.7% of *E. coli* isolates from male children were sensitive and 33.3% were resistant while 100% of *S. sonnei* isolates resistant.

Isolates from female children at Mukuru Kwa Njenga showed that 51.5% of *E. coli* was sensitive and 48.5% were resistant while 100% of *S. typhi* and *Salmonella sp* were sensitive and resistant, respectively. From Mukuru Kwa Reuben male children, 73.1% of *E. coli* isolates were sensitive and 26.9% were resistant. Hundred percent of *Salmonella sp* and *S. dysenteriae* were sensitive. From female children, isolates from Mukuru Kwa Reuben showed that 69.0% of *E. coli* were sensitive, 3.4% were intermediate and 27.6% were resistant while 100% of *S. sonnei* isolates were sensitive. From Sinai, 77.8% *E. coli* isolates from the male children were sensitive and 22.2% were resistant.

Hundred percent of of *Salmonella sp* and *S. sonnei* were resistant while *S. typhi* were sensitive. Isolates from female children in Sinai showed that 46.7% of *E. coli* were sensitive and 53.3% were resistant. Hundred percent of *Salmonella sp* and *S. dysenteriae* were resistant. About 66.7% of *S. typhi* were sensitive and 33.3% were resistant. About 33.3% of *S. sonnei* isolates were sensitive and 66.7% were resistant. Statistical analysis showed that there was no significant association between each variable (gender and area of residence) and response to the drug Tetracycline ($p>0.05$) as shown in Table 4.10.

4.3.5 Response of isolates to Ampicillin

Most *Escherichia coli* species (59.6%) were sensitive, 13.9% showed intermediate sensitivity while 26.5% were resistant to the drug. Hundred percent (100%) of *Salmonella spp* were resistant. Sixty percent (60%) of *Salmonella typhi* isolates were sensitive, intermediate and resistant were 20.0%, respectively. None of the *Shigella dysenteriae* isolates were sensitive, 66.7% were intermediate and 33.3% were resistant. About 12.5% of *Shigella sonnei* isolates were intermediate while 87.5% were resistant. In total, 24.7% of the isolates were sensitive to Ampicillin, 14.2% were

intermediate and 61.1% were resistant. The p-values showed that there were no significance association between the isolates and the response to ampicillin (Table 4.11).

Table 4.10: Response of isolates to Tetracycline by gender and residence

Gender	Residence	Isolate	Sensitive (%)	Intermediate (%)	Resistant (%)	p-value
Male	Mukuru Kayaba	*E.coli*	70.6		29.4	0.21
		S. spp			100.0	
		S.dysenteriae	100.0			
		S. sonnei			100.0	
	Mukuru Kwa Njenga	*E.coli*	66.7		33.3	0.17
		S. sonnei			100.0	
	Mukuru Kwa Reuben	*E.coli*	73.1		26.9	0.70
		S. spp	100.0			
		S.dysenteriae	100.0			
	Sinai	*E.coli*	77.8		22.2	0.17
		S. spp			100.0	
		S. typhi	100.0			
		S. sonnei			100.0	
Female	Mukuru Kayaba	*E.coli*	61.5		38.5	0.23
		S. sonnei			100.0	
	Mukuru Kwa Njenga	*E.coli*	51.5		48.5	0.14
		S. spp			100.0	
		S. typhi	100.0			
	Mukuru Kwa Reuben	*E.coli*	69.0	3.4	27.6	0.80
		S. sonnei	100.0			
	Sinai	*E.coli*	46.7		53.3	0.53
		S. spp			100.0	
		S.typhi	66.7		33.3	
		S.dysenteriae			100.0	
		S. sonnei	33.3		66.7	

Key: *S. Spp- Salmonella species, S. typhi – Salmonella typhi, E.coli- Escherichia coli, S.sonnei- Shigella sonnei, S. dysenteriae- Shigella dysenteriae*

Table 4.11: Response of isolates to Ampicilin

Isolate	Ampicilin	Percentage (%)	p-value
E. coli	Sensitive	26.5	0.778
	Intermediate	13.9	
	Resistant	59.6	
Salmonella spp	Resistant	100.0	
Salmonella typhi	Sensitive	60.0	0.659
	Intermediate	20.0	
	Resistant	20.0	
Shigella dysenteriae	Intermediate	66.7	0.083
	Resistant	33.3	
Shigella sonnei	Intermediate	12.5	0.408
	Resistant	87.5	
Total	Sensitive	24.7	0.715
	Intermediate	14.2	
	Resistant	61.1	

***Salmonella sp*- resistant only, *Shigella dysenteriae* and *Shigella sonnei* – not sensitive**

When compared by gender and residential area, 41.2% of *E. coli* isolates from male children at Mukuru Kayaba were sensitive, 11.8% were intermediate and 47.1% were resistant. Hundred percent (100%) of *Salmonella sp* and *S. sonnei* were resistant while *S. dysenteriae* were intermediate. Isolates from female children at Mukuru Kayaba showed that 15.4% of *E. coli* were sensitive, 23.1% were intermediate while 61.5% were resistant while 100% of *S. sonnei* isolates were resistant. From Mukuru Kwa Njenga, 16.7% of *E. coli* from male children were sensitive and intermediate, respectively while 66.7% were resistant. Hundred percent (100%) of *S. sonnei* isolates from male children were resistant. Isolates from female children showed that 30.3% of *E. coli* isolates were sensitive, 3.0% were intermediate while 66.7% were resistant. Hundred percent (100%) of *salmonalla sp* and *S. typhi* were resistant and intermediate, respectively. Isolates from Mukuru Kwa Reuben; 15.4% of *E. coli* from male children were sensitive, 23.1% were intermediate while 61.5% were resistant. Hundred percent (100%) of *Salmonella* sp and *S. dysenteriae* were resistant and intermediate, respectively. Isolates from female children showed that 31.0% of *E. coli*

were sensitive, 24.1% were intermediate and 44.8% were resistant while 100% of *S. sonnei* were intermediate. From Sinai, 44.4% of *E. coli* isolates from male children were sensitive while 55.6% were resistant. Hundred percent of *Salmonella* sp and *S. sonnei* isolates from male children were resistant while *S. typhi* were sensitive. Isolates from female children showed that 26.7% of *E. coli* were sensitive while 73.3% were resistant. Hundred percent (100%) of *Salmonella sp, S. dysenteriae* and *S. sonnei* were resistant. About 66.7% of *S. typhi* were sensitive while 33.35 were resistant. The isolates from female children from Mukuru Kwa Njenga had p-values of 0.00 hence had significant association to Ampicillin response. The other variables had p-values more than 0.05 hence there were no significant associations between the isolates and the response to Ampicillin (Table 4.12).

4.3.6 Response of isolates to Ciprofloxacin

All the isolates had varied response to ciprofloxacin. 89.8% of *Escherichia coli* isolates were sensitive, 0.6% were intermediates and 9.6% resistant. All the *Salmonella spp* and *Shigella dysenteriae* isolates were sensitive. Eighty percent (80%) of *Salmonella typhi* isolates were sensitive and 20% were intermediate. About 87.5% of *Shigella sonnei* isolates were sensitive and 12.5% were resistant. In total, 90.0% of the isolates were sensitive, 1.1% was intermediate and 8.9% were resistant. All the p-values were more than 0.05 hence there was no significant association between the isolates and their response to ciproflaxoxine (Table 4.13).

Table 4.12: Response of isolates to Ampicillin by gender and residence

Gender	Residence	Isolate	Sensitive (%)	Intermediate (%)	Resistant (%)	p-value
Male	Mukuru Kayaba	*E.coli*	41.2	11.8	47.1	0.24
		S. spp			100.0	
		S.dysenteriae		100.0		
		S. sonnei			100.0	
	Mukuru Kwa Njenga	*E.coli*	16.7	16.7	66.7	0.78
		S. sonnei			100.0	
	Mukuru Kwa Reuben	*E.coli*	15.4	23.1	61.5	0.45
		S. spp			100.0	
		S.dysenteriae		100.0		
	Sinai	*E.coli*	44.4		55.6	0.41
		S. spp			100.0	
		S. typhi	100.0			
		S. sonnei			100.0	
Female	Mukuru Kayaba	*E.coli*	15.4	23.1	61.5	0.74
		S. sonnei			100.0	
	Mukuru Kwa Njenga	*E.coli*	30.3	3.0	66.7	
		S. spp			100.0	
		S. typhi		100.0		
	Mukuru Kwa Reuben	*E.coli*	31.0	24.1	44.8	0.24
		S. sonnei		100.0		
	Sinai	*E.coli*	26.7		73.3	0.31
		S. spp			100.0	
		S.typhi	66.7		33.3	
		S.dysenteriae			100.0	
		S. sonnei			100.0	

Key: *S. Spp- Salmonella species, S. typhi – Salmonella typhi, E.coli- Escherichia coli, S.sonnei- Shigella sonnei, S. dysenteriae- Shigella dysenteriae*

Table 4.13: Response of isolates to Ciprofloxacin

Isolate	Ciprofloxacin	Percentage (%)	p-value
E. coli	Sensitive	89.8	0.642
	Intermediate	.69	
	Resistant	.6	
Salmonella spp	Sensitive	100.0	
Salmonella typhi	Sensitive	80.0	0.576
	Intermediate	20.0	
Shigella dysenteriae	Sensitive	100.0	
Shigella sonnei	Sensitive	87.5	0.408
	Resistant	12.5	
Total	Sensitive	90.0	0.415
	Intermediate	1.1	
	Resistant	8.9	

*** *Salmonella sp* and *Shigella dysenteriae* - all were sensitive, *Salmonella typhi* - no resistant, *shigella sonnei*-no intermediate sensitivity**

4.3.8 Response of isolates to Chloramphenicol

All the isolates had varied response to chloramphenicol. Ninety one (91%) of *E. coli* isolates were sensitive, 4.2% were intermediate and 4.8% were resistant. 87.5% of *Salmonella spp* isolates were sensitive while 12.5% were resistant. All the *Salmonella typhi* and *Shigella dysentariae* isolates in this study were sensitive to the drug. 87.5% of *Shigella sonnei* isolates were sensitive while 12.5% were resistant. In total, 91.1% of the isolates were sensitive 3.7% intermediate while 5.3% were resistant to chloramphenicol. Analysis showed that there was no significant association between the isolates and their response to the drug chloramphenical (Table 4.17).

Comparison in terms of gender and residence were also done and the results were varied. Mukuru Kayaba 82.4% of *E. coli* isolates from male children were sensitive while 17.6% were resistant while the rest were 100% sensitive. Isolates from female children showed that 100% of *E. coli* and *S. sonnei* were sensitive from Mukuru Kayaba. From Mukuru Kwa Njenga, 91.7% of *E. coli* isolates from male children were sensitive and 8.3% were resistant while 100% *of S. sonnei* were sensitive. Results of isolates from females showed that 81.8% of *E. coli* were sensitive, 3.0% were intermediate and 15.2% were resistant. Hundred percent of *Salmonella spp* and

S. typhi were sensitive. From Mukuru Kwa Njenga, 92.3% of *E. coli* isolates from male children were sensitive while 7.7% were resistant. Hundred percent of *Salmonella sp* and *S. dysenteriae* were sensitive. Isolates from female children from Mukuru Kwa Reuben showed that 93.1% of *E. coli* were sensitive and 6.9% were resistant while 100% of *S. sonnei* were sensitive. All the bacteria isolated from the male children from Sinai were sensitive to Ciprofloxacin. Isolates from female at this area showed that 86.7% of *E. coli* were sensitive while 13.3% were resistant. Hundred percent of *Salmonella sp* and *S. dysenteriae* were sensitive. About 66.7% of *S. typhi* and *S. sonnei* were sensitive while 33.3% of *S. typhi* were intermediate and 33.3% of *S. sonnei* were resistant. The p-values were more than 0.05 hence there were no significant association between the isolates from either gender or area of residence and their response to Ciprofloxacin (Table 4.14).

4.3.7 Response of isolates to Nalidixic acid

Susceptibility test of Nalidixic acid against the isolated enteric bacteria were as follows: about 86.1% of *E. coli* isolates were sensitive, 1.8% were intermediate and 12.0% were resistant. Hundred percent (100%) of *Salmonella spp* and *Shigella dysenteriae* isolates were sensitive. Eighty percent (80%) of *Salmonella typhi* isolates were sensitive and 20% were intermediate. About 87.5% of *Shigella sonnei* isolate were sensitive and 12.5% were resistant. In total, 86.8% of the isolates were sensitive, 2.1% intermediate and 11.1% were resistant. The p-values were more than 0.05 ($p>0.05$) therefore there was no significant association between the isolated bacteria and their response to the drug nalidixic acid (Table 4.15).

Table 4.14: Response of isolates to Ciprofloxacin by gender and residence

Gender	Residence	Isolate	Sensitive (%)	Intermediate (%)	Resistant (%)	p-value
Male	Mukuru Kayaba	*E.coli*	82.4		17.6	0.89
		S. spp	100.0			
		S.dysenteriae	100.0			
		S. sonnei	100.0			
	Mukuru Kwa Njenga	*E.coli*	91.7		8.3	0.76
		S. sonnei	100.0			
	Mukuru Kwa Reuben	*E.coli*	92.3		7.7	0.92
		S. spp	100.0			
		S.dysenteriae	100.0			
	Sinai	*E.coli*	100.0			
		S. spp	100.0			
		S. typhi	100.0			
		S. sonnei	100.0			
Female	Mukuru Kayaba	*E.coli*	100.0			
		S. sonnei	100.0			
	Mukuru Kwa Njenga	*E.coli*	81.8	3.0	15.2	0.93
		S. spp	100.0			
		S. typhi	100.0			
	Mukuru Kwa Reuben	*E.coli*	93.1		6.9	0.79
		S. sonnei	100.0			
	Sinai	*E.coli*	86.7		13.3	0.33
		S. spp	100.0			
		S.typhi	66.7	33.3		
		S.dysenteriae	100.0			
		S. sonnei	66.7		33.3	

Key: *S. Spp- Salmonella species, S. typhi – Salmonella typhi, E.coli- Escherichia coli, S.sonnei- Shigella sonnei, S. dysenteriae- Shigella dysenteriae*

Table 4.15: Response of isolates to Nalidixic acid

Isolate	Nalidixic acid	Percentage (%)	p-value
E. coli	Sensitive	86.1	0.905
	Intermediate	12.0	
	Resistant	1.8	
Salmonella spp	Sensitive	100.0	
Salmonella typhi	Sensitive	80.0	0.576
	Intermediate	20.0	
Shigella dysenteriae	Sensitive	100.0	
Shigella sonnei	Sensitive	87.5	0.408
	Resistant	12.5	
Total	Sensitive	86.8	0.707
	Intermediate	2.1	
	Resistant	11.1	

*** *Salmonella* and *Shigella dysenteriae*- were all sensitive, *Salmonella typhi* and *Shigella sonnei*- no intermediate sensitivities**

When compared by gender and residence, 82.4% of *E. coli* isolates from male children in Mukuru Kayaba were sensitive to Nalidixic acid while 17.6% were resistant. All the other isolates from male chidren in Mukuru Kayaba were sensitive to the drug. Isolates from female children at Mukuru Kayaba showed that 100% of *E. coli* and *S. sonnei* were sensitive. From Mukuru Kwa Njenga, 83.3% of *E. coli* isolates from male children were sensitive, 4.2% were intermediate and 12.5% were resistant. Hundred percent of *S. sonnei* from male children were sensitive. Isolates from female children at Mukuru Kwa Njenga showed that 78.8% of *E.coli* were sensitive, 3.0% were intermediate and 18.2% were resistant. Hundred percent of *Salmonella sp* and *S. typhi* were resistant. From Mukuru Kwa Ruben, 88.5% of *E. coli* isolates from male children were sensitive while 11.5% were resistant. All the other isolates were sensitive. Isolates from female children showed that 86.2% of *E. coli* were sensitive, 3.4% were intermediate and 10.3% were resistant while 100% of *S. sonnei* were sensitive. All the isolates from the male children in Sinai ware sensitive to Nalidixic acid. Finally from Sinai, 86.7% of *E. coli* isolates from female children were sensitive while 13.3% were resistant. Hundred percent of *Salmonella sp* and *S.*

dysenteriae were sensitive. About 66.7% of *S. sonnei* and *S. typhi* were sensitive. All the p values were >0.05 hence no significant association between isolates from either gender and area of residence verse their response to the drug (Table 4.16).

4.3.8 Response of isolates to Chloramphenicol

All the isolates had varied response to chloramphenicol. Ninety one (91%) of *E. coli* isolates were sensitive, 4.2% were intermediate and 4.8% were resistant. About 87.5% of *Salmonella spp* isolates were sensitive while 12.5% were resistant. All the *Salmonella typhi* and *Shigella dysentariae* isolates in this study were sensitive to the drug. Again 87.5% of *Shigella sonnei* isolates were sensitive and 12.5% were resistant. In total, 91.1% of the isolates were sensitive to, 3.7% were intermediate and 5.3% resistant resistant to chloramphenical. Analysis showed that there was no significant association between the isolates and their response to the drug chloramphenical (Table 4.17).

Table 4.16: Sensitivity test of bacteria isolates to Nalidixic acid

Gender	Residence	Isolate	Sensitive (%)	Intermediate (%)	Resistant (%)	p-value
Male	Mukuru Kayaba	*E.coli*	82.4		17.6	0.89
		S. spp	100.0			
		S. dysenteriae	100.0			
		S. sonnei	100.0			
	Mukuru Kwa Njenga	*E.coli*	83.3	4.2	12.5	0.91
		S. sonnei	100.0			
	Mukuru Kwa Reuben	*E.coli*	88.5		11.5	0.88
		S. spp	100.0			
		S. dysenteriae	100.0			
	Sinai	*E.coli*	100.0			
		S. spp	100.0			
		S. typhi	100.0			
		S. sonnei	100.0			
Female	Mukuru Kayaba	*E.coli*	100.0			
		S. sonnei	100.0			
	Mukuru Kwa Njenga	*E.coli*	78.8	3.0	18.2	0.90
		S. spp	100.0			
		S. typhi	100.0			
	Mukuru Kwa Reuben	*E.coli*	86.2	3.4	10.3	0.92
		S. sonnei	100.0			
	Sinai	*E.coli*	86.7		13.3	0.33
		S. spp	100.0			
		S. typhi	66.7	33.3		
		S. dysenteriae	100.0			
		S. sonnei	66.7		33.3	

Key: *S. Spp- Salmonella species, S. typhi – Salmonella typhi, E.coli- Escherichia coli, S.sonnei- Shigella sonnei, S. dysenteriae- Shigella dysenteriae*

Table 4.17: Response of isolates to Chloramphenicol

Isolate	Chloramphenicol	Percentage (%)	p-value
E. coli	Sensitive	91.0	0.348
	Intermediate	4.2	
	Resistant	4.8	
Salmonella spp	Sensitive	87.5	0.408
	Resistant	12.5	
Salmonella typhi	Sensitive	100.0	
Shigella dysenteriae	Sensitive	100.0	
Shigella sonnei	Sensitive	87.5	0.168
	Resistant	12.5	
Total	Sensitive	91.1	0.341
	Intermediate	3.7	
	Resistant	5.3	

*** *Salmonella typhi* and *Shigella dysenteriae* – were all sensitive, *Salmonella sp* and *s. sonnei* – had no intermediate sensitivities**

There was different variation in the response of the isolates to the drugs when compared in terms of gender and residence. About 88.2% of *E. coli* isolates from male children in Mukuru Kayaba were sensitive to Chloramphenicol while 11.8% were intermediate. Hundred percent of *Salmonella sp, S. dysenteriae* and *S. sonnei* were sensitive. Isolates from female children from Mukuru Kayaba showed that 92.3% of *E. coli* were sensitive while 7.7% were resistant. Hundred percent of *S. sonnei* from female children were sensitive. From Mukuru Kwa Njenga, 87.5% of *E. coli* isolates from male children were sensitive, 4.2% were intermediate and 8.3% were resistant while 100% of *S. sonnei* was sensitive. From Mukuru Kwa Njenga, 90.9% of *E. coli* isolates from female children were sensitive, 3.0% were intermediate and 6.1% were resistant. About 66.7% of Salmonella sp were sensitive and 3.3% were resistant while 100% of S. typhi were sensitive. From Mukuru Kwa Reuben, 88.5% of E. coli isolates from male children were sensitive, 7.7 intermediate and 3.8% were resistant while the rest were 100% sensitive. Mukuru Kwa Reuben, 89.7% of E. coli isolates from female children were sensitive, 3.4% were intermediate while 6.9% were resistant while 100% of *S. sonnei* were sensitive. From Sinai, 100% of *E. coli,*

Salmonella sp and *S. typhi* isolates from male children were sensitive to Chloramphenicol while 1005 of S. sonnei were resistant. The isolates from female children from Sinai were sensitive. The isolates from female children from Sinai had significant association with their response to Chloramphenicol ($p=0.01$) while the rest had no statistical significant association ($p>0.05$) as shown in Table 4.18.

4.3.9 Response of isolates to Gentamicin

Most of the *E. coli* isolates (94.6%) were sensitive, 0.6% were intermediate sensitive and 4.8% were resistant. All the *Salmonella spp, Salmonella typhi, Shigella dysenteriae* and *Shigella sonnei* isolates were sensitive to the drug under investigation. In total, 95.3% of the isolates were sensitive, 0.5% intermediate sensitive and 4.2% were resistant. From the results of this study, there was no significant association between the isolated organisms and their response to the drug Gentamicin (Table 4.19).

Table 4.18: Response of isolates to Chloramphenicol by gender and residence

Gender	Residence	Isolate	Sensitive (%)	Intermediate (%)	Resistant (%)	p-value
Male	Mukuru Kayaba	*E.coli*	88.2	11.8		0.94
		S. spp	100.0			
		S. dysenteriae	100.0			
		S. sonnei	100.0			
	Mukuru Kwa Njenga	*E.coli*	87.5	4.2	8.3	0.93
		S. sonnei	100.0			
	Mukuru Kwa Reuben	*E.coli*	88.5	7.7	3.8	0.99
		S. spp	100.0			
		S. dysenteriae	100.0			
	Sinai	*E.coli*	100.0			
		S. spp	100.0			
		S. typhi	100.0			
		S. sonnei			100.0	
Female	Mukuru Kayaba	*E.coli*	92.3		7.7	0.77
		S. sonnei	100.0			
	Mukuru Kwa Njenga	*E.coli*	90.9	3.0	6.1	0.57
		S. spp	66.7		33.3	
		S. typhi	100.0			
	Mukuru Kwa Reuben	*E.coli*	89.7	3.4	6.9	0.94
		S. sonnei	100.0			
	Sinai	*E.coli*	100.0			
		S. spp	100.0			
		S. typhi	100.0			
		S. dysenteriae	100.0			
		S. sonnei	100.0			

Key: *S. spp- Salmonella species, S. typhi – Salmonella typhi, E.coli- Escherichia coli, S.sonnei- Shigella sonnei, S. dysenteriae- Shigella dysenteriae*

Table 4.19: Response of isolates to Gentamicin

Isolate	Gentamicin	Total percentage (%)	p-value
E. coli	Sensitive	94.6	0.578
	Intermediate	0.6	
	Resistant	4.8	
Salmonella spp	Sensitive	100.0	
Salmonella typhi	Sensitive	100.0	
Shigella dysenteriae	Sensitive	100.0	
Shigella sonnei	Sensitive	100.0	
Total	Sensitive	95.3	0.606
	Intermediate	0.5	
	Resistant	4.2	

* ***Salmonella, Salmonella typhi, Shigella dysenteriae, Shigella sonnei*- were all sensitive**

When compared in terms of gender and residence, 88.2% of *E. coli* isolates from male children in Mukuru Kayaba were sensitive to Gentamicin while 11.8% were resistant. Hundred percent of *Salmonella sp*, *S. dysenteriae* and *S. sonnei* were sensitive. All the isolates from the female children in Mukuru Kayaba were sensitive to Gentamicin. From Mukuru Kwa Njenga, 95.8% of *E. coli* isolates from male children were sensitive and 4.2% resistant while 100% of *S. sonnei* were sensitive. Mukuru Kwa Njenga, 90.9% of *E. coli* isolates from female children were sensitive, 3.0% were intermediate and 6.1% were resistant while 100% of *Salmonella sp* and *S. typhi* were sensitive. All the isolates from the male children in Mukuru Kwa Reuben were sensitive. From Mukuru Kwa Reuben, 89.7% of *E. coli* isolates from female children were sensitive and 10.3% were resistant while 100% of *S. sonnei* were sensitive. All the isolates from the male and female children in Sinai were sensitive to Gentamicin. All the p-values were >0.05 hence there was no significant association between all the isolates from different gender/ area of residence and response to Gentamicin (Table 4.20).

Table 4.20: Response of isolates to Gentamicin by gender and residence

Gender	Residence	Isolate	Sensitive (%)	Intermediate (%)	Resistant (%)	p-value
Male	Mukuru Kayaba	*E.coli*	88.2		11.8	0.94
		S. spp	100.0			
		S. dysenteriae	100.0			
		S. sonnei	100.0			
	Mukuru Kwa Njenga	*E.coli*	95.8		4.2	0.84
		S. sonnei	100.0			
	Mukuru Kwa Reuben	*E.coli*	100.0			
		S. spp	100.0			
		S. dysenteriae	100.0			
	Sinai	*E.coli*	100.0			
		S. spp	100.0			
		S. typhi	100.0			
		S. sonnei	100.0			
Female	Mukuru Kayaba	*E.coli*	100.0			
		S. sonnei	100.0			
	Mukuru Kwa Njenga	*E.coli*	90.9	3.0	6.1	0.98
		S. spp	100.0			
		S. typhi	100.0			
	Mukuru Kwa Reuben	*E.coli*	89.7		10.3	0.74
		S. sonnei	100.0			
	Sinai	*E.coli*	100.0			
		S. spp	100.0			
		S. typhi	100.0			
		S. dysenteriae	100.0			
		S. sonnei	100.0			

Key: *S. Spp- Salmonella species, S. typhi – Salmonella typhi, E.coli- Escherichia coli, S.sonnei- Shigella sonnei, S. dysenteriae- Shigella dysenteriae*

4.3.10 Response of isolates to Ceftriaxone

About 97.6% of the *E. coli* isolates were sensitive, 1.2% intermediate while 1.2% were resistant to Ceftriaxone. All *Salmonella spp, Salmonella typhi, Shigella dysenteriae* and *Shigella sonnei* were sensitive to the drug. In total, 97.9% of the isolates were sensitive while 1.1% were intermediate and resistant, respectively.

There was no significant association between the isolated bacteria and their response to Ceftriaxone (p=0.132 and 0.130) as shown in Table 4.21.

Table 4.21: Sensitivity test of isolates to Ceftriaxone

Isolate	Ceftriaxone	Percentage (%)	p-value
E. coli	Sensitive	97.6	0.132
	Intermediate	1.2	
	Resistant	1.2	
Salmonella spp	Sensitive	100.0	
Salmonella typhi	Sensitive	100.0	
Shigella dysenteriae	Sensitive	100.0	
Shigella sonnei	Sensitive	100.0	
Total	Sensitive	97.9	0.130
	Intermediate	1.1	
	Resistant	1.1	

***Salmonella sp, Salmonella typhi, Shigella dysenteriae, Shigella sonnei*- all were sensitive**

In terms of gender and area of residence all the isolates from both male and female children in Mukuru Kayaba were sensitive to Ceftriaxone. From Mukuru Kwa Njenga, 95.8% of *E. coli* isolates from male children were sensitive and 4.0% were intermediate, while 100% of *S. sonnei* were sensitive. All the isolates from female children from Mukuru Kwa Njenga were sensitive. In Mukuru Kwa Reuben, 96.2% of *E. coli* isolates from male children were sensitive and 3.8% were intermediate while 100% of *Salmonella sp* and *S. dysenteriae* were sensitive. From Mukuru Kwa Reuben, 92.6% of *E. coli* isolates from female children in were sensitive and7.4% were resistant while 100% of *S. sonnei* were sensitive to Ceftriaxone. All the isolates from the male and female children from Sinai were sensitive to the Ceftriaxone. The p values were more than 0.05 hence there was no significant association between the isolated enteric bacteria from different gender and area of residence and response to Ceftriaxone (Table 4.22).

Table 4.22: Response of isolates to Ceftriaxone by gender and residence

Gender	Residence	Isolate	Sensitive (%)	Intermediate (%)	Resistant (%)	p-value
Male	Mukuru Kayaba	*E.coli*	100.0			
		S. spp	100.0			
		S. dysenteriae	100.0			
		S. sonnei	100.0			
	Mukuru Kwa Njenga	*E.coli*	95.8	4.2		0.84
		S. sonnei	100.0			
	Mukuru Kwa Reuben	*E.coli*	96.2	3.8		0.96
		S. spp	100.0			
		S. dysenteriae	100.0			
	Sinai	*E.coli*	100.0			
		S. spp	100.0			
		S. typhi	100.0			
		S. sonnei	100.0			
Female	Mukuru Kayaba	*E.coli*	100.0			
		S. sonnei	100.0			
	Mukuru Kwa Njenga	*E.coli*	100.0			
		S. spp	100.0			
		S. typhi	100.0			
	Mukuru Kwa Reuben	*E.coli*	92.6		7.4	0.78
		S. sonnei	100.0			
	Sinai	*E.coli*	100.0			
		S. spp	100.0			
		S. typhi	100.0			
		S. dysenteriae	100.0			
		S. sonnei	100.0			

Key: *S. Spp- Salmonella species, S. typhi – Salmonella typhi, E.coli- Escherichia coli, S.sonnei- Shigella sonnei, S. dysenteriae- Shigella dysenteriae*

4.3.11 Response of isolates to Kanamycin

Most (94.0%) of the *E. coli* isolates were sensitive, 2.4% were intermediate while 3.6% were resistant to Kanamycin. About 87.5% of the *Salmonella spp* isolates were sensitive while 12.5% were intermediate. Majority (80.0%) of the *Salmonella typhi* isolates were sensitive and 20% were intermediate. *Shigella dysenteriae* isolates were 66.7% sensitive, intermediates were 33.3% and there were no resistant isolates. About 87.5% of *Shigella sonnei* were sensitive while 12.5% were intermediate. In

total, 92.6% of the isolates were sensitive to Kanamycin, 3.2% were intermediate while 4.2% were resistant. There was no significant association ($p < 0.05$) between the isolates and their response to Kanamycin (Table 4.23).

Table 4.23: Response of isolates to Kanamycin

Isolate	Kanamycin	Percentage (%)	p-value
E. coli	Sensitive	94.0	0.233
	Intermediate	2.4	
	Resistant	3.6	
Salmonella spp	Sensitive	87.5	0.408
	Intermediate	12.5	
Salmonella typhi	Sensitive	80.0	0.576
	Intermediate	20.0	
Shigella dysenteriae	Sensitive	66.7	0.083
	Resistant	33.3	
Shigella sonnei	Sensitive	87.5	0.408
	Resistant	12.5	
Total	Sensitive	92.6	0.058
	Intermediate	3.2	
	Resistant	4.2	

****Salmonella sp, Salmonella typhi, Shigella sonnei, Shigella dysenteriae*** **– have no resistance or intermediate sensitivity**

Comparison in terms of gender and area of residence of the participants was also considered, and it showed that isolates from the male and female children in Mukuru Kayaba were susceptible to Kanamycin. From Mukuru Kwa Njenga, 95.8% of *E. coli* isolates from the male children were sensitive and 4.2% were resistant, while 100% of *S. sonnei* were sensitive to Kanamycin. From Mukuru Kwa Njenga, 87.9% of *E. coli* isolates from female children were sensitive, 9.1% were intermediate and 3.0% were resistant. About 66.7% of *Salmonella* sp were sensitive and 33.3% were intermediate while 100% of *S. typhi* were sensitive. In Mukuru Kwa Reuben 96.2% of *E. coli* isolates from male children were sensitive and 3.8% were intermediate while the rest are 100% sensitive. From Mukuru Kwa Reuben, 89.7% of *E. coli* isolates from female children were sensitive and 10.3% were resistant while 100% *S. sonnei* were

sensitive. All the isolates from the male children in Sinai were susceptible to Kanamycin. From Sinai, 93.3% of *E. coli* isolates from female children were sensitive while 6.7% were resistant. The p values were less than 0.05 hence no statistical significant association between the bacteria isolates from different areas and genders verse their response to Kanamycin (Table 4.24).

Table 4.24: Response of isolates to Kanamycin by gender and residence

Gender	Residence	Isolate	Sensitive (%)	Intermediate (%)	Resistant (%)	p-value
Male	Mukuru Kayaba	*E.coli*	100.0			
		S. spp	100.0			
		S. dysenteriae	100.0			
		S. sonnei	100.0			
	Mukuru Kwa Njenga	*E.coli*	95.8		4.2	0.84
		S. sonnei	100.0			
	Mukuru Kwa Reuben	*E.coli*	96.2	3.8		0.96
		S. spp	100.0			
		S. dysenteriae	100.0			
	Sinai	*E.coli*	100.0			
		S. spp	100.0			
		S. typhi	100.0			
		S. sonnei	100.0			
Female	Mukuru Kayaba	*E.coli*	100.0			
		S. sonnei	100.0			
	Mukuru Kwa Njenga	*E.coli*	87.9	9.1	3.0	0.76
		S. spp	66.7	33.3		
		S. typhi	100.0			
	Mukuru Kwa Reuben	*E.coli*	89.7		10.3	0.74
		S. sonnei	100.0			
	Sinai	*E.coli*	93.3		6.7	0.04
		S. spp	100.0			
		S. typhi	66.7	33.3		
		S. dysenteriae			100.0	
		S. sonnei	66.7		33.3	

Key: *S. Spp- Salmonella species, S. typhi – Salmonella typhi, E.coli- Escherichia coli, S.sonnei- Shigella sonnei, S. dysenteriae- Shigella dysenteriae*

4.3.12 Response of isolates to Streptomycin

A total of 77.7% of *E. coli* isolates were sensitive, 7.2% were intermediate sensitive while 15.1% were resistant to Streptomycin. Of the *Salmonella spp* isolated 25% were sensitive while 75.0% were intermediate sensitive. All the isolated *Salmonella typhi* were sensitive to Streptomycin antibiotic. About 33.3% of *Shigella dysenteriae* isolates were sensitive and 67.3% were resistant to Streptomycin. Response of *Shigella sonnei* was as follows; 37.5% were sensitive, 37.5 were intermediate sensitive while 25.0% were resistant to the drug. In total, 73.7% of the isolated enteric bacteria were sensitive to Streptomycin, 11.1% were intermediate sensitive while 15.3% were resistant. The p-values were more than 0.05 therefore was no significant association between the isolates and the response to the drug (Table 4.25).

Table 4.25: Response of isolates to Streptomycin antibiotic

Isolate	Streptomycin	Percentage (%)	p-value
E. coli	Sensitive	77.7	0.208
	Intermediate	7.2	
	Resistant	15.1	
Salmonella spp	Sensitive	25.0	0.673
	Intermediate	75.0	
Salmonella typhi	Sensitive	100.0	
Shigella dysenteriae	Sensitive	33.3	0.386
	Resistant	66.7	
Shigella sonnei	Sensitive	37.5	0.315
	Intermediate	37.5	
	Resistant	25.0	
Total	Sensitive	73.7	0.431
	Intermediate	11.1	
	Resistant	15.3	

***Salmonella sp, Salmonella typhi, Shigella dysenteriae*- they showed either resistant or intermediate sensitivities**

When compared by gender and area of residence of the participating children, 82.4.0% of *E. coli* isolates from male children in Mukuru Kayaba were sensitive, 5.9% were intermediate and 11.8% were resistant while 100% of *Salmonella sp* and *S. sonnei* were intermediate. From Mukuru Kayaba, 69.2% of E. coli isolates from

female children were sensitive, 23.1% were intermediate and 7.7% were resistant while 100% of *S. sonnei* were intermediate.

Salmonella sp were sensitive, 9.1% were intermediate and resistant, respectively. About 33.3% of *S. typhi* were sensitive while 66.7% were intermediate. From Mukuru Kwa Ruben, 84.6% of *E. coli* isolates from male children were sensitive, 7.7% were intermediate and resistant, From Mukuru Kwa Njenga, 66.7% of *E. coli* isolates from male children were sensitive and 33.3% were resistant while 100% of *S. sonnei* were intermediate. Isolates from female at Mukuru kwa Njenga showed that 100% of *E. coli* isolates were sensitive, 81.8% of the respectively while 100% of the *Salmonella sp.* were sensitive. Mukuru Kwa Reuben results revealed that, 100% of *E. coli* isolates from female children were sensitive, 82.8% of S. sonnei were sensitive, 3.4% were intermediate and 13.8% were resistant to Streptomycin. From Sinai, 77.8% of E. coli isolates from male children were sensitive and 22.2% were resistant, while 100% of Salmonella sp were intermediate and 100% of S. typhi and S. sonnei were sensitive. Finally Sinai slum showed that, 66.7% of Salmonella sp isolates from female children were sensitive, 13.3% were intermediate and 20.0% were resistant. Statistical analysis showed that Mukuru Kayaba and Mukuru Kwa Njenga isolates from male participants had statistical significant association response to streptomycin (p-values of 0.05 and 0.00, respectively). The female gender from Sinai slums had significant association at 95% confidence interval ($p \leq 0.05$) as shown in Table 4.26.

Table 4.26: Response of isolates to Streptomycin by gender and residence

Gender	Residence	Isolate	Sensitive (%)	Intermediate (%)	Resistant (%)	p-value
Male	Mukuru Kayaba	*E.coli*	82.4	5.9	11.8	
		S. spp		100.0		
		S. dysenteriae	100.0			
		S. sonnei		100.0		
	Mukuru Kwa Njenga	*E.coli*	66.7		33.3	
		S. sonnei		100.0		
	Mukuru Kwa Reuben	*E.coli*	84.6	7.7	7.7	
		S. spp	100.0			
		S. dysenteriae			100.0	
	Sinai	*E.coli*	77.8		22.2	
		S. spp		100.0		
		S. typhi	100.0			
		S. sonnei	100.0			
Female	Mukuru Kayaba	*E.coli*	69.2	23.1	7.7	0.26
		S. sonnei		100.0		
	Mukuru Kwa Njenga	*S. spp*	81.8	9.1	9.1	0.09
		S. typhi	33.3	66.7		
		E.coli	100.0			
	Mukuru Kwa Reuben	*S. sonnei*	82.8	3.4	13.8	0.90
		E.coli	100.0			
	Sinai	*S. spp*	66.7	13.3	20.0	
		S. typhi		100.0		
		S. dysenteriae	100.0			
		S. sonnei			100.0	

Key: *S. Spp- Salmonella species, S. typhi – Salmonella typhi, E.coli- Escherichia coli, S.sonnei- Shigella sonnei, S. dysenteriae- Shigella dysenteriae*

CHAPTER FIVE

DISCUSSION

5.1 Participants characteristics

A total number of 190 children below the age of five years presenting with diarrhoea in the Government health facility at Mukuru slums participated in this study. Intestinal enteropathogens which cause gastroenteritis are major public health problem in developing countries, especially among children (Palpasa *et al.,* 2010). The youngest child was 3 months old while the oldest was 72 months according to the findings of this study. Female children were 3.7 months older than the male children. Age can be a predisposing factor to diarrhoea in children below the age of five years (WHO, 2007). Living in the slums is also a predisposing factor to diarrhoeal infections because of the poor hygienic conditions coupled with poor sanitation (WHO, 2010). The reason for the high incidence of bacterial isolates in age group 25 – 36 months and 37 – 46 months could be due to the fact that children within this age group are most often than not unaccompanied and cannot differentiate between what to eat and what not to eat; they have not learnt the rules of adherence to aseptic or hygienic practice and they can barely express themselves (Sang *et al.,* 2012). Those below the age of twelve months are essentially under their mothers' care, feeding mainly on breast milk thereby reducing their susceptibility to these pathogens. Analysis of the participants gender, area of residence and prevalence of diarrhoea each showed that there was no significant difference ($P>0.05$) this is because the entire residential area is within Mukuru slums hence share a common environment.

The results of other studies concur with the current study. Chitnis *et al*., (2012) in their study observed that patients susceptible to Carbapenem-resistant

enterobacteriaceae (CRE) were more likely to be female. The results of the current study concurs with a study done by Sule *et al.,* (2011) in Kaduna Nigeria where they found the incidence between both sexes showing female children having the highest percentage (26%) compared to males (18%). Abdullahi *et al.,* (2010) reported that male children were more infected (22.33%) than female children (18.33%), although the difference was not statistically significant (χ^2 = 0.531, p>0.05) hence contradicting the finding of the current study. Most diarrhoeal episodes occur during the first two years of life due to a combination of factors; declining levels of maternal acquired antibodies, lack of active immunity in the infant, the introduction of food that may be contaminated with enteric bacteria or direct contact with human or animal faeces carrying enteric bacteria when the infant starts to crawl (Sang, 2007). Most enteric pathogens stimulate at least partial immunity against repeated infections or illness, which helps to explain the declining incidence of diseases in older children (Patwari *et al.,* 1993).

The participants attending Mukuru Kwa Njenga government health facility were noted to reside in four neighbouring slums namely; Mukuru Kwa Njenga, Mukuru Kwa Reuben, Mukuru Kayaba and Sinai. The roads within the slums are in a state of disrepair, with scarcity of clean water and electricity in many households and schools. Houses are built without proper planning hence result in both poor drainage systems and sanitation. There is no sewerage system in the slums hence the residents use pit latrines and 'flying' toilets. Due to the extreme poverty many of the residents are forced to do odd jobs and live in tiny, shared rooms with large families and dependants. Congestion is the order of the day and many families depend on less than one dollar per day (KDHS, 2010). The people in this area experience acute shortage

of water quite often hence rely on water vendors from unknown/suspect sources (KDHS, 2010). The majority of the participants were from Mukuru kwa Njenga (32.6%) followed by Mukuru Kwa Reuben (30.5%) then Sinai (18.9%) and the least were from Mukuru Kayaba (17.9%). Mukuru Kwa Njenga had the highest number of female children while Mukuru Kwa Reuben had the highest number of male children. There was no significant difference between participants characteristics and their area of residence (p= 0.144). This is because life in the four slums is basically the same in that the residents face similar challenges. In a study done in different geographical areas in Kenya, it was found that there was no statistical significant difference in the distribution of isolated pathogens or in the antimicrobial resistance by geographical region (Sang *et al.,* 2013). It was also observed that the residents of the slum keep pets like dogs, cats and livestock such as pigs, goats and cattle as well as poultry like chicken and other birds. Some of these domesticated animals and poultry are known to be reservoirs of pathogens and can be sources of several zoonotic diseases. This could explain the high prevalence of diarrhoea in this study. The salmonella species which was not identified to species level in this study could be non-typhoidal *Salmonella* species which is of zoonotic origin.

Some factors which were not captured in this study include; predisposing factors that enhance spread and increase in the risk of diarrhoea in young children like failure to breast feed exclusively for the first 4 – 6 months of life (Sule *et al.,* 2011). Studies have shown that the risk of developing diarrhoea is greater in non-breast fed infants than those exclusively breast fed (Sang *et al.,* 2013). Breast feeding until at least one year of age or prolonged breast feeding reduces the incidence and severity of diarrhoeal diseases (Abdullahi *et al.,* 2010). The uses of infant feeding bottles which

may be contaminated with bacteria; under nutrition, immunodeficiency or immune suppression and current or recurrent measles attacks are among the risk factors of diarrhoeal diseases in young children (Sang *et al.*, 2013).

5.2 Isolation and Identification of the Bacteria

Acute diarrhoea due to bacterial infections is an important cause of morbidity and mortality in infants and young children in most developing countries including Kenya especially in the slums (Adegunloye, 2005). Identification of the enteropathogens causing diarrhoeal diseases in the country is an essential step towards the implementation of effective primary health care activities against the disease (Olowe *et al.,* 2003). Poor sanitation in the study area could have also contributed to the high prevalence of bacteria isolated. The residents live in congested environments with their domesticated animals which could have contributed to the high prevalence of isolated enteric bacteria. According to a study done by Kariuki *et al.,* (2006), a significantly higher proportion of younger children (< 3 years of age) and those from the slums presented with invasive non- typhoidal *Salmonella spp* compared to older children and those from upper socio-economic groups ($p < 0.001$).

In terms of gender and area of residence, Mukuru kwa Njenga, had more *E. coli* isolated from female children (35.2%) compared to male children (29.4%) the rest of the isolates were uniform in both genders. In Mukuru kwa Reuben the trend was the same in that more *E. coli* were also isolated from female children (17.5%) than from male children (13.0%). *Salmonella spp* were 4.9% from female children and 0.0% from male children while *S. typhi* were more from male children (3.0%) compared to female children (0.5%). At Sinai the percentages of the isolates from both male and female children were almost equal (9.0% and 9.9%, respectively). *Shigella sonnei*

were more from (3.2) female than male children (1.0%). The rest were almost the same in both male and female children. At Mukuru Kayaba *E. coli* isolates were more from female (10.7%) than male children (7.2%). *S typhi* were 1.6% in females and 0.0% in males while the rest were 0.0%. There was no significant association between the gender and percentage isolates ($p>0.05$). There was also no significant association between the prevalence of the isolates and the area of residence of the children ($\chi^2 = 2.23$, $p=0.693$). The results of this study do not concur with what Sang *et al.,* (2013) found in their studies on the prevalence of bacteria in four provinces in Kenya where they had recruited 651 participants and isolated pathogenic bacteria in (17.7%) of the participants. Among the isolated bacteria were; pathogenic *E. coli* (11.2%), *Salmonella* (3.5%), *Shigella* (2.3%) and *Vibrio cholera* (0.6%) (Sang,2007). The reason for the different results could be because the study area was basically a slum hence the high prevalence of bacteria isolated especially the *E. coli.*

A similar study was done by Ifeanyi *et al.,* (2010) in Abuja Nigeria among cases of diarrhoea with potential bacterial pathogens detected being 65.8% of all patients screened. This was in contrast to a report of the prevalence of 83.1% from similar study in Abakaliki, south –eastern Nigeria (Ogbu *et al.,* 2008). Another study reported a prevalence of 63.3%-71.83% isolation of enteric bacteria in ifakara Tanzania (Vargas *et al.,* 2004). The variation in prevalence between the two Nigerian cities might be attributed to differences in infrastructural and socioeconomic indices (Ogbu *et al.,* 2012).

In a different study, the prevalence of bacterial aetiology of diarrhoea was 44% which follows the same trend with the research conducted in Kano State which was found to be 40.67% (Tsang *et al.,* 2009; Abdullahi *et al.,* 2010). In Gabon prevalence of diarrhoea with bacterial aetiology was 38% (Patwari *et al.,* 1993). In Tanzania it was

36% (Molbak *et al.,* 1997). The study showed that *Shigella spp* appears to be the predominant bacteria causing diarrhoea followed by *E. coli*, and *Salmonella* in that order. A total of 56% of the hundred diarrhoea cases investigated had no bacterial pathogen suggesting viral, protozoan or nonpathogenic factors (Abdullahi *et. al*., 2010).

Salmonella *spp* isolated in Mukuru slums could be non- typhoidal salmonella which is a zoonotic strain. The children could have been contaminated with faecal matter of the domesticated animals hence the acquisition of the bacteria. Occurrence of diarrhoeagenic bacteria in the current study showed that gram negative bacteria (*Shigella spp, Salmonella spp, Escherichia coli*) are the main cause of bacterial diarrhoea. Sule *et al.,* (2011) in Kaduna Nigeria conducted a similar study and found similar results. Generally, the aetiology of diarrhoea in young children could be attributed to a wide range of factors, but one of the main causes of diarrhoea is related to bacteria (such as *Salmonella spp, Shigella spp,Vvibrio, Escherichia coli, Aeromonas* and *Pseudomonas* (Abdullahi *et al.,* 2010).

Results from the current study shows that, though there are a number of causative agents of diarrhoeal diseases, bacteria still remain one of the major causes with *Shigella, Salmonella* and *Escherichia coli* being the most important pathogens among paediatric patients presenting with diarrhoea in Mukuru kwa Njenga Government health facility. Judicious use of antibiotic therapy requires education of health workers and patients, adequate laboratory diagnostic capabilities and government regulations. Antibiotic sensitivity paterns must be monitored, to effectively treat enteric bacteria such as pathogenic *E. coli, Shigella* and *Salmonella* (WHO, 2007). Emphasis should be placed on primary preventive health measures such as ensuring sewerage

management and safe drinking water in Kenya especially in the slums such as Mukuru.

5.3 Antibiotic sensitivity profiles of the isolated bacteria

5.3.1 Response of isolates to Amoxicillin/ Clavulanic Acid

The response of all the isolates from different sites and gender had varied response to Amoxicillin/ Clavulanic Acid. In total 34.2% of the isolates were sensitive, 16.3% were intermediate and 49.5% were resistant. The isolated *E. coli* showed varied response to Amoxicillin/ Clavulanic Acid. About 47.1% of the *E. coli* isolates at Mukuru Kayaba from male children were resistant while from female children, 46.2% were resistant, 100% of *Salmonella sp, S. typhi, S. dysenteria* and *S. sonnei* were resistant from male children. *Shigella sonnei* isolated from female children from this area were 100% resistant.

From Mukuru Kwa Njenga; 62.5% *E. coli* isolates from male children were resistant while *Shigella sonnei* were 100% resistant. *E. coli* isolates from female children were 54.5% resistant. *Salmonella sp* and Salmonella *typhi* isolates were 100% resistant. From Mukuru Kwa Reuben; 34.6% of *E. coli* isolates from male children were resistant. *Salmonella* sp were 100% resistant while *Shigella dysenteriae were* 100% intermediate. The *E. coli* isolates from female children were 41.4% resistant. From Sinai; 33.3% of *E. coli* isolates from male children were resistant. From female children, 53.3% of *E. coli* isolates were resistant while *Salmonella* sp and *Shigella dysenteria* were 100% resistant. The p-values more than 0.05 hence there was no significant association between the variables (age, gender and area of residence) verses the drug reaction against the isolates except the male gender and isolates response to the drug at Sinai which had a p-value of 0.02.

The study showed that there is an emerging resistance of enterobacteria to Amoxicillin/ Clavulanic Acid. This high resistance could be due to the fact that the drug is readly available over the counter hence high chances of missuse due to easy accessibility. Due to its high number of generics in the market, the drug is cheaply available hence leading to missuse. The emergence of multidrug resistance in Kenya especially in the slums could lead to high morbidity and mortality rates.

A similar study reported the highest resistance rate (54.4%) observed for Amoxicillin/ Clavulanic Acid in bacteria isolated from diarrhoeagenic children below five years (Nuke *et al*., 2012). Total average resistance of AM (76.3%) and AMC (36.9%) was also reported. Wide differences in resistance rates were observed for particular antibiotics among bacterial genera of enterobacteriaceae. It was also noted that resistance rates for different antibiotics greatly differed within the same bacteria. Resistance rates were significantly different between stations within the same bacterial genus, with the exception of *Serratia* and *Klebsiella sp.* isolates which were resistant to amoxicillin-clavulanic acid (Nuke *et al.*, 2012) and the percentage of the bacterial isolates showing resistance to aminoglycosides such as streptomycin (23.1%, 23.1%, and 17.3%) was quite high in frequency.

5.3.2 Response of isolates to Sulphamethoxazole/ trimethoprim

This study has showed that in total, sensitive isolates to Sulphamethoxazole/ trimethoprim (SXT) were 51.1%, 10% were intermediates while 38.9% were resistant.

The *E. coli* isolates from male children at Mukuru Kayaba were 64.7% sensitive and 35.3% intermediate. Hundred percent of *Salmonella sp* and *S. sonnei* were intermediate while *S. dysenteriae* were sensitive from male children. *E. coli* isolates from female children at Mukuru Kayaba were 46.2% sensitive, 23.1% were

intermediate and 30.8% of the *E. coli* isolates were resistant while 100% of *S. sonnei* isolates were intermediate.

From Mukuru Kwa Njenga; *E. coli* isolates from male children were 45.8% sensitive, 4.2% were intermediate and 50.0% resistant while 100% of *S. sonnei* isolates were resistant.

From the female children, the results showed that 51.5% of the *E. coli* isolates were sensitive and 48.5% were resistant. Of *Salmonella sp* from female children, 33.3% were intermediate and 66.7% were resistant while 100% of *S. typhi* were sensitive.

This drug was fairly sensitive when comapared in terms of gender, area of residence and isolate response. From Mukuru Kwa Reuben, isolates from male children showed that 50.0% of *E. coli* were sensitive and 34.6% were resistant while 100% of *Salmonella sp* and *S. dysenteria* were sensitive. About 51.7% of *E. coli* isolates from female children were sensitive and 41.4% were resistant while 100% of *S. sonnei* isolates were sensitive. From Sinai isolates from male children; 66.7% of the *E. coli* isolates were sensitive and 22.2% resistant while 100% of *S. sonnei* isolates were resistant. From the female children, 46.7% of *E. coli* isolate were sensitive and 46.7% were resistant while 100% of *Salmonella sp* and *S. typhi* were intermediate and sensitive, respectively.

The p-values of isolates from male and female children at Mukuru kayaba and Sinai were less than 0.05 hence had significant association while the rest had no significant association. From the findings of this study, this drug was active against Salmonella species in general and therefore could be recommended for treatment.

It has been reported by other studies that resistance to SXT among enteric bacterial pathogens has increased dramatically over the last 14 years (Von-Sonnenburg 2000).

Studies have reported that SXT should not be considered active against enteropathogens causing traveller's diarrhoea and should not currently be recommended for empirical treatment of traveller's diarrhoea regardless of the region of the world (Harumi *et al.,* 2001). Again it has been reported elsewhere that this antimicrobial agent was more effective as CIP in the therapy of traveller's diarrhoea (Dupont 2010).

5.3.3 Response of isolates to Ceftazidime

All the isolates had varied response to Ceftazidime (CAZ). The finding showed that 93.4% of *E. coli* isolates were sensitive, 3% were intermediate and 3.6% were resistant. In a nut shell Ceftazidime action to all the isolated bacteria were 94.2% sensitive, 2.6% intermediates and 3.2% resistant. There was no significant association between the isolates and their response to Ceftazidime. Majority (94.1%) of E. coli isolates from male children at Mukuru Kayaba, were sensitive and a few (5.9%) were resistant while 100% of *Salmonella sp, S. dysenteria* and *S. sonnei* were sensitive. Isolates from female children from Mukuru Kayaba showed that 92.3% of *E. coli* were sensitive and while 100% of *S. sonnei* isolates were sensitive.

Majority (91.7%) of *E. coli* isolates from male children at Mukuru Kwa Njenga were sensitive while 100% of *S. sonnei* isolates were sensitive. From female isolates 97.0% of E. coli isolates were sensitive while 100% of *Salmonnela* sp and *S. typhi* isolates were sensitive.

All the isolates from the male children from Mukuru Kwa Reuben were sensitive. Majority (86.2%) of *E. coli* isolates from female children at Mukuru Kwa Reuben were sensitive, while 100% of *S. sonnei* isolates were sensitive. From Sinai, 88.9% of *E. coli* isolates from male children were sensitive while the rest were 100% sensitive. From female children majority (93.3%) of *E. coli* isolates from Sinai were sensitive

while all the rest were 100% sensitive to the antibiotic Ceftazidime. There were no significant association between the isolates from the defferent gender and area of residence verses the response to Ceftazidime ($p>0.05$).

The resistant isolates were very few due to the fact that the drug is less accessible to the common people hence less misused. Most organisms have not been exposed to the drug owing to its control hence the high sensitivity rate reported in this study. In other similar studies it was reported that, more than 90% of *Shigella* isolates were susceptible to ceftazidime (Kariuki *et al.*, 2013).

Among the diarrhoeagenic isolates, 58% of *Shigella* species and 40% of *E. coli* were resistant to Ceftazidime antibiotics (Fereshteh *et al.,* 2009) which do not agree with the findings of this study. This could be due to the fact that this antibiotic is readily available in that country.

5.3.4 Response of isolates to Tetracycline

Isolates response to Tetracycline in this study were variable. The results showed that 33.3% of *Shigella spp* isolates were resistant and 66.7% sensitive. All the isolates tested were 60.5% sensitive, 0.5% intermediate and 38.9% resistant.

In Mukuru Kayaba, majority (70.6%) of *E. coli* isolates from the male children were sensitive while 29.4% were resistant. Results of female children from Mukuru kayaba showed that 61.5% of *E. coli* isolates were sensitive and 38.5% were resistant while 100% *S. sonnei* showed resistance. From Mukuru Kwa Njenga, most (66.7%) of *E. coli* isolates from male children were sensitive and a few (33.3%) were resistant while 100% of *S. sonnei* isolates were resistant. Isolates from female children at Mukuru Kwa Njenga showed that 51.5% of *E. coli* were sensitive while 100% of *S. typhi* and *Salmonella sp* were sensitive and resistant, respectively. From Mukuru Kwa Reuben

male children, majority (73.1%) of *E. coli* isolates were sensitive and 26.9% were resistant. From female children, isolates from Mukuru Kwa Reuben showed that majority (69.0%) of *E. coli* isolates were sensitive while 100% of *S. sonnei* isolates were sensitive. From Sinai, majority (77.8%) *E. coli* isolates from the male children were sensitive while 100% of *Salmonella sp* and *S. sonnei* were resistant. At Sinai isolates from female children showed that a few (46.7%0 of *E. coli* were sensitive while majority (53.3) were resistant. There was no significant association between gender/area of residence where the bacteria were isolated and response to the drug Tetracycline ($p>0.05$).

This drug is fairly accessible, fairly toxic and is not recommended to be taken with alkali for example milk therefore not preferred for children. It is also not a pediatric recommended drug. In a similar study done by Sang *et al.,* (2013) it was reported that 76 diarrhoeagenic *E. coli* isolates were 63% resistant to tetracycline. The traditional antibiotic Tetracyline (TC) showed low activity against these *E. coli* strains. Among the *Shigella* isolates 35% strains tested showed high levels of resistance (77%) to TC. The TC showed very low activity against *Shigella* strains (Bii *et al.,* 2004; Sang *et al.,* 2013).

5.3.5 Response of isolates to Ampicillin

In total 26.5% of *Escherichia coli* were sensitive, 13.9% were intermediate while 59.6% were resistant. All the *Salmonella spp* isolated were resistant to ampicillin. In total, 24.7% of the isolates were sensitive, 14.2% were intermediate and 61.1% were resistant to Ampicillin. The finding show that there was no significant association

between the isolates and their response to ampicillin ($p>0.05$). More than 50% of the isolates were resistant to ampicillin in this study. When compared in terms of gender and residencial area, 47.1% of *E. coli* isolates from male children at Mukuru Kayaba were resistant while 100% of *Salmonella sp* and *S. sonnei* were resistant. Isolates from female children at Mukuru Kayaba showed that 61.5% of *E. coli* were resistant while 100% of *S. sonnei* isolates were resistant. From Mukuru Kwa Njenga, majority (66.7%) of *E. coli* from both male and female children were resistant. and intermediate, respectively while 66.7% were resistant. Isolates from Mukuru Kwa Reuben; 61.5% of *E. coli* from male children were resistant while100% of *Salmonella* sp were resistant to the drug. Isolates from female children showed that 44.8% % of *E. coli* were resistant while 100% of *S. sonnei* were intermediate. From Sinai, 55.6% of *E. coli* isolates from male children were resistant 100% of *Salmonella* sp and *S. sonnei* isolates from male children were resistant. Isolates from female children showed that majority (73.3%) of *E. coli* were resistant. The isolates from female children from Mukuru Kwa Njenga had p-values of 0.00 hence had significant association to Ampicillin response, the rest were not significant ($p>0.05$). The current study shows that majority of the isolates are resistant to this drug regardless of where it was isolated by gender or area of residence. Amplicillin is easily accessible in Kenya over the counter therefore easily misused leading to emergence of resistance.

In a study done by Harumi *et al.,* (2001) the isolates were 5% resistant to AMP hence their results do not concur with the results of this study. Studies done by Kariuki *et al.,* (2001) reported similar pattern of resistance to these drug. This could be due to the fact that ampicillin has been used in the country for a long time and because of their easy availability and accessibility there is potential for misuse hence resistance.

Prats *et al.,* (2000) in their studies on antibiotic resistance trends in enteropathogenic bacteria isolated in 1985–1987 and 1995–1998 in Barcelona found results which are in agreement with the findings of this study. Studies done in the neighboring Eritrea (Naik, 2006) and Kenya (Brooks *et al.,* 2006), have reported *Shigella* isolates to be completely resistant to ampicillin (Roma *et al.,* 2000; Yismay *et al.,* 2006; Asrat, 2008; Tiruneh, 2009; Reda *et al.,* 2011).

5.3.6 Response of isolates to Ciprofloxacin

Sensitive *Escherichia coli* isolates to Ciprofloxacin were 89.8%, 0.6% were intermediate and 9.6% were resistant. Ciprofloxacin can be recommended for treating enteric pathogens because 90% of the isolated bacteria were sensitive, 1.1% were intermediate while 8.9% were resistant to CIP drug. Mukuru Kayaba majority (82.4%) of *E. coli* isolates from male children were sensitive, while the rest of the isolates from the other slums were 100% sensitive. Isolates from female children showed that 100% of *E. coli* and *S. sonnei* were sensitive from Mukuru Kayaba. From Mukuru Kwa Njenga, majority (91.7%) of *E. coli* isolates from male children were sensitive while 100% of *S. sonnei* were sensitive. Results of Mukuru Kwa Njenga isolates from females showed that 81.8% of *E. coli* was sensitive, while 100% of *Salmonella sp* and *S. typhi* were sensitive. From Mukuru Kwa Reuben, over 90.0% of *E. coli* isolates from both male and female children were sensitive while 100% of *Salmonella sp* and *S. dysenteriae* were sensitive. All the bacteria isolated from the male children from Sinai were sensitive to Ciprofloxacin while isolates from female had varied results. There were no significant association between the isolates from either gender or area of residence and their response to Ciprofloxacin ($p>0.05$).

The drug CIP though readily available, it is still under control in Kenya. It is only dispensed on prescription. This supports the fact that it was very active against the isolated bacteria in this study. The activity of CIP against the enterobacteria in this study are almost similar with that of Harumi *et al.,* (2001) who found that all the isolates causing diarrhoea in their study were about ninety percent (90%) sensitive to CIP. Ciproflaxoxin has been recommended as an effective therapy for traveller's diarrhoea (Harumi *et al.,* 2001; Sang *et al.,* 2006; Sang *et al.,* 2013). Studies of Ogbolu *et al.,* (2012) on enteric bacteria reported results that do not concur with the results of this study, using the E-test, 88.9% of bacterial isolates were resistant to ciprofloxacin, 92.6% were resistant using broth microdilution, 96.3% were resistant using agar dilution and 72.2% were resistant using disc diffusion methods.

5.3.7 Response of isolates to Nalidixic acid

In this study 86.1% of *E. coli* isolates were 86.1%, 1.8% were intermediate and 12% were resistant to Nalidixic acid. In total, 86.8% of the isolates were sensitive, 2.1% were intermediate and 11.1% were resistant to Nalidixic acid. There was no significant association between the isolated bacteria and their response to Nalidixic acid ($p>0.05$). When compared by gender and residence, majority (82.4%) of *E. coli* isolates from male children in Mukuru Kayaba were sensitive to Nalidixic acid while all the other isolates were sensitive to the drug from both male and female. From Mukuru Kwa Njenga, 83.3% of *E. coli* isolates from male children were sensitive, while 100% of *S. sonnei* were sensitive. Isolates from female children at Mukuru Kwa Njenga showed that 78.8% of *Ecoli* were sensitive, while 100% of *Salmonella sp* and *S. typhi* were sensitive. From Mukuru Kwa Ruben, 88.5% of *E. coli* isolates from male children were sensitive while while all the other isolates were sensitive. Isolates from female children showed that 86.2% of *E. coli* were sensitive, while 100% of *S.*

sonnei were sensitive. All the isolates from the male children in Sinai were sensitive to Nalidixic acid. Finally from Sinai, 86.7% of *E. coli* isolates from female children were sensitive while 100% of *Salmonella sp* and *S. dysenteriae* were sensitive. The p-values were >0.05 hence no significant association between the isolates from either gender and area of residence verse their response to the drug.

The current study showed that isolates were very sensitive to this drug.Like CIP this drug is not readily available, strictly controlled as a prescription only drug and has very few generics if any. Studies have reported using NA for dysenteric illness in developing countries (Vila *et al.,* 2000; Von-Sonnenburg *et al.,* 2000; Sang *et al.,* 2006) and it has been used in screening test for prediction of fluoroquinolone resistance (Rogerie *et al.,* 1986). Among the strains reported to be resistant to NA, three enterotoxigenic *E. coli* (ETEC) and four enteroaggregative *E. coli* (EAEC) showed resistance to fluoroquinolones as judged by the National Committee on clinical laboratory standards (NCCLS, 2003) breakpoints. The cross-resistance between these drugs raises concern about the emergence of future fluoroquinolone resistance (Von-Sonnenburg *et al.,* 2000). Nalidixic acid resistance in *Shigella* isolates increased from 43% in 2002 to 55% in 2004 (Sang *et al.,* 2006). Emergence of resistance, though still at low levels, was observed in nalidixic acid (Sang *et al.,* 2006).

5.3.8 Response of isolates to Chloramphenicol

In the current study 91% of *E. coli* isolates to Chloramphenicol were sensitive, 4.8% were resistant and 4.2% were intermediate in this study. This drug is the best for treating diseases caused by the isolated pathogens because it was 91.1% sensitive. All the p-values were greater than 0.05 hence no significant association between the isolated bacteria and their response to the drug. More than 85% of *E. coli* isolates

from both male and female children in Mukuru Kayaba, Mukuru Kwa Reuben, Mukuru Kwa Njenga and Sinai were sensitive to Chloramphenicol. All the other isolates were 100% sensitive for male children from all the slums studied. From Mukuru Kwa Njenga, 66.7% of *Salmonella* sp isolates from female children were sensitive while the rest of the isolates were 100% sensitive from all the slums among the female gender. The isolates from female children from Sinai had significant association with their response to Chloramphenicol ($p=0.01$) while the rest had no statistical significant association ($p>0.05$).

Studies have shown that among the *E. coli* isolated from animals and human beings varying degree of resistance to chloramphenicol exist. However, resistance to chloramphenicol was significantly higher in cattle isolates than in humans as reported (Sang *et al.,* 2013).

5.3.9 Response of isolates to Gentamicin

In this study 94.6% of *E. coli* isolates were sensitive to gentamicin, 0.6% were intermediate and 4.8% were resistant. In total, 95.3% of the isolates were sensitive, 0.5% were intermediate and 4.2% were resistant. The p-values showed that there was no significant association between the isolated bacteria and their response to Gentamicin ($p>0.05$). When compared in terms of gender and residence, more than 88.0% of *E. coli* isolates from male and female children in Mukuru Kayaba, Mukuru kwa Reuben, Mukuru kwa Njenga and Sinai were sensitive to Gentamicin while the other isolates were 100% sensitive. There was no significant association between all the isolates from different gender/ area of residence and response to Gentamicin ($p>0.05$) in this study. This drug was very sensitive because gentamicin is only

available in injectable preparation, hence not abused/misused like most oral antibiotics. This drug is available on prescription only, hence it is regulated.

Studies of Reda *et al.,* (2011) concurs with the current study. They reported high level of sensitivity (92.8%) of *Shigella spp* to gentamicin and 94.1% of the *Salmonella spp* isolates were sensitive. Other studies reported moderate resistance of 25% of all the enteric pathogens isolated and tested for response to Gentamicin (Tiruneh 2009). Other studies have reported high sensitivity of gentamicin to enteric bacteria (Assefa *et al.,* 1997; Roma *et al.,* 2000) including a report from Kenya (Brooks *et al.,* 2006).

5.3.10 Response of isolates to ceftriaxone

The current study showed that 97.6% of *E. coli* isolates were sensitive, 1.2% intermediate and resistant, respectively to ceftriaxone. All the isolates of *Salmonella spp, Salmonella typhi, Shigella dysenteriae* and *Shigella sonnei* were sensitive to ceftriaxone. In total, 97.9% of all the isolates were sensitive while 1.1% were intermediate and resistant, respectively.

In terms of gender and area of residence over 92% of all the isolates from both male and female children in all the slums (Mukuru Kayaba, Mukuru Kwa Reuben, Mukuru Kwa Njenga and Sinai) were sensitive to Ceftriaxone. There was no significant association between the isolated enteric bacteria from different gender and area of residence and response to Ceftriaxone ($p>0.05$). All the isolates were very sensitive to this drug probably because the drug is available only as injectable preparation hence less misuse. Likewise, the drug is administered by trained medical personnel hence not easily available over the counter especially in the slums.

The results concurs with studies of Harumi *et al.,* (2010), they found out that CRO was active against the 268 enteropathogens studied. Although CRO has been used parenterally, especially for peadiatric diarrhoeal diseases in developing countries, unfortunately there is no oral formulation of this drug, creating a limitation for its clinical use in the treatment of traveller's diarrhoea. Orally administered broad-spectrum cephalosporins should be evaluated for therapy of traveller's diarrhoea, based on the high CRO susceptibility pattern of the enteropathogens tested (Harumi *et al.,* 2001). Kariuki *et al.,* (2006) reported that Ceftriaxone was the only antibiotic tested to which all the nontyphoidal *Salmonella spp* (NTS) were 100% sensitive.

5.3.11 Response of isolates to Kanamycin

In the current study 94% of *E. coli* isolates were sensitive, 2.4% were intermediate and resistant were 3.6% to Kanamycin. About 87.5% of *Salmonella spp* isolates were sensitive while 66.7% of *Shigella dysenteriae* isolates were sensitive and 33.3% were resistant and there were no intermediate. In total, 92.6% of the isolates were sensitive to Kanamycin, 4.2% of them were resistant and 3.2% were intermediate. There were no significant association between all the isolates and their response to the drug ($p>0.05$). Comparison in terms of gender and area of residence of the participants were also considered, and it showed the isolates from the male and female children in Mukuru Kayaba were susceptible to Kanamycin. From all the slums (Mukuru Kayaba, Mukuru Kwa Reuben, Mukuru Kwa Njenga and Sinai) the results showed that over 95.0% of all *E. coli* isolates from the male children were sensitive while the other isolates were 100% sensitive.

From all the slums (Mukuru Kayaba, Mukuru Kwa Reuben, Mukuru Kwa Njenga and Sinai) the results showed that over 87.0% of all *E. coli* isolates from the female children were sensitive while the other isolates had varied sensitivities to Kanamycin.

The results showed that there were no significant association between the isolates from different gender/residence and their response to Kanamycin in this study ($p>0.05$). Again this drug is available in injectable form only contributing to less missuse, yet there was varied response from bacteria isolated from female children in all the slums.

In a different study all isolated enteric bacteria were sensitive to kanamycin (Getnet 2004). This could be because of the fact that these antibiotic is prescribed less frequently in treating diarrhoeal cases either due to lesser availability, route of administration or cost. As is indicated in another similar study, this could be either because they are not commonly prescribed or sold on the open market and private pharmacies (Kaba and Ayele (2000). Compared with other similar research findings conducted in Addis Ababa, this study showed increased sensitivity to most tested isolates. The drug Kanamycin is used for the treatment of *Campylobacter* species infection in most countries, but in some countries emerging drug resistant isolates have been reported. In Thailand, for example resistance of 11% and 46% for *C. jejuni* and *C. coli* respectively are reported and in Singapore it was 51% according to the studies of Getnet, (2004).

5.3.12 Response of isolates to Streptomycin

In this study a total of 77.7% *E. coli* isolates were sensitive, 7.2% were intermediate while 15.1% were resistant to Streptomycin. Of the *Salmonella spp* isolated 25% were sensitive while 75% were intermediate. In total, 73.7% of the isolated enteric bacteria were sensitive to Streptomycin, 15.3% were resistant while 11.1% were intermediate. There was no significant association between the isolated bacteria and their response to streptomycin in this study ($p>0.05$). From all the slums (Mukuru Kayaba, Mukuru

Kwa Reuben, Mukuru Kwa Njenga and Sinai) the results showed that over 66.0% of all *E. coli* isolates from the male children were sensitive while the other isolates had varied response to streptomycin. From all the slums (Mukuru Kayaba, Mukuru Kwa Reuben, Mukuru Kwa Njenga and Sinai) the results showed that over 64.0% of all *E. coli* isolates from the female children were sensitive while the other isolates had varied sensitivities to Kanamycin.

The study showed that there was significant association between isolates from Mukuru Kayaba, Mukuru Kwa Njenga and Sinai from male children and their response to streptomycin (p-values of 0.05 and 0.00, respectively). Likewise, the isolates from female gender in Sinai slums and response to the drug had significant association ($p<0.05$) while the rest and response to streptomycin had no significant association ($p\leq0.05$). Streptomycin is a controlled drug since it is only available on prescription. It is used as injectable only thus not readily available over the counter hence less misused. This supports the fact that it was very sensitive in this study. Reports from other studies indicate that percentage of the bacterial isolates showing resistance to streptomycin was high (Nuke *et al.,* 2012; Karim *et al.,* 2001) and does not agrees with the results of this study. Drugs sensitivity and resistance varies from one country to the other depending on its availability. The above studies were done in Turkey and India. Streptomycin drug could be readily available in these countries hence highly misused leading to resistance.

There were isolates which showed multidrug resistance in this study. The *E. coli* isolate was resistant to Amoxicillin/ Clavulanic Acid and Ampicillin, *Salmonella sp* was resistant to Amoxicillin/ Clavulanic Acid (100%), Ampicillin (100%) and Tetracycline (87.5%). The *S. dysenteriae* isolate was resistant to Amoxicillin/

Clavulanic and streptomycin (66.7%), Ampicillin, tetracycline and kanamycin (33.3%). The isolate *S. sonnei* was resistant to Amoxicillin/ Clavulanic Acid (50.0%), Ampicillin (87.5%), tetracycline (75.0%), Sulphamethoxazole/ trimethoprim (37.5%).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In this study the female participants were more than the males. Mukuru Kwa Njenga had the highest (35.2%) number of female children while Mukuru Kwa Ruben had the highest (32.9% number of male children. There was no statistical significant difference between the participants characteristics and their area of residence (p= 0.144). Age of the participants had significant association with the prevalence of diarrhoea (p=0.00).

The total prevalence of isolated bacteria among the participants was very high (90.6%). The *E. coli* bacteria showed the highest percentage of enteric pathogens isolated (35.2%) from female children at Mukuru Kwa Njenga and 29.4% from male children, *Salmonella spp* being second (4.9%) from female at Mukruru Kwa Reuben and the least was *Shigella sonnei* (3.2%) from female children at Sinai.

The *E. coli, Salmonella spp, Shigella dysenteriae* and *Shigella sonnei* isolates were either resistant or intermediate to Amoxicillin/ Clavulanic Acid (from both gender at all the slums) except *Salmonella typhi* from male children at Sinai and *Shigella sonnei* from female children at Mukuru Kwa Reuben. *Escherichia coli, Salmonella, Salmonella typhi, Shigella dysenteriae* and *Shigella sonnei* isolates were sensitive to Ceftazidime with the isolates from male children from Mukuru Kayaba, Mukuru Kwa Reuben, Mukuru Kwa Njenga and Sinai being more sensitive (over 90.0%) compared to isolates from female children (over 86.0%). *Escherichia coli, Salmonella* and *Shigella sonnei* from all the slums and both gender isolates were generally resistant to

Ampicillin. Multidrug resistant isolates were also identified in this study- The *E. coli* isolate was resistant to Amoxicillin/ Clavulanic Acid and Ampicillin, *Salmonella sp* was resistant to Amoxicillin/ Clavulanic Acid (100%), Ampicillin (100%) and Tetracycline (87.5%). The *S. dysenteriae* isolate was resistant to Amoxicillin/ Clavulanic and streptomycin (66.7%), Ampicillin, tetracycline and kanamycin (33.3%).

6.2 Recommendations

The study recommends the following antibiotics to be prescribed to patients diagnosed with the corresponding enteric bacteria as follows;

- Sulphamethoxazole/trimethoprim for *E. coli, Salmonella typhi* and *Shigella dysenteriae*,
- Ceftazidime for *E. coli, Salmonella sp, Salmonella typhi, Shigella dysenteriae* and *Shigella sonnei*,
- Tetracycline for *E. coli, Salmonella typhi* and *Shigella dysenteriae,*
- Ampicillin for *Salmonella typhi*,
- Ciprofloxacin for *E. coli, Salmonella sp, Salmonella typhi, Shigella dysenteriae and Shigella sonnei*,
- Nalidixic acid for *E. coli, Salmonella sp, Salmonella typhi, Shigella dysenteriae* and *Shigella sonnei*,
- Chloramphenicol for *E. coli, Salmonella sp, Salmonella typhi, Shigella dysenteriae* and *Shigella sonnei*,
- Gentamicin for *E. coli, Salmonella sp, Salmonella typhi, Shigella dysenteriae* and *Shigella sonnei*,
- Ceftriaxone for *E. coli, Salmonella sp, Salmonella typhi, Shigella dysenteriae* and *Shigella sonnei*,

- Kanamycin for *E. coli, Salmonella sp, Salmonella typhi, Shigella dysenteriae* and *Shigella sonnei* and Streptomycin for *E. coli, Salmonella typhi,* and *Shigella sonnei*.
- Antibiotics that showed broad spectrum activity by the study with > 90% senstivity include Gentamycin,Ceftriaxone and Ceftazidime.

Further studies should investigate social demographic characteristics of children, parents and their households in order to understand more the causes and predisposing factors of diarrhoea in the slums.

REFERENCES

Abbott, S. (2003). Klebsiella, Enterobacter, Citrobacter, Serratia, Plesiononas, and other Enterobactericeae. In: Manual of Clinical Microbiology, 8th Ed. ASM Press. New York, pg.1090

Abdullahi, M., Olonitola, S.O., and Inabo, I. H. (2010). Isolation of Bacteria Associated with diarrhoea among children attending some hospitals in Kano Metropolis, Kano State, Nigeria. *Bayer Journal of Pure and Applied Sciences* 3 (1): 10 – 15.

Adegunloye, D.V. (2005). Carrier rate of enteric bacteria associated with diarrhoea in children and pupils in Akure, Ondo State, Nigeria. *African Journal of Biotechnology* Vol. 5 (2), pp. 162-164,

Afia, A.N., Bachman, A.C. and Chicure, K. (2009). Oral Rehydration solutions in non - cholera diarrhoea: *American Journal of medicine*. 104 (1): 2596 - 2604

Agafitei, O., Kim, E.J., Maguire, T. and Sheridan, J. (2010). The role of Escherichia coli porins OMPC and OMPF in antibiotic cross resistance induced by subinhibitory concentration of Kanamycin. *Journal of Experimental Microbial Immunology*; 14(1): 34-39

Amyes, S.G.B. (2001). Magic bullets, lost horizons: the rise and fall of antibiotics. *Journal of Infectious Diseases and Immunity* Vol. 3(10): 34-39,

Asrat, A, (2008). Transposition and transposition immunity of transposon Tn3 derivatives having different ends. *European Molecular Biology Organization Journal*, 3(1): 1723–1729.

Banja, W. D, (2010). Antibiotic Use In Two Hospitals In West Wollega, Ethiopia. (Addis Ababa University), *Ethiopian Journal*; 5(1): 127-139

Banwell, J.C. and Sherr, H. (1973). Effect of bacterial enterotoxin on the gastrointestinal tract. *Gastroenterology Journal*, 65: 467 - 495.

Barrett, C.T. and Barrett, J.F (2003). Antibacterials. Are the new entries enough to deal with the emmerging resistant problems? *Currrent opinion on Biotechnology;* 14(1): 621 – 626

Bax, R., Mullan, N. and Verhoef, J. (2000). The millinium bag-the need for developing of new antibacterials; *International Journal of Antimicrobial Agents*; 16(1): 51- 59

Benna, M. (2002). *Manual of Clinical Microbiology*. American Society of Microbiology Press, Washington D.C. 6; 1-9

Bii, C.C., Taguchi, H, T. T Ouko, Muita, L.W., Wamae, N. and Kariuki, S. (2005). Detection of virulence-related genes by multiplex PCR in multidrug-resistant diarrhoeagenic Salmonnella typhi isolates from Kenya and Japan. *American Journal of Medicine*. 1(3): 627-633.

Bopp, C.A. (2003). Escherichia, Shigella, and Salmonella. In: *Manual of Clinical Microbiology*, 8th Ed. American Society of Microbiology Press. Washington, DC, p 189

Bradford, P.A., Bern, C., Martines, J., DeZosa, I. and Glass, R.I. (2001). Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clinical Microbiol Reviews*; 14:933-951.

Brooks, B., Jureen, Manji K.P., Tamin, B.S., Mwakagile, D.S., Urasa, W.K., Fataki, M., Msangi, V., Tellevik, M.G., Maselle, S.Y. and Langeland, N. (2006). High rate of fatal cases of pediatrics caused by gram-negative bacteria with extended-spectrum beta lactamases in Dar es Salaam, Tanzania. *Journal of Clinical Microbiology;* 43(1): 745-749.

Bryce, J.,Boschi-Pinto, C., Shibuya, K. and Black, R.E. (2005). WHO estimates of the causes of death in children. *Lancet* 365: 1147–1152.

Casburn-Jones, A.C., Farthing, M.J.G. and Guerout, A.M. (2007). Management of Infectious Diarrhoea. *Gastroentorology Journal*; 53(1): 296 – 305.

Chambers, J.M. (2001). Insertion sequences revisited. Craig, R. Craigie, M. Gellert, and A. M. Lambowitz (ed.), Mobile DNA II. American Society of Microbiology Press, Washington, DC. p. 305–366.

Chan, C.X., Beiko, R.G. and Ragan, M.A. (2011). Lateral transfer of genes and gene fragments in staphayllococcus extends beyond moltile elements'; *Journal of Bacteriology*, 193(15): 3964-3977

Chart, H. (1998). Toxigenic Escherichia coli: *Journal of Applied Microbiology*; 84(1): 775 - 865.

Cheesbrogh, M. (2005). Mode of action and mechanisms of bacterial resistance. In V. Lorian (ed.), Antibiotics in Laboratory Medicine, 4th ed, Williams and Wilkins, American Press, Baltimore pp. 502–577.

Chitnis, A.S., Caruthers, P.S., Rao, A.K., Lamb, J., Lurvey, R., Beau. D. R., Kitchel, B. and Cancio B., (2012). "Outbreak of carbapenem-resistant enterobacteriaceae at a long-term acute care hospital: Sustained reductions in transmission through active surveillance and targeted interventions". Infection control and hospital epidemiology: *Journal of the Society of Hospital Epidemiologists of America*; 33 (10): 984–92.

Chopra, J., Lurvey, R., Beau, De-Rochars, V. and Kitchel, B (1998). Protein synthesis as a target for antibacterial drugs: current status and future opportunities. *Expert opinion on investigational drugs*; 7(1,):1237-1244.

Cirz, R.T., Chin, J.K., Andes, D.R. and Romesberg, F.E. (2005). Inhibition of mutation and combating the evolution of antibiotic resistance; *Public Library of Science Biology*; 3(6): e176

CLSI, (2011). Performance Standards for Antimicrobial Disk Diffusion Susceptibility Tests: 8th Informational Supplement. Document M100S8. Villnova, PA: National Committee for Clinical Laboratory Standards. Informational Supplement; 940 West Valley Roa; M100-S17; 27(1): 26- 30

Cowan, W. and Stell, K. (2002). Effects of Cryptosporidium parvum infection in Peruvian children: growth faltering and subsequent catch-up growth. *American Journal of Epidemiology*; 148(1): 497–506.

Cundiliffe, K.P. (1989). Mixed infections in childhood diarrhoea: results of a Community Study in Kiambu District, Kenya; *East African Medical Journal*; 66(1): 715-23.

Dupont, H.L. (2000). Shigella species (bacilliary dysentry). In: Mandell, Doughlas, and Bernett's principles and practice of infectious Diseases 5th Ed. University press, London, pg. 710

Dupont, S. (2010). Carbapenem Hydrolysing Metallo ¬ ß ¬ Lactamases: *International Journal of Current Pharmaceutical Research;* 3(3):1-9.

English, M., Dennis, Schr, Der, H., Helga, S. and Frank, H. (2003). Hypothetical performance of syndrome-based management of acute paediatric admissions of children aged more than 60 days in a Kenyan district hospital. *East African medical Journal*; 81(2): 166-173.

Eugene, W., Denise, G., Evanns, C. and Martha, T. (2012). Microbiology- A human perspective, 7th edition, American press, Washington D.C; pg 472

Falagas, M.E., Grammatikos, A.P.and Michalopaulos A. (2008). Potential of old generation antibiotics to address current need for new antibiotics. *Antimicrobials Infectious Journal*; 6(5): 593-600

Falagas, M.E., Mohammad, H. and Mohammad R.Z. (2010). Fosfomycin for the treatment of multidrug-resistant extended-spectrum β-lactamase producing Enterobacteriaceae . *Lancet Infectious Dieases*; 10(1):43–50

FAO, (2008). World Food Summit, Medecins Sans Frontiers Malnutrition Fact Sheet -[http://issuu.com/msf_australia/docs/malnutritionfactsheet].

Farmer, J.J. (2003). Enterobactericeae: Introduction and identification: In Manual of Clinical Microbiology, 5th Ed. American Society of Micribiology Press, New york, pg. 1020

Feikin, D.R., (2010). Evaluation of the optimal recall period for disease symptoms in home-based morbidity surveillance in rural and urban Kenya. *American Journal for Microbiology*; 4(1): 450-458.

Fereshteh, J., Mohammad, H. and Mohammad R. (2009). Prevalence and antimicrobial resistance of diarrhoeagenic Escherichia coli and Shigella species associated with acute diarrhea in Tehran, Iran; *The Canadian Journal of Infectious Diseases and Medical Microbiology;* 20 (3)56 – 62

Fischer, D., Elofsson, A., Rice, D. and Eisenberg, D. (1986). Assessing the performance of fold recognition methods by means of a comprehensive benchmark. In *Pacific Symposium on Biocomputing, Hawaii.*, pp. 300-318.

Forsberg, B.C., Petzold, M.G., Tomson, G. and Alleback, P. (2007). Diarrhoea case management in low and middle income countries. *World Health Organization Bulletin*; 85(1): 42 - 48

Fre´de´rique M.B.I., Podglajen, I.B.P. and Ekkehard, C. (2008). Enzyme structural plasticity and the emergence of broad spectrum antibiotic resistance. *European Molecular Biology Organization reports*, 9(4): 1- 15.

Gebreyes, N.D.F. (2002). The movement of Tn3-like elements: transposition and cointegrate resolution. Mobile DNA II (Craig NL, Craigie R, GellertM& Lambowitz AM, eds),. American Society for Microbiology Press,Washington, DC. pp. 272–302

Geo, R. L., Oriá, R. B., and Moore, S. R. (2004). Nutrition for children with diarrhoea; NIH *Malnutrition Public Access journal;* 66(9): 487-505.

Getnet, B. (2004). Antimicrobial sensitivity pattern of Campylobacter species among children in Jimma University Specialized Hospital, Southwest Ethiopia; *Ethiopian Journal of Health Development*;18(3): 1 - 29

Gianella, R.A., Haile, A. and Drake, K.W. (2009). Effect of purified *E. coli* heat stable enterotoxin on intestinal cyclic nucleotide metabolism and fluid secretion infect. *Immunology Journal*; 24(1): 19 - 23.

Gniadkowski, M. (2008). Evolution of Extended-Spectrum Beta-Lactamases by Mutation: *Clinical Journalof Infectious Microbiology*; 14 (1):11-32.

Graham, D.I. (2005). Association of early childhood diarrhoea and .cryptosporidiosis with impaired physical fitness and cognitive function four- seven years later in a poor urban community in northeast Brazil. *American Journal of Tropical Medicine and Hygiene*; 61(1): 707–713.

Green, D.W. (2002). The bacterial cell wall as a source of antibacterial targets. *Expert Opinion on Therapeutic Targets*. 6(1): 1- 19.

Guerrant, R.L., Hughes, J.M., Lima, N.L. and Crane, J. (1990). Diarrhoea in developed and developing countries: Magnitude, special settings and aetiologies. *Reviews on Infetious Diseases*, 12(1): 541-50.

Guerrant, R.L., Schorling, J.B., McAuliffe, J.F. and De-Souza M.A. (2001). Diarrhoea as a cause and an effect of malnutrition: diarrhoea prevents catch-up growth and malnutrition increases diarrhoea frequency and duration. *American Journal of Tropical Medicine and Hygiene*; 47(1): 28–35.

Hall, R.M. and Stokes, H.W. (2004). Intergrons or superintergrons? *Microbiology Journal*; 150(1): 3-4

Hare, R. (1970). The birth of pennicillins; Carbapenem-hydrolyzing β -lactamases. *Journal of Antimicrobial Agents and Chemotherapy*; 41(1): 223 -32

Harumi, G., Zhi-Dong J., Javier, A., Adachi, D., Brett L., Mangala, P., Robert S. and Herbert L. (2001). In Vitro Antimicrobial Susceptibility Testing of Bacterial Enteropathogens Causing Traveler's Diarrhea in Four Geographic Regions; *Journal of Antimicrobial Agents and Chemotherapy*; 45(1): 212–216.

Higgins, C.F. (2007). Multiple Molecular Mechanisms for Multidrug Resistance Transporters. *Nature Pubmed*; 446(7137): 749-57.

Ifeanyi, C., Ifeanyichukwu, C., Isu R., Akpa A. and Ikeneche N. (2010). Enteric Bacteria Pathogens Associated With Diarrhoea of Children in the Federal Capital Territory Abuja, Nigeria; *Science Journal*; 3(1) 1-28.

Irena, A.H., Mwambazi, M. and Mulenga, V. (2011). Diarrhoea is a Major killer of Children with Severe Acute Malnutrition Admitted to Inpatient Set-up in Lusaka, Zambia. *Nutrition Journal*, 10(1): 110 - 140

Jana, S. and Deb, J.K., (2005). Molecular targets for Design of Novel inhibitors to Circumvent aminoglycoside resistance. *Current Drug Targets Journal;* 6(3): 353-61

Johnsborg, O., Eldholm ,V. and Havarstein, L.S. (2007). Natural genetic transformation: prevalence mechanisms and functions; *Research Journal of Microbiology*; 158(10): 767 -778

Kaba, M. and Ayele, F. (2000). Ethnographic study of diarrhoeal diseases among under-five children in Mana district, Jimma Zone, Southwest Ethiopia. *Ethiopian Journal of Health Development*; 14(1):77-83.

Karim, A., Poirel, L., Nagarajan, S. and Nordmann, P. (2001). Plasmid- mediated extended-spectrum beta-lactamase (CTX-M-3 like) from India and gene

association with insertion sequence ISEcp1. *Journal Microbiol Letters*; 201(1): 237–241.

Kariuki, S., Revathi, G., Kariuki, N., Kiiru, J., Mwituria, J. and Hart, C. (2006). Characterisation of community acquired non-typhoidal Salmonella from bacteraemia and diarrhoeal infections in children admitted to hospital in Nairobi, Kenya; *British Microbiology Council*; 6(1): 101- 120.

Kariuki, S., Kariuki N., Kiiru J., Mwituria, J. and Hart, C. (2013). Genotype Analysis of Escherichia coli Strains Isolated from Children and Chickens Living in Close Contact. *British Microbiology Council*; 65(2): 472-476.

Kariuki, S., Kariuki, N., Kiiru, J., Mwituria, J. and Hart, A. (2001). Characterisation of community acquired non-typhoidal Salmonella from bacteraemia and diarrhoeal infections in children admitted to hospital in Mbagathi District Hospital-Kenya *Britich Microbiology Council;* 11(5):41-50.

KDHS. (2010). Kenya Demographic and Health Survey, Government press, Nairobi, Kenya, page 120.

Kelesidis, T., Yurieva, O., Minakhina, S. and Nikiforov, V. (2008). Tigecycline for the treatment of multidrug-resistant Enterobacteriaceae; *Journal of Antimicrobial and Chemotherapy*; 62(1): 895–904.

Kelly, J.A., Moews, P.C., Knox, J.R., Fre're, J.M. and Glunysen, J.M. (2000). Penicillin target enzyme and the antibiotic binding site. *Science Journal*; 218(1): 479-481.

Knothe, G.P., Shah, P., Kremery, V., Antai, M. and Mitssuhashi, S. (1983). Transferable resistance to cefotaxime, cefoxitin, cefamadole and cefuroxime in clinical isolates of Klebsiella pneumoniae and Serratia marcescens. *Journal of Infections*; 11(1): 315-317.

Kumar, A. and Schweizer, P. (2005). Bacterial resistance to antibitotics: Active Efflux and reduced uptake. *Journal of Advanced Drugs Delivery Reviews*; 57(1): 1486-1513

Lakshmi, R., Nusrin, K.S., Georgy, S.A. and Sreelakshmi, K.S., (2014). The Role of Betalactamases in Antibiotic Resistance; *International Journal of Pharmacy*; 5(2): 37-40

Lambert, P.A. (2005). Bacterial resistance to antibiotics: modified targets sites. *Advanced Drug Delivery Reviews*. 57(1): 1471-1485

Law, D. and Chart, H., Bryan, L.E. and Godfrey, A.J. (1998). A Review on Enteroaggregative *Escherichia coli*. *Journal of Applied Microbiology*; 84(1): 685 - 697.

Li, W. and Rufs, S. (2000). Plant molecular biology. *Advances in Applied Science Research*; 76(3): 443 -419

Li, X.Z., Nikaido, H. and Nikaidol, A., (2009). Efflux–Mediated Drug Resistance in Bacteria: *Drug Resistance Journal*; 69(12): 1555-623

Lima, A.M., Moore, S.R. and Barboza, M.S. (2000). Persistent diarrhoea signals a critical period of increased diarrhoea burdens and nutritional shortfalls: a prospective cohort study among children in North-eastern Brazil. *Journal of Infectious Diseases*; 181(1): 1643–1651.

Mandigan, M., Martinko, J., Stahl, D., Clark, D. and Brook, P. (2012). Biology of Microorganisms; 13th Edition; Pearson Education press, Berlin, p 797

Mandomando, I.M., Macete, E.V., Ruiz, J., Sanz, S. and Abacassamo, F. (2007). Etiology of diarrhoea in children younger than 5 years of age admitted in a rural hospital of southern Mozambique. *American Journal of Tropical Medicine and Hygiene*; 76: 522–527

Martinez, J.L. and Baquero, F., (2000). Mutation Freguencies and antibiotic resistance. *Antimicrobial Agents and Chemotherapy Journal* ; 44(7): 1771-1777

Martinez, L. Pascual, A. and Jacoby, G. (1998). Quinolene resistance from a transferable plamid; *American Medical Journal*; 351(1): 797 - 9.

Mazel, D., (2006). Intergrons; Agaents of Bacterial Evolutions, *Nature Reviews Microbiology*; 4(1): 608-620

Mcauliffe, F. and Souza, .A.E. (1992). Diarrhoea as a Cause and an Effect of Malnutrition : Diarrhea Prevents Catch-Up Growth And Malnutrition Increases Diarrhea Frequency And Duration. *Journal of Malnutrition*; 47(1): 28-35.

Miller, P., Hirschhorn, N., Bryan, L. and Godfrey, A. (2003). The Effect of a National Control of diarrhoeal diseases Program on Mortality: The case of Egypt. *Social Science and Medicine*; 40(1): 1-30.

MOH. (2010). Ministry of Health; Rwanda, National Institute of Statistics and Research: Demographic and Health Survey of Rwanda Kigali, Rwanda. Page 102.

Molbak, K., Jeasen, H., Ingholt, L. and Aaby, P. (2002). Risk factors for diarrheal disease incidence in early childhood; a community cohort study form Guinea – Bissau. *American Journal of Epidemiology;* 14(6):273 – 82.

Mondal, D., (2009). Attribution of Malnutrition to Cause-Specific Diarrheal Illness : Evidence from a Prospective Study of Preschool Children in Mirpur, Dhaka ,

Bangladesh. Short Report: *Journal of Tropical Medicine and Hygiene;* 80(5): 824-826.

MSF. (2008). REVIEW IRT and CMT b-lactamases and inhibitor resistance. *Journal of Tropical Paediatrics*; 37(2): 293-99.

Murray, B., Tenover, P., Pfaller, B. and Godfrey, J. (2003). Manual of clinical microbiology. American society of microbiology Press, Washington DC; 6(1): 190-199

Naffalines, R.J., (2001). The role of Intracellular calcium in the induction of intestine section. *Clinical Microbiology Research*; 1(1): 63 – 71

Naik, R. J. (2006). The role of intracellular calcium in the induction of intestinal secretion. *Clinical Research Reviews;* 1(1): 63-71.

Nathisuwan, S., Burgess, D. and Lewis, J. (2001). Extended- spectrum beta-lactamases: epidemiology, detection and treatment. *Journal of Pharmacotherapy;* 21(1): 920-928

Neu, H.C., (1993). The Crisis in Antibiotic Resistance, *WHO reviews and reports*, 257.

Nuke, S., Cemal, S., Osman, B., Feyza, C. and Dilek, D. (2012). Antibiotic Resistance Profiles of Enteric Bacteria Isolated from Kucukcekmece Lagoon (Istanbul–Turkey). Turkish *Journal of Fisheries and Aquatic Sciences*; 12(1): 699-707.

Obimbo, E.M., Mbori-Ngacha, D., Ochieng, J., Richardson, B. and Otieno, P. (2004). Predictors of early mortality in a cohort of human immunodefi- ciency virus type 1-infected African children. *Pediatrics Infectious Diseases Journal*; 23(1): 536–543.

Ogawa, N., Nakamura, A. and Nakaya, R. (2009). Cinemicrographic study of tissue cultures infected with shigella flexneri. *Journal of Medical Sciences and Biology;* 21(1): 259 - 273.

Ogbolu, D.O., Terry-Alli, O.A., Daini, O.A., Olabiyi, F.A. and Igharo, E.A. (2012). Comparison of E-test with other conventional susceptibility testing methods for ciprofloxacin and gentamicin against gram negative enteric bacilli. *African Journal of Medical Sciences*; 41(2): 135-40.

Olowe, O.A., Olayemi, A.B., Eniola, K.T. and Adeyeba, O.A., (2003). Aetiological agents of diarrhoea in children under five years of age in Osogbo, Osun State. *African Journal of Clinical and Experimental Microbiology*; 4 (2):62-66

Opondo, C. (2011). Effect of a multi-faceted quality improvement intervention on inappropriate antibiotic use in children with non-bloody diarrhoea admitted to

district hospitals in Kenya. *Britich Medical Council of Pediatrics*, 11(1):109 - 111.

Palpasa, K., Pankaj, B., Sarala, M. and Gokarna, R. (2010). Antimicrobial susceptibilities of enteric bacterial pathogens isolated in Kathmandu, Nepal, during 2002-2004; *Journal of Infections in Developing Countries*; 5(3):163-168.

Patterson, D.L. and Bonomo, R.A. (2005). Extended-spectrum b-lactamases: A clinical update. *Clinical Microbiology Reviews*; 18(1): 657–686.

Patwari, A. K., Manorama, D. and Ridie, D. (1993). Clinical and Laboratory prediators of invasive diarrhea in children less than five years old. *Journal of Diarrhoeal Diseases Research;* 11(4): 211 – 216.

Pfeifer, S.R., (2010). Gene cassettes and cassette arrays in mobile resistance integrons. *Microbiol Reviews;* 33(1): 757–784.

Queenana A.M. and Bush K. (2007). Carbapenemases: the versatile B-lactamases. Clinical Microbial Rev; 20(1): 440 - 458

Reda, A., Berhanu, S., Jemal, Y., Gizachew, A., Sisay, F. and Jean-Michel, V. (2011). Antibiotic susceptibility patterns of Salmonella and Shigella isolates in Harar, Eastern Ethiopia; *Journal of Infectious Diseases and Immunity*; 3(8): 134-139,

Rhode, J. E. and Northrup, R.S. (2001). Taking science where diarrhoea is. Acute diarrhoea in children. GBA Foundation symposium. *Amsterdam Elselvier Excerpta*. 42(1): 339 - 366

Robicsek, A., Jacoby, G.A. and Hooper, D.C. (2006). The world wide emergence of plasmid- medicted quinolone resistance. *Lancet Infectious Diseases*; 6(1): 629 - 640.

Rohde, R.S. (1984). The Etiology of Diarrhea Studied in the Community in Young Urban Gambian Children. *Journal of Diarrhoeal Diseases Research*; 3(1): 7-13.

Roma, S., Lloyd-Evans, N., Williams, K. and Rowland, M. (2000). The Etiology of Diarrhea Studied in the Community in Young Urban Gambian Children. *Journal of Diarrhoeal Diseases Research*; 3(2):27-43.

Rusi, H., Michod, R.E., Bernstein, H. and Nedelcu, A.M., (2008). Molecular Machinery for DNA Translocation in Bacterial Conjugation; Plasmids; Current Research and Future Trends; Laister Academic Press. London; pg 178

Saidi, S., Iijima, Y., Sang. K., Mwangudza, A., Oundo, J., Taga, K., Aihara, M., Nagayama, K., Yamamoto, H., Waiyaki, P. and Honda, T. (2005).

Epidemiological study on infectious diarrheal diseases in children in a coastal rural area of Kenya. *Microbiol Immunology*; 41(1): 773-778.

Sanders, R. L. (1987). A novel integrative and conjugative element (ICE) of Escherichia coli: the putative progenitor of the Yersinia high-pathogenicity island. *Molecular Journal of Microbiology* ; 51(1): 837–848.

Sandosturn, S., Arinthip T. and Broker, P. (2010). Conjugation in *Escherichia coli*. *The International Union of Biochemistry and Molecular Biology*; 35(6): 440-445.

Sang, W.K. (2007). Serotypes and virulence properties of Shiga toxin producing *E. coli* from patients with diarrhea in Kajiado and Narok districts of Kenya, PhD Thesis.

Sang, W.K., Mwangudza, A.K., Oundo, J.O., Taga, K. and Aihara, M. (2013). Serotypes and virulence properties of Shiga toxin producing E. coli from patients with diarrhea in Kajiado and Narok districts of Kenya. *Molecular Microbiology Journal*; 53(1): 937–948.

Sang, W. K., Valerie, O. and David, S. (2013). Prevalence and antibiotic resistance of bacterial pathogens isolated from childhood diarrhoea in four provinces of Kenya; *Journal of Infectious Diseases in Developing Countries*; 6(7):572-578.

Skippington, E. and Ragan, M.A., (2011). Lateral genetics transfer and the constructions of genetic exchange communities. *Microbial Reviews*; 1(25):707-735

Skold, Ö. (2000). Sulfonamide Resistance: Mechanisms and Trends. *Drugs Resistant Updates*; 3(1): 155-160

Snyder, J. D. and Merson, M. H. (1982). The Magnitude of the Global Problem of Acute Diarrheal Disease: A Review of Active Surveillance Data. *Bulletin of the World Health Organization*; 60:605-13.

Spratt, B. G. (1994). Resistance to antibiotics mediated by target alterations. *Science Journal;* 264(1): 388–393

Subha, A., Ananthan, S. and Poirel, L. (2002). Extended spectrum Beta - Lactamase (ESBL) Mediated resistance to third generation Cephalosporins among Klebsiella Pneumoniaea in Chennai. *Indian Journal of Medical Microbiology*; 20 (2): 92 - 95

Sule, E.I., Aliyu, A.M. and Abdulaziz, B.M. (2011). Isolation of Diarrhoeagenic Bacteria in Children Attending Some Selected Hospitals Within Kaduna Metropolis, Kaduna State, Nigeria. *Continental Journal Applied Sciences;* 6 (1): 1 - 6

Tadesse, D.A., Shaohua, Z., Emily, T., Sherry, A., Aparna, S., Mary, J.B. and Patrick, F. (2012). Antimicrobial Drug Resistance in *Escherichia coli* from Humans and Food Animals, United States, 1950–2002; *Journal of Emerging Infectious Diseases*; 18(5): 15-29

Takeuchi, A. (2008). Penetration of the Intestinal Epithelium by vairous Micro-organisms. *Current Journal of Tropical Pathology*; 54(1): 1-27.

Tharian, J.A., Padmapriya, R. and Thirunalasundari, T. (2013). Biochemical Mechanisms of Bacterial Resistance; *Advances in Applied Science Research*; 4(4): 413 -419

Tiruneh, W.A. (2009). The Cognitive Effects of Diarrhoea, Malnutrition, and Entamoeba histolytica Infection on school-age children in Dhaka Bangladesh. *American Journal of Tropical Medicine and Hygiene*; 74(1): 475-481.

UNICEF/WHO. (2009). Diarrhoea: Why children are still dying and what can be done. WHO Press, Geneva; pp. 1–68.

Vargas, M., Gascon, J., Casals, C., Schellenberg, D., Urassa, H., Kahigwa, E., Ruiz, J. and Vila, J. (2004). Etiology of Diarrhoea in Children less than 5 years of age in Ifakara, Tanzania. *American Journal of Tropical Medicine and Hygiene*; 70(1): 536 - 539.

Vila, J., Vargas, M., Ruiz, J., Corachan, M., De-Anta, M. and Gascon, J. (2000). Quinolone resistance in Enterotoxigenic Esherichia coli causing diarrhoea in travelers to India in comparison with other geographical areas. *Antimicrobial Agents Chemotherapy Journal;* 44(1): 1731–1733.

Von-Sonnenburg, F., Tornieporth, N., Collard, F., Waiyaki, P., Lowe, B., Peruski, L.F., Chatterjee, S., Costa-Clemens, S., Cavalcanti, M., DuPont, L., Mathewson, J. and Steffen, R. (2000). Risk and etiology of diarrhoea at various tourist destinations. *Lancet*; 356(1): 133–134.

Walsh, C. (2000). Melecular Mechanisms that confer antibacterial drugs resistance. *Nature reviews Journal*; 406(1): 775-781

WHO. (2013). Pneumonia and Diarrhoea progress report .Ending Preventable Child Deaths From Pneumonia and Diarrhoea by 2025.The intergrated Global Action Plan for Pneumonia and Diarrhoea (GAPPD).World Health Organization, Geneva; 22

WHO. (1988). Persistent diarrhoea in children in developing countries: memorandum from a WHO meeting. *World Health Organization Bulletin*; 66:709–717.

WHO. (2007). The World Health Organization Report 2002: Reducing Risks, Promoting Healthy Life, WHO, Geneva; 198

WHO. (2009). A manual for physicians and other senior health workers. Global Water Supply and Sanitation Assessment; WHO, Geneva. Page 69

WHO. (2014). Global report on antibiotic resistance and threats to public health. *World Health Organization Bulletin*; 51: 1-15

WHO. (2005). Diarrhoea Treatment Guidelines Including new recommendations for the use of ORS and zinc supplementation for Healthcare Workers. *World Health Organization Bulletin* ; 612:70–71

Yismay, C.G., Huttly, S.R., Fuchs, S.C., Barros, F.C., Garenne, M. Leroy, O., Fontaine, O., Beau, J.P., Fauveau, V. and Chowdury, H. R. (2006). International Difffences in Clinical Patterns of Diarrhoeal Deaths: A Comparison of Children from Brazil, Senegal, Bangladesh, and India. *Journal of Diarrhoeal Diseases Research*; 11 (1):25-29

APPENDICES

Appendix 1: Patient written consent form

The study wishes to determine bacterial causes of diarrhoea and the best treatment options. The duration of participation is short and ends by bringing of stool specimen to the laboratory for processing. If the results for culture are to be relied upon diarrhoea may worsen. So, treatment options are reviewed after specimen collection without awaiting results. Benefits of this study will include free medical services, although other benefits are not instantaneous, results of the study will greatly help other children in future with diarrhoea by administering effective antibiotic treatment. Information obtained will be highly confidential with use of patient code rather than name. Injuries sustained in the Hospital will be compensated. In case of further clarifications call Samuel whose mobile telephone numbers are; 0726988298.

To be filled by the Parent/Guardian

I/We (Names of Parent(s)/ Guardian of child) .....

Child's name .....

AGREE: ☐ (Tick where appropriate)

DISAGREE: ☐

To take part in this study by providing the stool specimen of my/our Child

Kiswahili translated informed consent

(Kiswahili version)

Ridhaa

Utafiti huu unataka kubaini ni viini gani vya bacteria ambavyo huasababisha kuhara kwa watoto wachanga. Muda wa utafiti huu ni mfupi na utaisha wakati ambapo kinyesi cha mtoto kitaletwa mahabarani. Matokeo ya mahabara hayatategemewa, sababu kuhara kutakidhiri. Kwa hivyo matibabu ya kuondoa kuhara yataendelea bila ya kungoja matokeo ya mahabara. Manufaa ya utafiti huu yatakuwa kuhudumiwa hospitalini bila malipo, manufaa mengine hayatakuwa sasa hivi, lakini yatawafaidi watoto wengine wanaohara kwa kuwapa dawa bora zaidi siku za usoni. Ujumbe wowote utahifadhiwa kwa njia ya siri kwa kuhifadhi namabri ya usajili wala sio jina. Ajali yoyote itakayo mpata mtoto akitibiwa itagaramiwa. Kwa maelezo zaidi, uliza Samuel ambaye simu yake ya mkononi ni; 0726988298.

Sehemu ya kujazwa na Mzazi/Mlezi

Mimi/Sisi (Majian ya Mzazi/Mlezi wa mtoto) .....

Jina la Mtoto .....

NIMEKUBALI: ☐ (Weka alama panapo stahili)

NIMEKATAA: ☐

Kujihusisha na utafiti huu kwa kupeana kinyesi cha mtoto kifanyiwe utafiti wa viini

Appendix 2: Patient information sheet

Date of Hospital visit: ...../ ...../ 2013

Names of the child: .....

Gender: (Tick where appropriate) Male ☐ Female ☐

Age of the child in Months.....

Residence:

.....

Name of Mother/ Legal guardian: .....

Appendix 3: Permission to carry out the research

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

RE: PERMISSION TO CARRY OUT THE STUDY

Mr Samuel Soi is a Master of Science student at the University of Eldoret. His study "Antibiotic Susceptibility Profile of Enterobacteriaceae Isolated from Children aged under Six years Presenting with Diarrhoea at Mukuru Slums, Nairobi- Kenya" is nested within a bigger study (survey) which is funded by CDC and is carried out in KEMRI and MoH. The student was recruited to carry out filed work in Mukuru slums during long rains in April 2013 and later analysed the samples in KEMRI laboratories. He was allowed to use the bacterial data for his thesis. The main survey is investigating diarrhoea diseases caused by parasites, bacteria and viruses at the slums in Nairobi County during long and short rains over a period of five years beginning from 2011 and ends in 2015. The study covers all the main slums within the County.

Kindly accord him any necessary assistance. Thank you.

Yours Sincerely

Richard Kipserem Korir

Research Officer and Investigator of the main study – KEMRI

CENTRE FOR MICROBIOLOGY KEMRI 14 FEB 2014 P.O. Box 18464 - 00202 NAIROBI

Appendix 4: Culture Media Preparation

MacConkey Agar without salt: 47 g of the medium will be suspended in one litre of purified water. Heating with frequent agitation will be done and boiling for one minute to completely dissolve the medium. This solution will be autoclaved at 121^{o}C for 15 minutes. Media will be poured into petri-dishes and allowed to cool. Media will then be packed and stored refrigerated at 4^{o}C for future use.

Salmonella shigella Agar preparation: 60 g of the medium will be suspended in one liter of purified water. Heating with frequent agitation will be done and boiling for one minute to completely dissolve the medium. The mixture will not be autoclaved.

Mueller Hinton agar preparation: 38 g of the medium will be suspended in one litre of purified water. Heating with frequent agitation and boiling for one minute to completely dissolve the medium will be done. Autoclaving will be done at 121°C for 15 minutes and cooling to room temperature when poured into petri-dishes. Checking of prepared Mueller Hinton Agar to ensure the final pH is 7.3 ± 0.1 at 25^{o}C will be done. Storing in refrigerator at 4^{O}C for future use will be done.

Luria-Bertani (LB) broth preparation: Ten grams of tryptone, five grams of yeast extract and 5 grams sodium chloride will be reconstituted in one liter of sterile water.