ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF COMMON CIRCULATING ENTERIC BACTERIA PATHOGENS IN HIV POSITIVE AND NEGATIVE CHILDREN IN DANDORA, KENYA

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Antimicrobial Susceptibility Patterns Of Common Circulating Enteric Bacteria Pathogens in HIV Positive And Negative Children in Dandora Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I dedicate this work to my loving family: My husband, Mr. Juma Motha ,My mother, Mrs. Maimuna B. Tsuma, My father, Mr. Said Rashid , My sister, Mrs. Umi S. Rashid ,My caring brothers, Abdallah S. Rahid, Hassan S. Rashid ,Yusuf S. Rashid and Rashid S. Rashid and My friend Dr.Catherine K. Kaluwa for their love, encouragement and support.

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AST	Antimicrobial Sensitivity Testing
Bp	Base Pairs
DNA	Deoxyribo -Nucleic Acid
dNTP	deoxy-Nucleotide Tri-Phosphate
eaeA	Intimin gene
EAEC	Enteroaggregative *E. coli*
EHEC	Enterohemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E.coli*
KEMRI:	Kenya Medical Research Institute
MAC:	MacConkey media
MDR:	Multi Drug Resistance
MgCl:	Magnesium Chloride
NaCl	Sodium Chloride
PCR:	Polymerase Chain Reaction
SERU:	Scientific and Ethical Review Unit

SC: Simmon's Citrate

SIM Sulphur Indole Motility

SMAC Sorbital- MacConkey agar culture

ST Heat stable toxin

STEC Shiga-toxigenic *E.coli*

Stx1 Shiga toxin 1

Stx2 Shiga toxin 2

SNP Single nucleotide polymorphism

SS Salmonella Shigella

SSA Sub Saharan Africa

TSI Triple Sugar Iron

Vt1 Verotoxin type 1

Vt2 Verotoxin type 2

Vte2 Verotoxin type 2e

XLD Xylose Lysine Deoxy-cholate

W/V Weight per Volume

WHO World Health Organisation

ABSTRACT

There is an increasing trend in antibiotic resistance among enteric bacterial pathogens, particularly in developing countries, where bacterial diarrhoea is one of the main causes of morbidity and mortality, especially in children. It is documented that bacterial pathogens in HIV patients may manifest differently from infections in immune-competent hosts. Most studies on enteric bacterial pathogens and HIV co-infection have focused on children under five years of age. This study aimed at evaluating the distribution of common circulating enteric bacterial pathogens; *Escherichia coli* (*E. coli), Shigella, Salmonella*, and resistance patterns of these isolates among HIV positive and negative children aged between five and twelve years living in Dandora. This was analytic cross-sectional study of HIV positive children enrolled at Nyumbani Lea Toto HIV/AIDS outreach program in Dandora, while HIV negative children were from the same area (preferably sibling). After obtaining informed consent and assent forms, stool samples were collected and sent to the Microbiology laboratory in Kenya Medical Research Institute for processing. The samples were cultured using differential media for enteric bacteria. Suspected isolates were further identified using conventional biochemical methods and serotyping. Multiplex PCR was done on *E. coli* isolates to detect virulence factors responsible for different *E. coli* pathotypes. Antimicrobial susceptibility testing was done using Kirby Bauer disc diffusion method. The overall prevalence of pathogenic *E. coli*, *Shigella* and *Salmonella* were 44 (28%), 31 (19.7%) and 0 (0.0%), respectively. Enteroaggregative *E. coli* (43.2%) was the main *E. coli* pathotypes observed. The distribution of pathogenic *E. coli* from HIV positive and negative children was 12.7% and 15.3%, respectively, while that of *Shigella* was 6.4% and 13.4%. Antimicrobial Susceptibility testing was done against commonly prescribed antibiotics in the clinic that provide medical services for HIV positive children. The levels of resistance vary with each drug and HIV status as follows; STX (95%$^{HIV\ +ve}$ Vs 96%$^{HIV\ -ve}$), Amp (70 %$^{HIV\ +ve}$ Vs 75%$^{HIV\ -ve}$) and Nal (55%$^{HIV\ +ve}$ Vs 50%$^{HIV\ -ve}$) in *E. coli* isolates. Among *Shigella* isolates the levels of resistance were as follows, STX (100% Vs 81%), Amp (60% Vs 62%) and Nal (30% Vs 48%). The results portrayed in this study are striking in that the prevalence of pathogenic *E. coli* and *Shigella* was high among HIV negative children as compared to HIV positive children. The antimicrobial susceptibility test showed a slight difference in resistance patterns. However, resistance to Gentamicin and Ciprofloxacin was higher in HIV positive compared to HIV negative children, which indicates emerging resistance

CHAPTER ONE

INTRODUCTION

1.1 Background information

Diarrheal disease is a significant health problem, particularly in the developing world, where adequate sanitation facilities are lacking (Iruka *et al*., 2000). In Sub-Saharan Africa, diarrheal disease is a major cause of morbidity and mortality, accounting for an estimated 16% of deaths among children less than five years of age (Bryce *et al.*, 2005) and is compounded by HIV/AIDS epidemic (Prasad *et al*.,2000). It is generally estimated that about 100% of HIV-positive patients in the developing world may suffer from chronic diarrhoea, as estimated on a cumulative lifetime incidence, but the situation in the developed world is better, where a lower percentage of HIV-positive patients suffer from diarrhoea .In Kenya, human immunodeficiency virus (HIV) epidemic has aggravated diarrheal illness, which is the main cause of morbidity and mortality among HIV-infected patients. It is believed that bacterial infections in AIDS patients manifest differently from immune-competent hosts (Navaneethan *et al*.,2008). The prevalence of HIV in Kenya stands at 7.4 per cent with an estimated 70,000 - 100,000 infants exposed to HIV every year (WHO 2009) with one third of total pediatric admissions and 16% of all deaths among pediatric inpatients are diarrhea-related (Demographic. K., 2010).

A broad range of etiologic agents are responsible for acute and chronic diarrheal disease. The prevalence of such agents varies greatly by geographical region, season, patient age, immune status, and socioeconomic conditions. Majority of the enteric bacterial pathogens are transmitted through the fecal-oral route especially in developing countries where access to clean water and proper sanitation are lacking. Several enteric bacterial pathogens cause diarrhea but the most commonly circulating that are often associated with diarrheal illness in Kenya are; Pathogenic *E. coli*, *Salmonella* and *Shigella* species (Sang *et al*.,2012).

The bacterial pathogen most commonly associated with childhood diarrhea is *Escherichia coli* and at least six categories have been described: enteropathogenic *E. coli* (EPEC), which causes childhood diarrhea, enterotoxigenic *E. coli* (ETEC) which is associated with childhood and travelers' diarrhea , enteroinvasive *E. coli* (EIEC) that causes dysentery, enterohemorrhagic *E. coli* (EHEC) which leads to hemorrhagic colitis (HC) and haemolytic uremic syndrome(HUS), enteroaggregative *E. coli* (EAggEC) which is typically associated with persistent diarrhoea in children, especially in developing countries, enteroadherent *E. coli* (EAEC) which is a key cause of traveler's diarrhoea in North America; and Shiga toxin-producing *E. coli* (STEC), commonly associated with foodborne diseases, and diffusely adherent *E. coli* (DAEC) (Nataro *et al*., 1998; Mamun et al., 1993).

Most studies in Kenya have indicated a high prevalence of resistance to commonly used antimicrobials, such as ampicillin and trimethoprim-sulfamethoxazole (Chattaway *et al*.,2016, Sang *et al.,* 2012). However, resistance patterns are often regionally –specific, yet little data describes how these patterns have changed over time during HIV era. In Kenya, there is plausible data on the epidemiology of the diarrheal disease and the antimicrobial resistance of causative agents on HIV – positive children above five years of age. This study aimed at bridging the gap to improve diarrhea management protocols in HIV-infected children in the region.

1.2 Problem Statement

Diarrhea remains the second biggest infectious diseases killer after respiratory diseases of children under the age of five years. Globally, there are nearly 1.7 billion cases of diarrheal disease every year, many with acute and chronic effects-malnutrition, stunted growth, and impaired cognitive development, leading to diminished productivity over a lifetime for millions of people (Lorntz *et al*.,2006; Moore *et al*., 2010). In Kenya, diarrhoea continues to be a major cause of morbidity and mortality in infants and children. It is ranked as the third cause of death, behind malaria and pneumonia (Rono Salinah *et al*., 2014). The increase in bacterial resistance has compounded the challenges posed by a high incidence

of enteric bacterial infections. Studies conducted within the East African region from mid-70s show an increasing trend in antimicrobial resistance (Omulo et al., 2015).

Most studies on common enteric bacterial pathogens and HIV co-infection focus on children <5 years (Rono *et al.*, 2014; Van Eijk *et al*., 2010; Wilcox *et al*.,1996). Therefore, there is a need to ascertain the distribution of these enteric pathogens and their antimicrobial susceptibility in this population.

1.3 Justification

Several studies worldwide have shown that diarrhoea is one of the major causes of morbidity and mortality in HIV-infected persons (Fletcher *et al*.,2013). Success in managing diarrhoea in HIV-infected persons can be attained once the causative agents are established and their resistance patterns are monitored. In Kenya, studies have been carried on enteric bacterial pathogens in HIV-infected and non-infected children aged less than five years and not above(Van Eijk *et al*., 2010) Hence there is a need to establish the distribution of these causative agents and their antimicrobial-resistant patterns to the commonly prescribed antimicrobials to the patients with diarrhoea to ensure appropriate treatment and control of infection.

1.4 Research Question

1. Among HIV-positive and negative children, what is the distribution of common enteric bacteria, pathogenic *E. coli, Shigella*, and *Salmonella* species?
2. Which are the virulence factors associated with diarrhoea in *E.coli* isolates from HIV positive and HIV negative children?
3. Are enteric bacterial pathogens in HIV-positive and negative children susceptible to antimicrobial agents?

1.5 Null Hypothesis

There's no difference in the proportion of children with enteric bacterial pathogens among HIV positive and HIV negative children aged 5-12 years.

1.6 Objectives

1.6.1 General objective

To evaluate the distribution and antimicrobial susceptibility patterns of common circulating enteric bacterial pathogens among HIV positive and negative children (5-12 yrs) in Dandora, Kenya.

1.6.2 Specific objective:

1. To determine the distribution of pathogenic *E.coli*, *Salmonella,* and *Shigella* species among HIV positive and HIV negative children (5-12 yrs.) in Dandora.
2. To determine the virulence factors in *E. coli* isolated from HIV positive and HIV negative children (5-12 yrs) in Dandora.
3. To determine the antimicrobial susceptibility patterns of enteric bacterial pathogens in HIV positive and HIV negative children (5-12yrs) in Dandora

CHAPTER TWO

LITERATURE REVIEW

2.1 Common Enteric Bacterial Pathogens

Several bacterial pathogens can invade and colonize the human gut. However, a few of them are known to be pathogenic, often causing disease regularly. These include pathogenic *Escherichia coli*, *Salmonella,* and *Shigella* (Colombara *et al.,* 2016). The genus *Escherichia* is also associated with numerous bacterial infections, including urinary tract infection (UTI), traveler's diarrhea, cholangitis, bacteremia, and cholecystitis. Enteritis, defined as swelling or inflammation of the intestines, is commonly associated with *E. coli* (Colombara *et al*., 2016). At least six categories of diarrheagenic *E. coli* have been described: enteropathogenic *E. coli* (EPEC), which causes childhood diarrhea, enterotoxigenic *E. coli* (ETEC) which is associated with travelers' diarrhea , enteroinvasive *E. coli* (EIEC) that causes dysentery, enterohemorrhagic *E. coli* (EHEC) which leads to hemorrhagic colitis (HC) and haemolytic uremic syndrome(HUS), enteroaggregative *E. coli* (EAggEC) which is typically associated with persistent diarrhoea in children, especially in developing countries, enteroadherent *E coli* (EAEC) which is a key cause of traveler's diarrhoea in North America; and Shiga toxin-producing *E. coli* (STEC), commonly associated with foodborne diseases, and diffusely adherent *E. coli* (DAEC) (Nataro *et al*., 1998; Kelly *et al*., 1985; Mamun et al., 1993). Two additional categories, cell-detaching *E. coli* (CDEC) (Gunzburg *et al*., 1993) and cytolethal distending toxin-producing *E. coli* (CLDTEC), have been proposed. Classification is based on the presence of different chromosomal or plasmid-encoded virulence genes in *E. coli* enteropathogens that are absent in most commensal strains, as well as their pattern of interaction with epithelial cells and tissue culture monolayers (Nataro *et al*., 1998).

The pathogenicity of Escherichia coli is a complex multi-factorial mechanism involving a large number of virulence factors, which vary according to the pathotype. The virulence factors include attachment functions, host cell surfaces, modifying factors, invasion

characteristics, toxins, adhesion, and capsule production, as well as secretion systems, which export other virulence factors and pilot them to the target cells.

The genus *Salmonella* is made up of numerous species and serotypes. *Salmonella* are some of the most common enteric pathogens and are a known cause of bacterial foodborne diseases. *Salmonella* has also been implicated in several conditions, including typhoid or enteric fever (mainly due to *Salmonella typhi* and *Salmonella paratyphi*), endovascular infections, bacteremia, and enterocolitis (typically caused by *Salmonella typhimurium*, *Salmonella heidelberg*, and *Salmonella eneritidis*).

The genus *Shigella* comprises bacteria divided into four major O antigenic groups, including *S. dysenteriae*, *S. flexeneri*, *S. boydii*, and *S. sonnei*. *Shigella spp.*are invasive bacteria that cause shigellosis that can be spread from person to person. Shigellosis can be mild to severe, depending on several factors such as an individual's HIV status, and symptoms can range from diarrhoea (watery and sometimes bloody), fever and nausea. Cases of bacterial diarrhoea due to *Shigella spp*. occur worldwide but are more prevalent in developing countries. *Shigella spp*. is a major cause of bacterial dysentery, accounting for an estimated 165 million cases and up to 1 million deaths each year worldwide (Livio *et al.,* 2014).

2.2 Prevalence of bacterial diarrhoea

Recent data indicates that 1 in 9 deaths that take place in children around the world are due to diarrhoea. This is even worse in HIV-infected children. In developing regions, such as Africa, a higher burden of diarrheal disease is experienced. Socioeconomic inequalities experienced in developing regions of the world have contributed to poor health care access, maternal education, and poor water and sanitation infrastructure, all of which impact children's health (Fletcher *et al.*, 2013). It is estimated that up to 50% of Africans lack access to safe water, with 66% lacking access to hygienic sanitation practices. There are data limitations in estimating accurate diarrhoea cases in children aged between 5 and 12 years, however, available data indicates the importance of diarrheal disease as a key

morbidity and mortality cause in children aged less than five years within the African region (Fletcher *et al.,* 2013).

The challenges posed by a high incidence of enteric bacterial infections have been compounded by the increase in bacterial resistance. Studies conducted within the East African region from mid- 70s show an increasing trend in antimicrobial resistance (Omulo *et al.,* 2015).

In Kenya, diarrhea continues to be a major cause of morbidity and mortality in infants and children. It is ranked as the third cause of death, behind malaria and pneumonia (Rono *et al.,* 2014).

2.3 Modes of Contamination and Effects of Common Enteric Bacterial Pathogens

Enteric bacterial pathogens are usually transmitted through several routes that include contaminated food and water, person to person, and through the fecal oral route. A large proportion of the infections, about 36%, take place through contaminated food and water (Fletcher *et al.,* 2013). In the developing world, over 80% of foodborne illness attributable to non-typhoid S*almonella*, pathogenic *E. coli*, *Shigella*, and *Campylobacter*. Majority of the infections take place through the fecal-oral route. Up to 63% of children in low and middle income countries who suffer from persistent diarrhea have been found to harbor *E. coli* infection, often a marker of poor hygiene (Abba *et al.,* 2009).

2.4 Diagnosis and Treatment

In most HIV-negative individuals, gastroenteritis is usually self-limiting, and even the care offered is usually supportive to prevent dehydration and control symptoms. Blood and stool tests are often conducted when symptoms persist. Physical examination to determine important clues such as travel history (in the case of *E. coli* infection), exposure to contaminated water, change in diet (*Salmonella* and Staphs), abdominal cramps, malaise, fever, diarrhoea and /or dysentery (in case of shigellosis) and medication, among others. Other tests can include complete blood count, electrolytes, and kidney function

tests. Blood and mucus in the stool are common manifestation of shigellosis, faecal leukocytes are usually noted on examination due to inflammatory and invasive characteristics of the organism.

Diagnosis is confirmed by culturing a stool sample, using differential media for enteric bacterial that inhibit the growth of Gram-positive bacteria, such as MacConkey, Sorbitol-MacConkey for entero haemorrhagic *E.coli*, *Salmonella Shigella* (SS)Agar to distinguish between the *Salmonella* species, *Shigella* species, or xylose-lysine-deoxycholate (XLD), are necessary for isolation of *E.coli*, *Salmonella* and *Shigella* from clinical specimens. After overnight incubation at 37 °C *E.coli* appears pink while *Salmonella* and *Shigella* appear as pale, non-lactose fermenters colonies on MacConkey agar and as pink with a black dot in the colonies on XLD or SS for *Salmonella* and pink colonies for *Shigella.* Further identification of suspect colonies can be done to confirm using standard methods such as biochemical test and agglutination with species specific antisera **(**Edward *et al.*, 1972*).* For the detection of *E. coli* virulence genes, a polymerase chain reaction should be done after a biochemical test on *E. coli* suspect colonies.

The treatment of bacterial diarrheal disease in children is often done through oral rehydration therapy using balanced electrolyte solutions such as Gatorade. Clear fluids should also be provided. In the event of persistent infection, various antimicrobial agents are used to effectively treat enteric bacterial infections, and they include Beta-lactams, quinolones, macrolides, and others such as sulfonamides, cotrimoxazole, and tetracycline (Levy & Marshall, 2004).

2.5 Antimicrobial Susceptibility pattern of common enteric bacterial pathogens

2.5.1 General Antimicrobial Resistance

Antimicrobial resistance in gram-negative enteric bacteria such as *E.coli*, *Salmonella* and *Shigella* has previously been established. While a number of factors have been found to associate with resistance, investigations in HIV positive patients have generally reported

high rates of resistance (Marbou&Kuete, 2016). Studies show that *Staphylococcus aureus* and *Salmonella cholereasuis* were more frequently observed in the HIV positive, with a high resistance rate to commonly used antibiotics was observed, and this including a high *E.coli* resistance (Phe *et al.,* 2013). An evaluation of bacteremia causative agents and antimicrobial susceptibility among HIV-1-infected children on antiretroviral therapy in Uganda and Zimbabwe in 2013 established the following: *Streptococcus pneumoniae* (28.3%), *Staphylococcus aureas*(8.7%), *Klebsiella pneumoniae* (4.7%), *Pseudomonas aeruginosa* (4.7%), *Salmonella spp* (4.7%), *E.coli* (3.9%), *Haemophilus influenz*a (0.8%), other bacteria (42%). Majority of the tested isolates were highly susceptible to ceftriaxone, ciprofloxacin, and Cefotaxime; a very few of them were found to be susceptible to cotrimoxazole (Musiime *et al.,* 2013)

Earlier investigations of bacterial isolates among severely malnourished children infected and uninfected with the human immunodeficiency virus -1 in Kampala, Uganda, established that 58% were gram-negative consisting of 27.6% *S. typhimurium*, 26.3% *Staph aureus,* 11.8% *S. enteriditis,* and 13.2% *Strep. pneumoniae*; severely immune-compromised children were likely to grow (Bachou *et al.,* 2006).

2.5.2 Antimicrobial Resistance Patterns in Salmonella species

Salmonella Typhimurium, a common chicken meat contaminant that causes significant health problems in humans, has been evaluated for antimicrobial susceptibility in various antimicrobial agents. In children, less than two years of age with known HIV status in Kisumu, Kenya, a high incidence of diarrhea in HIV positive children compared to HIV negative, accompanied with a low susceptibility of *Salmonella* and *Shigella* to standard antibiotic treatments were established (Van Eijk *et al.,* 2010).

An assessment of the phenotypic properties of clinically isolated *S. Typhimurium* exposed to ciprofloxacin and ceftriaxone showed that *S. Typhimurium* CCARM 8009 was highly resistant to ampicillin, penicillin G, Kanamycin, and streptomycin, with a minimum inhibitory concentration of value of more than 512 μg/ml; *S. Typhimurium* ATCC 19585

was only resistant to erythromycin. Additionally, *S. Typhimurium* ATCC 19585 showed the highest β-lactamase when exposed to ceftriaxone (8.2 μmol/min/ml); in regard to ethidium bromide (EtBr), a considerable increase *S. Typhimurium* ATCC 19585 when treated with eflux pump inhibitors (Kim & Ahn, 2017).

2.5.3 Antimicrobial Resistance Patterns in *E. coli*

E.coli, the most common Gram-negative bacterial pathogen affecting humans, has been found to be resistant to a number of antimicrobial agents in various studies. It is resistant to Cefotaxime and tetracycline, though exposure to chlortetracycline significantly decreases the proportion of Cefotaxime resistant *E.coli* (Platt *et al.,* 2008).

A study by (Sang *et al.,* 2012) in Kenya indicated that pathogenic *E.coli* strains were resistant to locally prescribed antibiotics as follows: Chloramphenicol 24%, Ampicillin 25%, Tetracycline 63%, Fosfomycin 54% and Trimethoprim/Sulphurmethoxazole 84%. Various food types or items may also harbour *E. coli*. This includes raw egg surface, raw chicken, raw meat, and unpasteurised milk. Possible contamination has been established in the following order, raw chicken (23%), followed by vegetables at a salad (13.3%), raw egg surface (10%) and unpasteurised milk (6.7%) (Rasheed et al., 2014). Reasons for the high resistance rates could be contamination of abattoirs with cattle and poultry products, in addition to the use of untreated sewage in irrigating vegetables.

2.5.4 Antimicrobial Resistance Patterns in Shigella species

Shigella species are the bacterial agents responsible for invasive acute intestinal infections that clinically appear as bloody or watery diarrhoea. Shigellosis has been identified as a major disease burden in developing countries. A number of antimicrobial resistance cases have been identified in relation to *Shigella*. Resistance to the broad spectrum the broad-spectrum β-lactam ampicillin has previously been observed, in addition to emerging resistance to ciprofloxacin and plasmid mediated azithromycin resistance in multidrug-resistant *Shigella* isolates (Nuesh-Inderbinen *et al.,* 2016).

HIV-positive individuals are at an increased risk of acquiring infections resulting from antimicrobial-resistant *Shigella spp.* An increase in the risk of acquiring and transmitting Shigellosis may occur in HIV infected individuals as a result of alteration in the immune system or an increase in the carriage and shedding time (Murray *et al.,* 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

This study focused on children served by the Nyumbani Lea Toto HIV/AIDS community outreach program. It is one of the largest non-governmental pediatric HIV/AIDS providers in Kenya that extends care of HIV-positive children into the community through providing medical services such as nutrition, counselling, and capacity building. This was a Nested study ongoing protocol No KEMRI/SERU/CMR/0049/3387 by Matey *et al*. The study was conducted in one of the centers served by the Nyumbani Lea Toto HIV/AIDS community outreach Program (Dandora). In this facility, research has not addressed the infection of pathogenic *E.coli, Salmonella* and *Shigella* and their resistance to the drugs commonly used to manage bacterial diarrhoea in these children (5-12 yrs). Samples collected from this site were processed at KEMRI –CMR laboratories.

3.2 Study Design

Analytic cross-sectional study design was adapted to compare the prevalence of common enteric bacterial pathogens, their antimicrobial susceptibility and virulence patterns among HIV positive and HIV negative children (5- 12 yrs) from the same locality.

3.3 Study Population

The study population comprised of HIV positive and HIV negative children aged between 5 and 12 years, residing at Dandora estate. The HIV positive children targeted for this study were those enrolled in Nyumbani Lea Toto HIV/AIDS outreach program and receiving free ART while HIV negative children from same locality (preferably sibling). The children live with their parents or guardians in the aforementioned study sites and the HIV positive children together with their parents visit the study clinics every 3 months for medical check-ups. During these routine visits, the clinicians were requested

to randomly select, consent and assent participants for the study. Willing participants were encouraged to also bring one of their previously confirmed HIV uninfected children/sibling. A recent HIV test (within three months) on the child based on the clinic medical records was used to confirm the HIV status. One of the staff member at the Nyumbani clinics was accessing the children's medical records.

3.3.1 Inclusion criteria

- Children aged 5 - 12 years who are HIV positive and enrolled at Nyumbani Lea Toto HIV/AIDS outreach programin Dandora with diarrhoea symptom whose parents /guardian were willing to consent.
- Children aged 5 - 12 years who are HIV negative and living in Dandora during the study period with diarrhoea symptoms whose parents /guardian were willing to consent.

3.3.2 Exclusion criteria

- Children aged 5- 12 years who are HIV positive and enrolled at Nyumbani Lea Toto HIV/AIDS outreach program in Dandora whose parents /guardian were unwilling to consent.
- HIV positive children who have not been enrolled in Nyumbani Lea Toto HIV/AIDS outreach program in Dandora and shows diarrhoea symptoms.

3.4 Sample size determination

The aim of the study was to compare the prevalence of enteric bacteria pathogens and antimicrobial susceptibility in HIV-positive and HIV-negative children. The sample size was estimated using the formula for comparison between two groups (Fleiss, 1981).

$$n \geq \frac{2\bar{p}(1-\bar{p})(Z_{\beta}+Z_{\frac{\alpha}{2}})^2}{(p_1-p_2)^2}$$

Where;

n = minimum sample size for one group

Z_{β}= critical value corresponding to 80% power

$Z_{\frac{\alpha}{2}}$ = critical value corresponding to 0.05 type I error

$p_1 - p_2$ = difference in proportion of the even between two groups (HIV positive and negative).

p_1 is the estimated prevalence of antimicrobial resistance in enteric bacterial pathogens among HIV positive children and p_2 is the prevalence of resistance in enteric bacterial pathogens among HIV negative children. p_1= 0.410 and p_2= 0.117 based on Gentamycin (GEN) resistance (Rono *et al.*, 2014)

$\bar{p}$ = pooled prevalence = (prevalence in HIV positive group P_1+ prevalence in HIV negative group P_2) / 2

Estimated pooled prevalence ($\bar{p}$) = 0.264

Using this formula, the minimum sample size to detect the difference of 30% (p_1-p_2=0.293) in antimicrobial resistance is 36 children (with enteric bacterial pathogens) per group. The estimated proportion of HIV positive children with enteric bacterial pathogens is 0.347 (Rono *et al.,* 2014). The sample size to obtain 36 HIV positive children with enteric bacterial pathogens was 80. The total sample size of 160 children derived from the above formula.

3.5 Sampling Method

To control for the heterogeneity of prevalence of common enteric bacterial pathogens in the study population due to demographic factors (age and sex), stratification was done

based on sex and age. Simple random sampling was used to select children from each stratum.

3.6 Specimen Collection

After obtaining ethical approved from KEMRI -Scientific Ethical Review Unit (SERU) and Informed consent from parents/guardians of the study participants. A single stool sample was collected in a sterile plastic container labeled with their unique study number and transported within 6 hours at ambient temperature to KEMRI (CMR) laboratories for processing.

3.7 Isolation and characterization

3.7.1 Specimen Processing

All stool samples were plated onto differential media for enteric bacterial pathogens; MacConkey agar, Sorbitol-MacConkey agar (for detection of entero haemorrhagic *E.coli*) and *Salmonella Shigella* Agar (SS) (to distinguish between the *Salmonella* and *Shigella* species) The plates were incubated aerobically at 37ºC for 18-24 hours.

The presence of *E. coli* was identified by the appearance of pink colonies on MacConkey agar, *Salmonella* was identified by the appearance of pink dark centered colonies on SS medium, and *Shigella* small pale/ clear colonies.

3.7.2 Biochemical Identification

After overnight growth at 37ºC a single colony suspected as *Salmonella* species, *Shigella* species and approximately five *E.coli* colonies of different morphologies were picked and inoculated onto biochemical media; Triple Sugar Iron Agar(TSI), Sulphur Indole Motility medium (SIM), Simmons citrate agar and Urea agar as follows:

a. In TSI agar, inoculation was done by stabbing the bottom of the tube with a single down and up motion. After stabbing, streaking the slant portion of agar was done immediately and the tube was loosely cupped.
b. In SIM tube, inoculation was done by stabbing in a single down and up motion in the center of the agar going three –fourths of the way down the tube by keeping the wire straight as possible, and the tube was loosely cupped.
c. In Simmons citrate agar tube, inoculation was done by streaking the slanted surface of the agar, and the tube was loosely cupped.
d. In Urea agar tube, inoculation was done by stabbing 2-3 times into the agar later the tube was loosely cupped.

All the tubes were placed in a test tube rack and incubated aerobically at 37ºC for 18-24 hours. Thereafter, all the tubes were examined for typical biochemical reactions as indicated on the table 3.1.

Table 3.1: Biochemical identification

SPECIES	**TSI**				**SIM**		**CIT**	**UREA**
	SLOPE	**BUTT**	**H_2S**	**GAS**	**MOT**	**IND**		
Salmonella typhi	R	Y	+ Weak	_	+	_	_	_
Salmonella paratyphi A	R	Y	_	+	+	_	_	_
Other *Salmonella*	R	Y	+	D	+	_	D	_
Shigella	R	Y	_	_	_	D	_	_
E. coli	Y	Y	_	+	+	+	_	_

Key: R= Red- pink (Alkaline reaction), Y= Yellow (Acid reaction), H_2S =Hydrogen sulphide (blackening), Mot =Motility, Ind= Indole test, Cit= Citrate test, d= different strains give different results.

All confirmed E. coli strains were plated onto Muller – Hinton Agar plates and incubated at 37ºC for 18-24 hours.

3.7.3 Serotpe identification

The strains identified as *Salmonella* and *Shigella* by their colonial morphology and biochemical properties were serotped using O antigen and H antigen antisera (Denka Seiken Co LTD, Tokyo-Japan) by slide agglutination assay .

The strains were sub-cultured onto nutrient agar plates and tested for agglutination on glass slides that had been divided into four sections using an indelible marker. A drop of 2% (W/V) NaCl solution was used as a negative control, and a drop of appropriate antiserum (Denken Seiken) was used in

other sections to serotype test bacteria. By using a sterile inoculation loop, a single representative colony was emulsified with the NaCl solution. The same procedure was repeated with the other sections of the slide containing antiserum. The slide was gently rocked and examined for visible agglutination within one minute using the naked eye (Bettelheim and Thampson, 1987).

3.8 Multiplex Polymerase Chain Reaction (PCR)

A multiplex PCR assay that allowed detection of eleven trait genes or virulence factors that characterise *E. coli* based on the method of Pass *et al.*,(2000). Primers for amplifying segments of Shiga toxins (Stx_1, Stx_2 and $Stx2_e$, Cytotoxin necrotising factors (CNFI and CNF2) attaching and effacing mechanisms (*eaeA*), enteroaggregative mechanism (Eagg), enteroinvasive mechanism (Einv), and heat-labile (LT) and heat-stable (ST1 ad ST2)

toxins were tested (Pass *et al*., 2000). Vero toxin assay was carried out according to Konowalchuk method of Konowalchuk *et al*., (1977).

Four multiplex primer sets were prepared in four tubes i.e. Set A amplify *vt1, vt2, and vt2e* and *eaeA* gene , Set B amplify *CNF1* and *Eagg*, Set C amplify *CNF2* and *Einve gene* and Set D amplify *st1, st2* and *lt* gene.

3.8.1. Colony PCR

PCR was performed in 0.2ml Eppendorf tubes in a PTC-200 thermal cycler (MJ Research Inc, Watertown, Massachusetts, U.S.A.) in a reaction volume of 25µl. A colony of *E.coli* isolate was picked from Muller-Hinton Agar plate and suspended in 20µl of nuclease free water and vortex. From this suspension DNA template of 2µl was added to a 25µl reaction mixture containing 2.0µl of 10mM mix deoxynucleiotide triphosphate (dNTPs), 2.5µl of $MgCl_2$ (25mM), 2.5µl 10X buffer solution and 1.25µl of each of the PCR primer with concentration of (0.5 pmol/µl) (Bioserve Biotechnologies, Laurel, MD.USA). 0.3µl of Taq Polymerase(5U/µl), (Applied Biosystems, Roche Molecular, Inc, and Branchbury, New Jersey, USA) was added to this reaction mix. Base sequences and predicted sizes of amplified products for the specific oligonucleotide primers was used in this study are as shown in table 3.2.

Table 3.2: Sequences for DEC Multiplex PCR primers; forward (fp) and reverse (bp)and their respective product sizes

Target gene	Amplicon size	Primers	Sequence (5'-3')
MEinv a	140	invasive	fp: TGG AAA AAC TCA GTG CCT CTG CGG
MEinv b			bp: TTC TGA TGC CTG ATG GAC CAG GAG
mVT1 a	121	Verotoxin-1	fp: ACG TTA CAG CGT GTT GCA GGG ATC
mVT1 b			bp: TTG CCA CAG ACT GCG TCA GTG AGG
mVT2a	102	Verotoxin-2	fp: TGT GGC TGG GTT CGT TAA TAC GGC

mVT2b			bp: TCC GTT GTC ATG GAA ACC GTT GTC
MEagga	194	Aggregative	fp: AGA CTC TGG CGA AAG ACT GTA TC
mEaggb			bp: ATG GCT GTC TGT AAT AGA TGA GAA C
mST1a	160	Heat-stable	fp: TTT CCC CTC TTT TAG TCA GTC AAC TG
mST1b		toxin1	bp: GGC AGG ATT ACA ACA AAG TTC ACA G
mST2a	423	Heat-stable	fp: CCC CCT CTC TTT TGC ACT TCT TTC C
mST2		toxin 2	bp: TGC TCC AGC AGT ACC ATC TCT AAC CC
MEaeA	241	Attaching	fp: TGA GCG GCT GGC ATG AGT CAT AC
mEAEAb		and effacing	bp: TCG ATC CCC ATC GTC ACC AGA GG
mLT1a	360	Heat-labile	fp: TGG ATT CAT CAT GCA CCA CAA GG
mLT1b		toxin 1	
			bp: CCA TTT CTC TTT TGC CTG CCA TC
mCNF1a	552	Cytotoxic	fp: GGC GAC AAA TGC AGT ATT GCT TGG
		necrotizing-1	
mCNF1b			bp: GAC GTT GGT TGC GGT AAT TTT GGG
mCNF2a	839	Cytotoxic	fp: GTG AGG CTC AAC GAG ATT ATG CAC TG
mCNF2b		necrotizing-2	
			bp: CCA CGC TTC TTCTTC AGT TGT TCC TC

* *Ampli- Amplicon size* source: (Pass et al., 2000).

The assay was set as follows:

The PCR program consists of an initial denaturation cycle at 95°C for 30 s, followed by 20 cycles each at 95°C for 30 s (denaturation), 63°C for 30 s (annealing), 72°C for 30 s (polymerisation) and a final extension of 72°C for 5 mins.

Reaction products are separated by agarose gel electrophoresis on a 2% (Sigma) high-resolution agarose stained using AZ in gel vision dye in Tris Borate (TBE) buffer at 100V for one and half hours.A molecule size marker (100bp DNA ; Promega, Madison,Wisconsin,USA) was added to every agarose gel to estimate the size of amplicons.DNA in the gel were visualised on a UV trans illuminator and photographed using a B/W instant Polaroid film.

3.9. Antibiotic Susceptibility Testing

Antibiotic Susceptibility Testing: Antibiotic susceptibility was done using Kirby-Bauer disk diffusion method (Bauer *et al*., 1966). The following antimicrobial agents were used to test for susceptibility for bacterial pathogens: Tetracycline, Chloramphenicol, Ampicillin, Erythromycin, Gentamicin, Ciprofloxacin, Cefotaxime, Trimethoprim/Sulfamethoxazole, and Nalidixic acid.

3.9.1 Inoculum preparation

All isolated colonies of enteric pathogens from overnight growth on Muller – Hinton agar plates were emulsified in 3ml of sterile distilled water to achieve the correct inoculum turbidity. The inoculum for susceptibility testing was compared against the McFarland 0.5 turbidity standard. *E. coli* ATCC 25922 and *Shigella flexneri ATCC* 12022 strains were used as the test quality control organisms .

Sterile, non-toxic swabs were dipped into the inoculum suspension and excess fluid was removed by pressing the swab agaist the iside wall of the test tube. The swab was then used to streak the the entire MHA surface of 150mm plate, rotating the plate approximately 90^0 each time to ensure even distribution of the inoculum. Before applying the antibiotic disk, the agar surface was left to be compeletely dry by allowing the absorption of excess moisture for 10-15 minutes. *E. coli* ATCC 25922 was used as a quality control for drug potency and growth. All the plates were incubated at 37^0 C for 18 hours, zones of inhibitions were measured and the interpretation of results was done according to Clinical Laboratory Standard Institute guidelines (CLSI 2017) as shown on the table 3.3 below.

Table 3.3: Interpretation table zone diameter and breakpoints

Antimicrobial Agents	Disk Content	Interpretive Criteria and Zone Diameter Breakpoints (Nearest whole mm)	QC ATCC® 25922

		S	I	R	Passed
Ampicillin (AMP)	10μg	≥17	14-16	≤13	16
Chloramphenicol (C)	30μg	≥18	13-17	≤12	22
Ciprofloxine (CIP)	5μg	≥21	16-20	≤15	30
CIP *Salmonella* spp	5μg	≥31	21-30	≤20	34
Cefotaxime(CTX)	30μg	≥26	23-25	≤22	32
Erythromycin (ERY)	15μg	≥21	16-20	≤15	25
Gentamicin (CN/GEN)	10μg	≥15	13-14	≤12	20
Trimethoprim/Sulfamethoxazole (TMP-SMX)	1.25/23.75μg	≥16	11-15	≤10	28
Tetracycline (TET)	30μg	≥15	12-14	≤4	18
Nalidixic Acid	30μg	≥19	14-18	≤13	23

QC- Quality control strain . CLSI-Clinical Laboratory Standards Institute guideline 2017

3.9.2 Data Analysis

Data collected was entered, cleaned and analysed using Microsoft excel 2010 (Microsoft corporation, USA).Using Stata version 14, Chi -square test was used in computing the *p*-value for the distribution of enteric bacteria pathogens, and differences were considered significant at $p < 0.05$. Binary logistic regression model was used to compute the odds ratios, and CI's.

CHAPTER FOUR

RESULTS

4.1 Distribution of the study population in terms of HIV status

A total of 160 children aged 5 -12 years were recruited for this study , out of these 157 stool samples were analysed of which 50.3% (79) were from HIV negative while 49.7% (78) were from HIV positive children (figure 4.1). The mean age of these children was 10.48 years with a standard deviation (SD) of 3.18 years.

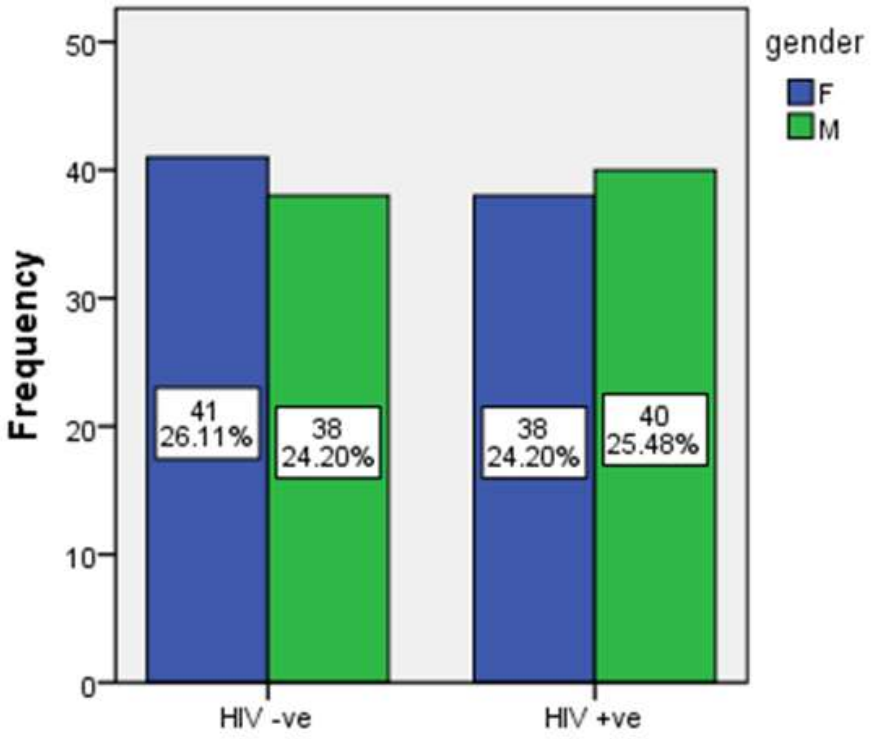

Figure 4.1: The number of samples processed from both arms

4.2 Prevalence of diarrheagenic bacteria among HIV positive and negative children

The overall prevalence of diarrheagenic bacteria was 47.8% (75), out of this 45 were from HIV negative while 30 were from HIV positive children. Pathogenic *E. coli* was the highest diarreagenic bacteria isolated (28.0%), followed by Shigella species (19.7%) and no *Salmonella* species (spp) was isolated. The *P* value of pathogenic *E. coli* was greater than 0.05, this indicates that there was no significant association between HIV status and the distribution of E. *coli* pathogens (Table 4.1).

This was not the case with Shigella infection, where the P value was less than 0.05, indicating that there was a significant relationship between HIV status and distribution of *Shigella* pathogens among these children.

Table 4.1: Prevalence of the enteric bacteria among HIV positive and negative children

Enteric Pathogens	Bacterial	HIV Negative (N=79)	HIV Positive (N=78)	P-Values	95% Confidence Intervals
Shigella		21	10	**0.033**	0.17 - 0.93
E.coli **Path types**	ETEC	1	0		
	EPEC	3	2		
	STEC	1	1		
	EIEC	8	9		
	EAEC	11	8		
	sub total	**24**	**20**	0.300	0.32 - 1.38
Salmonella		0	0	-	-

****Shigella:*** **P value <0.03**

4.3 Multiplex reaction for the detection of E.coli virulence related- genes

The combination of the primers was used in multiplex reaction to amplify genes in a single reaction, which provided a specific and suitable amplification of their respective target virulence genes for pathogenic *E. coli* as shown in figure 4.2 below.

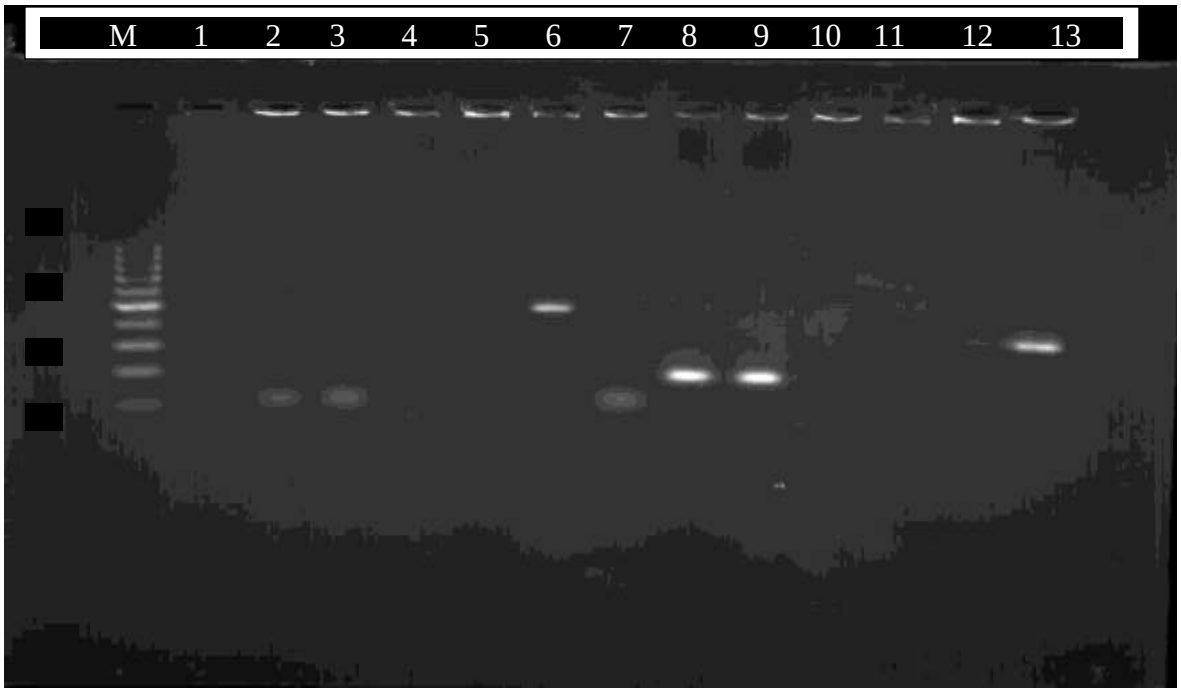

Figure 4.2: Agarose gel electrophoresis of DNA fragments of E.coli virulence genes. Lane M shows molecular weight marker (100bp), Lane 1 is a negative control containing dH2O, lane 2 is a positive control containing vt1 gene, Lane 3 and 7 have vt1 gene, Lane 6 has cnf1 gene, Lane 8 and 9 have eagg gene. Lane 10,11and 12 no gene was detected and 13 shows lt1 gene.

4.4 Prevalence of diarrheogenic *E.coli* virulence factors

A total of 44 participants were infected with diarrheogenic *E.coli*, the distribution of E. coli infection with various genetic components were as follows: Enteroaggregative *E.coli* (EAEC) strain harbouring Eagg or cnf1 was the most detected (43.2%). Seventeen (38.6%) isolates harboring cnf2 and invasive genes were grouped as enteroinvasive *E.coli* (EIEC). Five (11.4%) isolates with intimin genes (eae) and without Vt genes were grouped as enteropathogenic *E.coli* (EPEC). Shiga toxin-producing *E.coli* (STEC) stains harbouring vt1, vt2, vt1vt2 and with or without intimin (eae) and enterotoxigenic *E.coli* (ETEC) producing either ST or LT was the least detected as shown in table 4.2 below.

Table 4.2: Virulence factors associated with E. coli pathotypes

E. coli *Pathotype*	Virulence *genes*	HIV- Negative	HIV- Positive	Totals	P-values
		Freq (%)	**Freq (%)**	**Freq (%)**	
STEC/VTEC	*lt1*	1 (1.3)	1 (1.3)	2 (1.3)	0.993
ETEC	*st2*	1 (1.3)	0 (0)	1 (0.6)	-
EIEC	*einves*	8 (10.1)	7 (9.0)	15 (9.6)	0.776
	cnf2	0 (0)	2 (2.6)	2 (1.3)	
EAEC	*eagg*	12 (15.2)	7 (9.0)	19 (12.1)	-
	cnf1	0 (0)	1(1.3)	0 (0)	
EPEC	*eaeA*	3 (3.8)	2 (2.6)	5 (3.2)	0.659

4.5 Distribution of diarrheagenic bacteria among different age groups

The age distribution of participants was ranged between 5-12 years, the mean age of these children was 10.48 years with a standard deviation (SD) of 3.18 years. The children were clustered into three distinct age groups, 5-7years, and 8-10years and above 10 years. The distribution of diarrheagenic bacteria was high in children above 10 years of age with P = 0.319, and this indicates that there was no significant association between age of the children and the distribution of diarrheagenic bacteria among these children , the distribution of case with diarrheagenic bacteria according to age- group is show in table 4.3 below.

Table 4.3: Distribution of diarrheagenic bacteria among different age groups

Enteric bacterial pathogens		Age group (years)			P-value
		5-7	8-10	over 10	
E.coli	STEC	0	0	2	
pathotypes	EPEC	2	0	3	
	ETEC	0	0	1	
	EIEC	4	5	8	
	EAEC	4	2	13	
					0.319
Shigella		8	5	18	0.828

4.6 Distribution of distribution of diarrheagenic bacteria by gender

From the few diarrheagenic *E. coli* isolates, 47.7% were female, while 52.3% were male. Enteroinvasive *E. coli* (EIEC) was the highest *E.coli* pathotype isolated from female while in male cases enteroaggregative *E. coli* (EAEC) was the highest. The *P* value of diarrheagenic bacteria was greater than 0.05; this indicates no significant in association between gender and the distribution of diarrheagenic bacteria . Thirty-one participants had *Shigella* infection of which 58.1% were from females while 41.9 % were from male children.The *p*-value for shigella infection was 0.334 as show in the table 4.4.

Table 4.4: Distribution of distribution of diarrheagenic bacteria by gender

Enteric bacterial pathogens		Female (N=79)	Male (N=78)	P-Values	95% CI
E.coli pathotypes	ETEC	0	1	0.441	0.67 - 2.85
	EPEC	1	4		
	STEC	1	1		
	EIEC	11	6		
	EAEC	8	11		
Shigella		18	13	0.334	0.32-1.60

4.7 Antimicrobial susceptibility patterns of enteric bacterial pathogens

Antimicrobial susceptibility testing was done on all *E.coli* pathotypes which were previously detected by PCR and all *Shigella* isolates using disk diffusion method with the same antibiotics which were used in the clinic to treat infection. Nine antibiotics were distributed evenly as shown in figure 4.3 below and zone of inhibition were measured and interpreted as Sensitive (S), Intermediate (I) and Resistance (R) on the basic of CLSI guideline (2014).

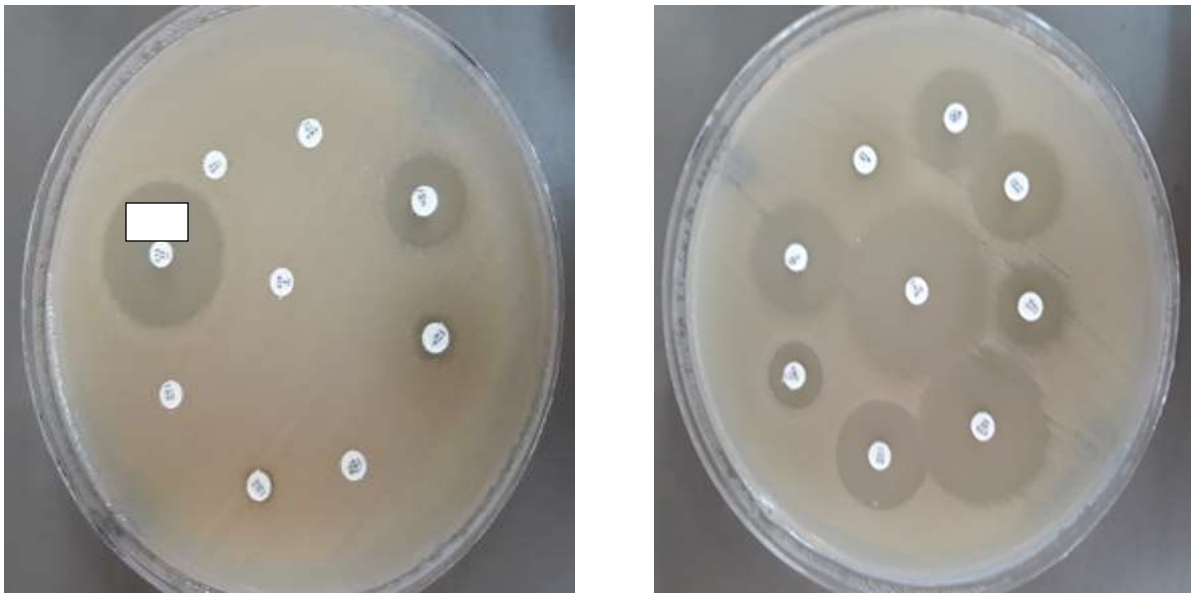

Plate A **Plate B**

Figure 4.3: Antimicrobial susceptibility test done using Kirby-Bauer disk diffusion method: Plate A &B shows the nine antimicrobial agents used for susceptibility test; Tetracycline, Chloramphenicol, Ampicillin, Erythromycin, Gentamicin, Ciprofloxacin, Cefotaxime, Trimethoprim/Sulfamethoxazole, and Nalidixic acid.

Of the 44 diarrheagenic *E.coli* isolate 75.0% were resistant to AMP, 4.5% were resistant to CHL 13.6% were resistant to CIP 56.8% were resistant to TET 38.6% were resistant to NAL 6.8% were resistant CTX 20.5% were resistant Gen/CN 93.2% were resistant to ERY and 86.4% were resistant to STX. On the other hand, out of 31 *Shigella* isolate 61.3% were resistant to AMP 3.2% were resistant to CHL 19.4% were resistant to CIP 41.9% were resistant to TET 45.2% were resistant to NAL 12.9% were resistant CN 96.7% were resistant ERY 87.1% were resistant to STX and all were sensitive to CTX .

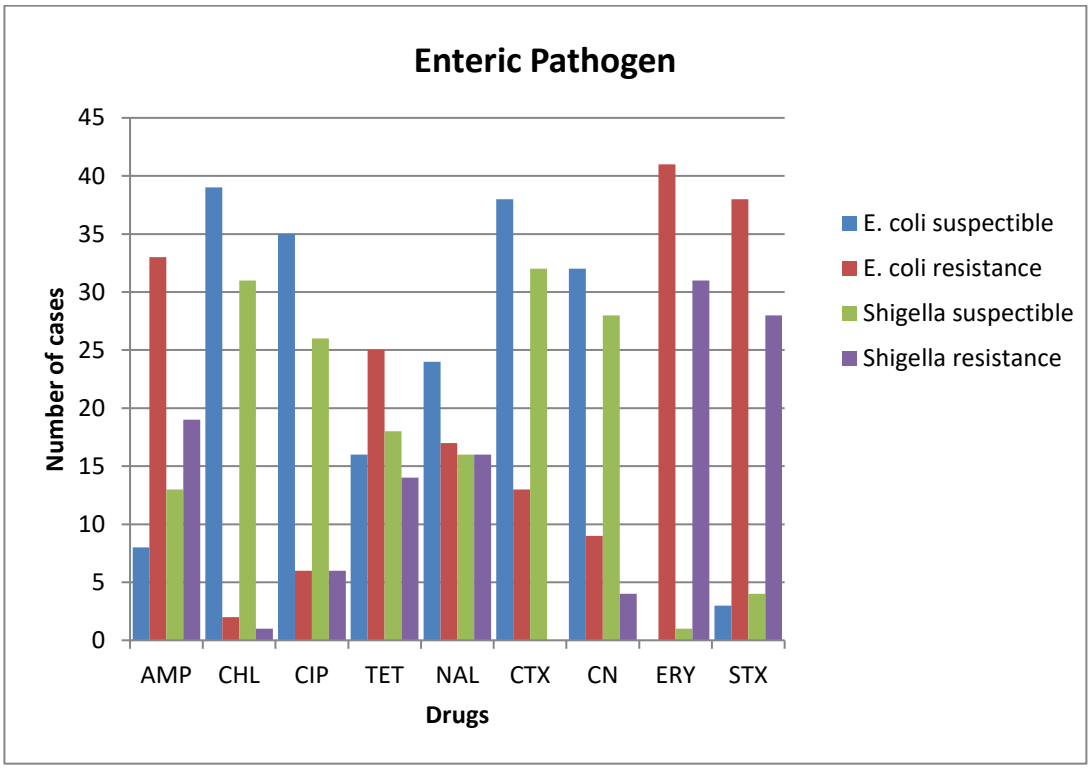

Figure 4.4: Antimicrobial susceptibility patterns of diarrheagenic bacteria isolated among HIV positive and HIV negative children (5-12yrs) in Dandora.

Among the children who had diarrheagenic bacterial infections (n=75), the level of resistance to most of the antibiotics tested was high among HIV-negative children compared to HIV-positive. HIV negative children who had diarrheagenic *E. coli* infections (n=24), the level of resistance were high for ERY 100% (n=24), STX 95.8% (n=23), AMP 79.0% (n=19) and TET 66.7% (n=16) as compared to HIV positive. While with Shigella infections, the resistance level for ERY 95.2% (n=20) and STX 81.0% (n=17) were high and all of them were susceptible to CTX as shown in table 4.5 below.

Table 4.5: Antimicrobial resistance of diarraheagenic bacteria among HIV positive and HIV negative children (5-12 years) in Dandora

	HIV status	AMP	CHL	CIP	TET	NAL	CTX	CN	ERY	STX
		n (%)	n (%)	n (%)	n(%)	n (%)	n (%)	n (%)	n (%)	n (%)
E.coli (44)	HIV $^{-ve}$ (24)	19 (79)	2 (8)	4 (17)	16 (67)	11 (46)	1 (4)	3 (13)	24 (100)	23 (96)
	HIV $^{+ve}$ (20)	14 (70)	0	2 (10)	9 (45)	6 (30)	2 (10)	6 (30)	20 (100)	19 (95)
Shigella (31)	HIV $^{-ve}$ (21)	12 (57)	1 (5)	2(10)	10 (48)	10(48)	0	2 (10)	20 (95)	17 (81)
	HIV $^{+ve}$ (10)	7 (70)	0	1(10)	3 (30)	3 (30)	0	1 (10)	10 (100)	10 (100)

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The human digestive tract represents a very attractive environment for bacteria to colonise, and it is therefore not surprising that most of the bacteria live in the gut. Although most of these gut bacteria are harmless, gastrointestinal mucosal repair and regeneration are decreased in HIV-positive populations, allowing the pathogens that could have been controlled by a mucosal barrier to cause disease. Previous study indicates that 1 in 9 deaths that occure in children around the world are due to diarrhoea, this is even worse in HIV-infected children (Fletcher et al., 2013). Other studies in Kenya have documented the prevalence of bacterial diarrhea among HIV-positive and negative children below five years of age (Rono *et al*.,2014; Van Eijk *et al*., 2010). This study is one of the first to address the prevalence of common circulating bacterial enteric pathogens among HIV positive and negative children above five years old in Dandora settlement.

In this study, the overall prevalence of diarrheagenic bacteria (pathogenic *E. coli* and *Shigella spp*) was 47.8%. Of these 28.7% was from HIV-negative and 19.1% was from HIV-positive cases. Whereas there was no case of *Salmonella* infection detected. This is in agreement with earlier findings where most of diarrheagenic bacterial pathogens cases were from HIV negative as compared to HIV positive children (Rono *et al.*, 2014).

The distribution of *Shigella* infections in this study was more significant in HIV negative cases than in HIV positive cases (P=0.03). This may be due to the frequent administration of antibiotics used to treat other infections among HIV-positive children. However, this study did not seek to isolate other diarrheagenic bacteria apart from those mentioned above.

Diarrheagenic *E .coli* (DEC) is major public health risk in children in developing countries, causing persistent diarrhoea, (Abba *et al*., 2009) and classification is based on

their virulence factors. In this study multiplex PCR showed that aggregative and CNF1 virulence genes found in EAggEC (43.18%) was the major pathotype isolated; this was consistent with the findings of several studies (Bii *et al*., 2005; Sang *et al.,* 1997; Rono *et al.,* 2014) which reported that EAggEC is the main *E. coli* pathotype commonly associated with persistent diarrhoea in children in Kenya.

In this study, the prevalence of infection was high in children above ten years old. Younger children aged less 10years had less risk of getting the bacterial diarrhoeal illness. This maybe because these children are closely monitored by their parents/guardians on the type of food they eat and water they drink thus reducing the rate of acquiring infection, this is strongly supported by previous studies done (Mengistu *et al* 2014). Although there was no significance in the distribution of diarrheagenic bacteria by gender with *P* value greater than 0.05, the prevalence of infection was slightly high (52%) in female compare to male (48%). This is unlike previous studies that reported a higher prevalence in males (Rathaur et al., 2014: Moyo *et al*., 2011).

All *E. coli* and *Shigella* isolates from this study displayed high levels of resistance to one or more antimicrobial agents, including Erythromycin, Trimethoprim/S ulphamrthoxazole, and Ampicillin. This trend had been observed in previous studies, Brander *et al* 2017., Langendorf *et al.,* 2015. However a study done in Sudan by Saeed *et al.,* 2015; and in Tanzania, Moyo *et al.,* 2011 observed higher levels of resistance to antibiotics in *Shigella*.

The resistance levels to antibiotics observed in this study was more elevated in HIV-negative children than in the HIV -positive counterparts,this is as result of wide use of antibiotics in the treatment of infections, which has raised a serious concern among general practitioners and pediatricians in the developing world. Antibiotic resistance for *E. coli* and *Shigella* infections was higher in this study.The high prevalence of resistance to these drugs could be explained by the long-term use and misuse of this antibiotic to treat enteric bacterial infection, ensuring selection pressure and maintenance of this resistance (GabreSilasie *et al*., 2018)..

For *Shigella* isolates as depicted in (table 4.5), where high level of resistance was observed in Ampicilin, Nalidixic acid, Erythromycin and Trimethoprim/Sulfamethoxazole, unlike Chloromphenicol and Cefotaxime which were very sensitivity. This agrees with previous studies in Kenya, (Sang *et al.,* 2012; Sang *et al*., 2019). In this study like previous work also points out the worrying of emerging resistance of Ciprofloxacin and Gentamycin that should be urgently addressed .

E.coli isolates exhibit a multiresistance to Ampicilin, Tetracyclin, Nalidixic acid, Erythromycin and Trimethoprim/Sulfamethoxazole as documented by GabreSilasie *et al*., (2018). This multidrug resistance was also observed in pathogenic *E.coli* isolates from both HIV positive and HIV negative children. The emerging resistance to Gentamycin, Cefotaxime, Ciprofloxacin and Chloromphenical is increasing at an alarming rate which was also reported (Nguyen *et al.,* 2005: Sang *et al.,* 1997). This is in contrast with a study from Ethiopia (GabreSilasie *et al.,* 2018) ,which suggested a high sensitivity of DEC to ciprofloxacin and Cefotaxime.

(WHO 2010), recommends the use of ciprofloxacin in case of bloody diarrhoea for both HIV positive and HIV negative cases in children; however, there has been incidences of misuse of antibiotics in case of diarrhoea resulting to AMR as reported in earlier studies, (Efunshile *et al*., 2019, Tulu *et al*., 2018, Osatakul *et al*., 2007). Brooks *et al.,* 2003, explain that antibiotics are recommended for treating bloody diarrhea to shorten the duration of illness, but the general practice of issuing antibiotics indiscriminately has contributed to the problem of AMR, making treatment of diarrhoea problematic.

Fluoroquinolones have been proved to be the preferred drug by most physicians because of their oral active, broad-spectrum, and heat stability. Thus chances of being overprescribed and subsequent misuse is common. Chattaway *et al.,* 2016 observed that the initial high cost of fluoroquinolones made them unavailable in most resource-constrained nations, especially in sub-Saharan Africa, however the expiry of the patent in 2003 gave way to cheaper generics in the African market. The need to prescribe more

fluoroquinolones has been escalated by a parallel increase in the prevalence of resistant bacteria showed by the findings of Laminkara *et al.*, 2011.

5.2 Conclusion

Diarrheagenic *E. coli* and *Shigella* were the main cause of diarrheal illnesses among HIV-positive and negative children in Dandora settlement. Enteroaggrigative *E. coli* was the major pathotype identified in both HIV-positive and negative children. This study shows a steady positive correlation between age of the children, demography and HIV status on the prevalence and etiology of diarrheagenic bacteria.

Antibiotic resistance data showed that all isolates were resistance to three or more antibiotics, including at least one first line treatment drug used in Kenya. The overall antibiotic resistance patterns are at much lower levels than those which have been reported from the rest of Kenya, except for Erythromycin and Trimethoprim/Sulfamethoxazole which range from 81%-100% for both population.

This study failed to accept the null hypothesis on the distribution of *Shigella* infection among HIV positive and negative children in Dandora.

5.3 Recommendation

Substantial gaps in knowledge about the infection of Enteroaggrigative *E. coli* and Shigella in HIV positive and negative children aged 5- 12 years in developing countries particulary in our case in Kenya, exist. Public health awarteness is needed as well as diagnostic facitities for Enteroaggrigative *E. coli* and *Shigella* infection.

The goal towards setting up a national surveillance program, would help determine the incidence rates, epidemiology risk factors, interaction of HIV/AIDS with *Shigella spp* and *E. coli* pathotypes, seasonal variation and current state of resistance to antimicrobial agents used in Kenya.

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APPENDICES

Appendix I: Informed Consent Form for specimen collection and Treatments

STUDY TITLE: Antimicrobial susceptibility patterns of common circulating enteric bacteria pathogens in HIV positive and negative children (5-12 years) in Dandora Kenya.

Principal investigator: Samya Said Rashid

Inclusion criteria: Parents/guardians consenting for children aged 5 to 12 years who are HIV positive and enrolled in the Nyumbani Lea Toto HIV/AIDS community outreach program and living in Dandora during the study period with diarrhoea symptoms.

Exclusion criteria: Parents / guardian of children who were unwilling to participate.

Informed consent

Your child is being asked to take part in a medical research study being performed by the Kenya Medical Research Institute (KEMRI), Nyumbanichildrens home and Kanazawa UniversityFaculty of Medicine, Institute of Medical, Pharmaceutical and Health Sciences, Japan. It is very important that you understand the following general principles that apply to all participants in our studies:

1) You and your child's participation is entirely voluntary;
2) You may withdraw from participation in this study or any part of this study at any time with no penalty, harm, or loss of access to treatment and care;
3) After you read about the study please ask any questions that will allow you to understand the study more clearly.

What are enteric bacteria?

These are organisms normally found inhibiting intestinal tract of humans and animals. However there are some types of bacteria capable of causing diseases in human, known

as pathogens. The most common enteric bacteria pathogens are *E.coli, Salmonella* and *Shigella* species which can multiply inside the human body allowing serious infections to develop. They get transmitted when someone comes in contact with infected feces (for example, through contaminated soil, food, or water).

Why do we want to conduct this study?

To find out the causes of diarrhoea among the HIV infected and non-infected children in Dandora, this could lead to control, treatment and management of diarrhoea illness in the two populations.

What is important for you to know?

To do this study, we will need to study some of your child's feces, the stool samples will be taken to the laboratory for preparation and other tests.we will test for presence of common enteric bacteria pathogens, virulence strain and antimicrobial susceptibility patterns. If we find that your child has these pathogens, he /she will be offered treatment, counselling and how to prevent infections (good sanitation, proper handling of food and water).Your child will be assigned a study number, and the links between the name and number, and all data collected will be kept confidential. We will just use the information to find out about the intestinal bacterial infections and how to manage them.

You and your family may not get any direct benefits from being in this study but what we find out will help us determine the best approach for management of intestinal pathogens in HIV infected and uninfected children. Although you will receive treatments, this treatment is also available at the government hospital.

You can decide if you want to take part in this study. Taking part in this study will not cost you or your family anything. You may also leave the study at any time. You can leave for any reason without any problems.

Who Can Participate In The Study?

We can include your child in the study only if you give consent to participate, and if your child agrees to participate.

Questions about research

If you have any questions about this study, you may contact Dr.willie Sang at the Kenya Medical Research Institute, (KEMRI) Nairobi Tel; +254720950 385 during the study and in the future. If you have concerns about human rights, ethics and welfare issues you may contact the scientific and ethics review unit (SERU) at KEMRI P.O Box 54840-00200, Nairobi; telephone +254717719477, email address: seru@kemri.org.

INFORMED CONSENT AGREEMENT

I, Mr./Mrs./Miss _____, being a person aged 18 years and over and being the lawful/legal guardian of: Msr/Miss (Child's name) ---------- voluntarily agree that my child may be included in the study which I have read or has been read to me. . I been made to understand the implications and benefits of the study. I accept the tests to be carried out. I understand that I may withdraw him/her from the research at any time, for any reason, without any penalty or harm. All the above conditions have been explained to me in the _____ language in which I am fluent.

_____ Name of Child

_____ Age of child

_____ Parent's/Guardian's name

_____ Parent's/Guardian's signature

_____ Date

_____ Place

_____ Person Obtaining Consent

_____ Witness

Treatment Consent

If your child has common enteric bacteria pathogens, he/she can be offered treatment. The treatments are free. Is it okay for your child to receive treatment if he/she has a worm infection?

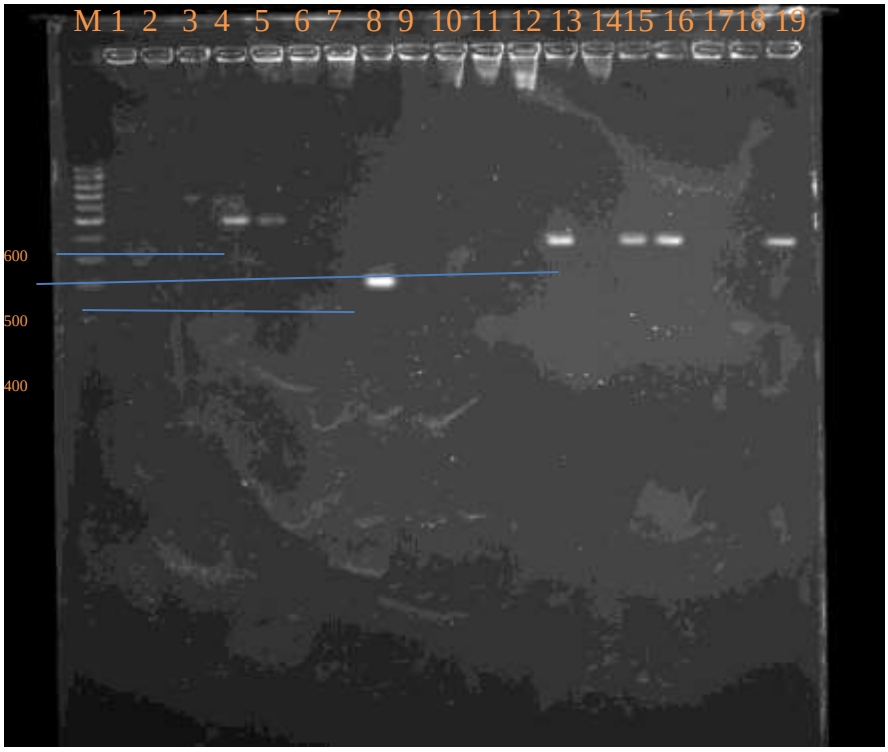
_____ Yes

_____ No

_____ Parent's/Guardian's signature

OFFICIAL STAMP

Appendix II: Gel images of PCR assay for *E.coli* pathotypes



Agarose gel electrophoresis of DNA fragments of E.coli virulence genes. Lane M shows molecular weight marker (100bp), Lane 4 and 5 have *cnf1* gene, Lane 8 has *vt2* gene, Lane 13,15 and 16 have *lt1* gene. Lane 18 shows negative control and Lane 19 shows positive control containing *lt1* gene.

Appendix III: MAP