INTERNATIONAL HEALTH SCIENCES UNIVERSITY

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PREVALENCE OF BACTERIAL ENTERIC PATHOGENS AMONG CHILDREN UNDER FIVE YEARS IN KAMPALA DISTRICT

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DECLARATION

I declare that the work presented in this book is entirely original, out of my efforts and has never been submitted for award of Bachelor of Medical Laboratory Science or any other award of any university, college or institution known to me.

Signature……..... Date…….

NANDALA MICHAEL WANZILA

APPROVAL

This is to certify that this research report has been done under my supervision and has never been presented anywhere for academic purpose or any other purpose.

Signature……..... Date…….

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DEDICATION

I dedicate this work to my dearest wife Ms. Prosy Muduwa, my children Prudence and Krystel. Without whose continued support and counsel, I could not have completed this study. May God bless my family and my footstep while climbing academic ladder.

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ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
CDD	Control of Diarrheal Diseases
CFU	Colony Forming Units
CLSI	Clinical and Laboratory Standards Institute
CPHL	Central Public Health Laboratories
HEA	Hekton Enteric Agar
HIV	Human Immunodeficiency Virus
IHSU	International Health Sciences University
KCCA	Kampala Capital City Authority
MAC	MacConkey Agar
MDG	Millennium Development Goals
MHA	Muller Hinton Agar
MIC	Minimal Inhibitory Concentration
MoH	Ministry of Health
OR	Odds Ratio
ORS	Oral Rehydration Salts
PCR	Polymerase Chain Reaction
QC	Quality Control
TSI	Triple Sugar Iron agar
UDHS	Uganda Demographic and Health Survey

UNICEF	United Nations International Children's Emergency Fund United Nations
WHO	World Health Organisation
XLD	Xylose Lysine Deoxycholate agar.

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ABSTRACT

Introduction: Diarrhoea is the second leading cause of child mortality worldwide and a major problem in developing countries. Though precise data on childhood mortality associated with diarrhoeal diseases in Uganda is not available, it has been estimated that 19,700 children under 5 years die each year from diarrhoea. The purpose of this study was to assess the prevalence of bacterial pathogens causing acute diarrhea in children under 5 years and identify the associated risk factors.

Materials and methods: A total of 113 children under 5 years with acute diarrhea in Kisenyi HCIV and Komamboga HCIV were enrolled between January and April 2014. Fecal specimens for culture were sent to Central Public Health Laboratories and cultured on conventional media. The organisms were identified by different biochemical tests and confirmed by serotyping. The bacteria identified were subjected to antimicrobial susceptibility test using Kirby-Bauer's disc diffusion method.

Results: The prevalence of bacterial enteric pathogens was 23.9% (27/113). The prevalence was greatest in the age group 10 – 29 months (14.2%). Major pathogenic organisms isolated were *Escherichia coli* 11 (9.7%), *Shigella flexneri* 7(6.2%), *Salmonella typhi* 1(0.9%), *Salmonella paratyphi* 4(3.5%), *Shigella boydii* 1(0.9%), *Shigella dysentriae* 2(1.8%) and *Enterobacter* species 1 (0.9%). Ciprofloxacin (85.2%) was the most sensitive antibiotic followed by Chloramphenical (37%). Among the isolates, 88.9% were resistance to Ampicillin and Sulphamethoxazole, 96.3% resistant to Tetracycline and 85.2% resistant to Nalidixic Acid.
The major predisposing factors to enteric bacteria in children under 5 years were; Children drinking un treated water; not exclusively breastfed, not washing hands before eating and after visiting toilet and Children fed on leftover food.

Conclusion: The prevalence of bacterial enteric pathogens was 23.9% in the under 5 year children. Commonly isolated pathogens were *Escherichia coli* and *Shigella* species. Therefore awareness on prevention of infectious diseases and efforts to improve personal and domestic hygiene should be encouraged.

Keywords: diarrhoea, bacteria enteric pathogens, children under five.

CHAPTER ONE

1.0 INTRODUCTION AND BACKGROUND

Diarrhea is defined as having 3 or more loose or liquid stools per day or having more stools than is normal for that person (WHO, 2007).

Diarrhoea is a significant health problem worldwide, especially in the developing world where adequate sanitation facilities are lacking (Kosek, et al.; 2003). Globally diarrhoeal diseases account for almost a fifth of all deaths of children below five years of age, with an estimated 2.2 million deaths annually (Boschi, *et al.*, 2009).

Around 90% of all the all diarrhoea-related deaths occur in children under five years of age living in low income countries. Similarly to all-cause mortality, the global estimate of the deaths due to diarrhoea have shown a steady decline, from 4.6 million in the 1980s to 3.3 million in the 1990s to2.5 million in the year 2000 (Kosek, et al ., 2003). It has been estimated that 88% of all diarrhoeal diseases are as a result of contaminated water and inadequate hygiene and sanitation (Kosek, et al., 2003).

Of the estimated total 10.6 million deaths among children younger than five years of age worldwide, 42% occur in Africa (Bryce, *et al.*; 2003). Although mortality rates among these children have declined globally from 146 per 1,000 in 1970 to 79 per 1,000 in 2003, the situation in Africa is strikingly different compared with other regions of the world. African region shows the smallest reductions in mortality rates (WHO, 2008).

The under-five mortality in the African region is seven times higher than that in the European region (Cynthia, et *al*., 2005). One of the contributing factor that may contribute to this situation is the HIV/AIDS epidemic in the region, but an underlying weakness of the implementation capacity of the health systems is also likely to blame (Walker, *et al.*, 2002).

New virulent enteric pathogens are emerging throughout the world, Africa included. A multi-drug resistant enteroaggregative E. coli, O44, which is associated with acute and persistent diarrhoea, has been reported in Kenyan children (Jill, *et al.*, 2010). Very recently, E. coli O157 was reported for the first time as the etiologic cause of a large dysentery outbreak in Swaziland (Morris, et al., 2012). .

In Uganda, children aged less than five years constitute 17% of the total population, and mortality of children aged less than five years is currently estimated at 90 per 1,000 live births. Malaria, diarrhoea, and acute respiratory tract infections are the leading causes of mortality. Childhood mortality is generally higher among children of less educated mothers and those in the poorest households (Uganda DHS, 2011).

According to results of the Uganda Demographic and Health Survey 2011, every two weeks, one in four children in Uganda are affected by diarrhoea. More than 75% of the overall burden of diseases is preventable, including malnutrition. Access to safe water, sanitation, hygiene, nutrition and living conditions are still poor, resulting in poor health in women and under five children (Uganda Demographic and Health Survey, 2011). An estimated 14,000 children under the age of five die of diarrhoea each year in Uganda.Unfortunately, many hospitals in Uganda are lacking clinical microbiology laboratories to diagnose the cause (Källander, *et al*., 2008).

1.1 Statement of the problem

Diarrhoea is the second most frequent cause of death amongst children aged five and bellow and is globally responsible for killing around 760000 children every year (WHO, 2010).

More than 80% of child deaths due to diarrhoea occur in Africa and South Asia, with only fifteen countries including Uganda contributing to almost three quarters of the total (UNICEF, 2009).

In Uganda, diarrhoea continues to be a major cause of childhood morbidity and mortality due to its syndromic nature despite great advances in the management of diarrhoeal diseases (Mshana. *et al*., 2009).

The prevalence of diarrhoeal diseases in Kampala is estimated to be 12 % (Tumwine, *et al*., 2003) but a comprehensive study on the actual significance of the bacterial etiological agents has not been performed. Therefore this study was conducted to identify bacterial enteric pathogens and risk factors associated with diarrhoeal diseases among children under 5 years attending Komamboga and Kisenyi Health centres in Kampala district.

1.2 Justification of the study

To effectively prevent diarrhoea, it is imperative that common pathogens and important risk factors associated with diarrhoea be identified first in communities. Identification of pathogens and risk factors, and then recommendations of simple, immediate, and effective risk-reduction measures will help local health care services to reduce morbidity and mortality due to diarrhoea among young children in Kampala District. The study report shall benefit future researchers by providing reference information to those interested in the similar study. Public health policy makers can also make use of the study findings to design possible interventions against diarrhoeal diseases and related conditions.

1.3 Study Objectives

1.3.1 General Objective

To determine the prevalence of enteric bacteria among children less than 5 years in Kisenyi and Komamboga Health Centres and identify risk factors associated diarrhoea.

1.3.2 Specific Objectives

i. To determine the prevalence bacterial enteric pathogens from faecal samples in children under 5 years.

ii. To determine the antibiotic susceptibility of isolated bacterial organisms among children under 5 years.

iii. To identify risk factors for diarrhoea among children under 5 years.

CHAPTER TWO: LITERATURE REVIEW

2.0 Epidemiology

Diarrheal disease is the second leading cause of death in children under five years old, and is responsible for killing around 760 000 children every year (WHO, 2010). Diarrhoea is defined as the passage of 3 or more loose or liquid stools per day or more frequent passage than is normal for the individual. It is usually a symptom of gastrointestinal infection. Diarrhoea due to infection constitutes a major burden of disease (Jill, *et al.,* 2010). The most dangerous complication is dehydration that occurs when there is excessive loss of fluids and minerals (electrolytes) from the body. With vomiting, dehydration becomes more severe. Dehydration is especially dangerous in infants and young children due to rapid body water turnover, high body water content and relatively larger body surface (Jensen, *et al.*, 2004). The median incidence of diarrhoea is greatest for infants aged 6 to 11 months (5 episodes /child /year). Where episodes are frequent children may spend 15% of their days with diarrhoea (UNICEF, 2008).

2.1The causative agents for diarrhea

Diarrhoea is a common symptom of gastrointestinal infections caused by a wide range of pathogens, including bacteria, viruses and protozoa (Boschi, *et al*., 2009). Infection may be spread through contaminated food or drinking water, or from person to person as a result of poor hygiene (Curtis, *et al*., 2000).

2.2 Bacterial infections

Diarrhoea caused by enteric bacteria is the most important worldwide, especially in tropical countries where it is a serious problem among older children as well as infants. The range of causative organisms is wide; it includes *Escherichia coli, Salmonella, Shigella, Campylobacter, Yesinia, vibrio,* and *Clostridium difficile.* (Hyams, *et al*., 1993). A study done by Haqo *et al* (2010) to investigate the enteric bacterial causing diarrhoea amongst urban refugee children in Eastleigh County Council Health Centre, Nairobi, Kenya found that, *Shigella* species, *Salmonella* species and Enterotoxigenic *Escherichia coli* were found to be responsible for diarrhoea amongst the urban refuge children.

The mechanism by which invasive bacteria evokes intestinal secretion is uncertain but is probably a multifactorial process involving products elaborated by the mucosal acute inflammatory reaction and enterotoxins elaborated by the bacteria (Behrens, 1991). Establishment of enteric infection depends upon a complex interplay between the host defence mechanism and bacterial virulence factors adapted to overcome these defences. Bacterial enteropathogens cause diarrhoea primarily by elaborating enterotoxins and by invading the intestinal mucosa. Enterotoxins cause intestinal secretion and diarrhoea by stimulating the adenyl cyclase system or the guanyl cyclase system and by other mechanisms. The ability of enterotoxigenic bacteria to adhere to the intestine involves a specific binding interaction between bacterial structures called pilli or fimbriae and specific receptors on the surface of intestinal cells (Warren, 2003).

2.3 Transmission

Infectious diarrhoea is acquired by faecal-oral transmission that includes consumption of contaminated food or water, person-to-person contact, or direct contact with faecal matter. With regard to water-borne-diarrhoea, transmission patterns occur when in-house water storage facilities or/and water sources are contaminated. Most of transmission of diarrhoea occurs in the domestic domain (Jensen, *et al*., 2004).

Modes of transmission can occur through two principle routes namely; Direct transmission; occurs when microorganisms are transferred from one infected person to another person without a contaminated intermediate object or person. Indirect transmission; involve the transfer of an infection through a contaminated object or person (Curtis, *et al*., 2000).

2.4 Types of diarrhoea

According to WHO:2007, based on clinical syndromes, diarrhoea could be classified into four types, each reflecting a different pathogenesis, including acute watery diarrhoea, dysentery, persistent or prolonged diarrhoea and chronic diarrhoea.

2.4.1 Acute watery diarrhoea

This term refers to diarrhoea characterized by abrupt onset of frequent, watery, loose stools without visible blood, lasting less than two weeks. Usually, acute watery diarrhoeal episodes

subside within 72 hours of onset. It may be accompanied by flatulence, malaise and abdominal pain. Nausea, vomiting may occur and also fever may be present. The most important causes of this diarrhoea in developing countries are *Rotavirus, Shigella, Enterotoxigenic E. coli* (ETEC), *Vibrio cholerae, Campylobacter jejuni, Enteropathogenic E. coli (EPEC), Salmonella spp.* and *Cryptosporidium* (Fewtrell, *et al.*, 2005).

2.4.2 Dysentery

Is defined as any diarrhoeal episode in which the loose or watery stools contain visible red blood. The illness also includes abdominal cramps, fever and rectal pain. The most important cause of bloody diarrhoea is *Shigella* species. In developing countries, the main causative agents of dysentery are *S. flexneri, S. boydii* and *S. dysenteriae*, whereas *S. sonnei* is the main cause in developed countries (Abram, 1999). Other pathogens causing endemic dysentery in children include: *Campylobacter jejuni*, invasive strains *of E. coli* (EIEC), non-typhoid *Salmonella* strains and *Entamoeba histolytica. Entamoeba histolytica* usually causes less than two percent of episodes of bloody diarrhoea in children under 5 years old.

2.4.3 Persistent diarrhoea

This term refers to diarrhoeal episodes that have an unusually long duration and last at least 14 days (Molbak.*et al.*, 200*0*). The episode may begin acutely either as watery diarrhoea or dysentery. The pathogenesis of persistent diarrhoea is not fully known. Several causes, probably in combination, include: infections with, EPEC and *Cryptosporidium*; intolerance to foods; delayed recovery of intestinal mucosal damage due to protein-energy malnutrition or Vitamin A or zinc deficiency; immunodeficiency; and inappropriate use of antibiotics (Jensen, 2004).

2.4.4 Chronic diarrhoea

This term refers to diarrhoea which is recurrent or long lasting due to mainly non-infectious causes. Chronic diarrhoea may be caused by gastrointestinal disease, may be secondary to systemic disease, or psychogenic in nature (Jane and Swanson, 2001). Physiologically, chronic diarrhoea may be categorized as inflammatory diarrhoea, osmotic diarrhoea, secretory diarrhoea and factitious diarrhoea (Armon, *et al.*, 2001).

2.5 Risk factors for diarrhea

Factors underlying increased risk of diarrhoea morbidity and mortality are: low socio-economic status; poor personal and domestic hygiene; low family income; living in a crowded room; lower maternal education; lack of breastfeeding and malnutrition. Malnutrition increases severity and duration, also some studies recently (Sudan and Mexico) have suggested that malnutrition increases the risk of frequent diarrheal episodes (WHO, 2009).Others include Immunodeficiency or immunosuppression, this may be temporary, after certain viral infections (e.g. measles) or it may be prolonged as in AIDS.

Demographic factors

Many studies have established that the diarrhoea prevalence is higher in younger children. The prevalence is highest for children 6- 11 months of age, remain at a high level among the one year old children, and decrease through to third and fourth years of life. Higher rate of diarrhoea has been observed in boys than girls (Molbak, *et al.,* 1997). Other demographic factors, like mothers' younger age, low level of mother's education, were significantly associated with more diarrhoea occurrence in children less than five (Tumwine, *et al.,* 2002).

Socio-economic factors

Some studies have shown that the association between socio-economic factors, such as poor housing, crowded conditions, low income and higher rate of diarrhoea was statistically significant (Etiler, *et al.,* 2004).

Water-related factors

As diarrhoea is acquired via contaminated water and foods, water-related factors are very important determinants of diarrhoea occurrence. Increasing distance from water sources, poor storage of drinking water , use of unsafe water sources ,water storage in wide mouthed containers have been found to be risk factors for more diarrhoea occurrence among children less than five years (Girma,*et al.,* 2007).

Sanitation factors

Sanitation plays a key role in reducing diarrhoea morbidity. Some sanitation factors, like indiscriminate or improper disposal of children's stool and household garbage, non-existence of

latrine or unhygienic toilet, house without sewage system, increased the risk for diarrhoea in children (Black, *et al.*, 2003)

Hygiene practices

Children not washing hand before meals or after defecation, mothers not washing hands before feeding children or preparing foods, children eating with their hands rather than with spoons, eating of cold leftovers, dirty feeding bottles and utensils unhygienic domestic places, unsafe food storage, presence of animals inside the house, presence of flies inside the house are associated with risk of diarrhoea morbidity in children (Curtis, *et al.*, 2000).

Breast feeding

In general, the morbidity of diarrhoea is lowest in exclusively breast-fed children; it is higher in partially breast-fed children, and highest in fully-weaned-children. A high concentration of specific antibodies, cells, and other mediators in breast milk reduces the risk of diarrhoea following colonization with enteropathogens (Molbak, 2000).

Malnutrition

The association between diarrhoea and malnutrition is so common in low income societies that the concept of a vicious circle is appealing, with diarrhoea leading to malnutrition and malnutrition predisposing to diarrhoea (Brown, 2003). Children whose immune systems have been weakened by malnutrition are the most vulnerable to diarrhoea. Diarrhoea, especially persistent and chronic diarrhoea, undermines nutritional status, resulting in malabsorption of nutrients or the inability to use nutrients properly to maintain health.

Immunodeficiency

Immunodeficiency is not only a cause of persistent or chronic diarrhoea, but also a risk factor for chronic diarrhoea. Due to innate or acquired immunodeficiency, patients are vulnerable to pathogens that cause infectious diseases including diarrhoea. Diarrheal incidence, duration, severity and mortality are higher in children with HIV/AIDS than in others (WHO, 2008).

2.6 Treatment of diarrhoeal illness

Reducing deaths depends largely on delivering life-saving treatment of Oral Rehydration Salts (ORS) solution and Zinc tablet to all children in need (WHO/UNICEF, 2009).

Rehydration and its correction of any electrolyte imbalance are critical in the treatment of diarrhea. Symptomatic relief is a second therapeutic goal (Warren, 2003). The new ORS formula is the gold standard for treating childhood diarrhoea, as well as treating dehydration once it occurs. Symptomatic anti-diarrhoeal drugs are usually not recommended for the treatment of acute diarrhea in children (Warren, 2003). Antimicrobials are not effective in uncomplicated acute diarrhoea and their use should be discouraged. Evidence from studies in other countries demonstrates a high prevalence of multiple antimicrobial resistances in normal bowel flora, which suggests that they may act as reservoir for resistance available to enteric pathogens. A study of commensal gut flora of children in Sudan by shears, et al (1998) found that 39% of children had strains resistant to six antimicrobials and over 70% of the children had strains resistant to at least 4 out of 6 antimicrobials commonly used in the country. Adequate dietary management during and after diarrheal disease is very important in order to reduce or prevent the damage of intestinal functions induced by withholding foods; to prevent or decrease the nutritional damage caused by the disease; to shorten the duration of the disease; and to allow catch-up growth and a return to good nutritional condition during convalescence (Fewtrell, *et al.,* 2005).

2.7 Prevention and control of diarrhea

Reducing childhood diarrhea requires interventions to make children healthier and less likely to develop infections that lead to diarrhea. A long-term, sustainable solution to childhood diarrheal disease must combine treatment with actions to eliminate diarrheal disease through prevention. (WHO/UNICEF, 2009). Primary preventive interventions reduce environmental risk factors and high-risk behaviors for whole communities by interrupting the disease transmission cycle. For diarrhoeal disease this means promoting changes in hygiene behaviour to protect people from ingesting diarrhoeal disease pathogens and providing sanitation solutions to protect the environment from faecal contamination. Strategies for comprehensive prevention and control of diarrhoea include: good personal and domestic hygiene; use of safe water; improved nutrition; immunization; and effective case management. (Guerrant, 2003).

CHAPTER THREE

METHODOLOGY

3.0 Study area

This study was conducted at Komamboga Health centre IV and Kisenyi Health Centre IV, in Kampala District between January 2014 and April 2014.

3.1 Study population

The population in this study comprised of children below five years with diarrhoea who received medical care from Komamboga Health centre IV and Kisenyi Health Centre IV at the time of study.

3.2 Study design

Cross sectional study was carried out to determine common bacterial aetiology of diarrhoeal diseases in the children under 5 years old and their antibiotic susceptibility. The study was conducted in the period of January to April 2014.

3.3 Sample size

The sample size was determined using the formula by Martin *et al.*, (1997).

$$n = \frac{Zt^2pq}{d^2}$$

Where n is the sample size and d is the allowable error.

Z is the standard normal deviation corresponding to 95% which is 1.96

P is the prevalence

Q is 1-P

Prevalence is 12.0 % and allowable error is 5%

$$n = \frac{1.96 \times 1.96 \times 0.12 \times 0.892}{0.05 \times 0.05}$$

n= 132 patients.

3.4 Sampling procedure/method

None probability sampling was under taken. Convenient samples of 132 patients were asked to participate in the study.

3.5 Inclusion criteria

Children with history of diarrhea and whose care givers consented

3.6 Exclusions criteria

- Children whose care givers that did not consent.
- Children that did not have diarrhoea.
- Children on antibiotic treatment at the time of the study.

3.7 Data collection

Data was collected by direct interview of parents and guardians of eligible children using structured questionnaire that addressed demographic and epidemiological data, duration of breastfeeding, hygiene practices, sources and treatment of drinking water, and disposal of feces.

3.8 Training of research assistants

Two research assistants fluent in English and the local language (Luganda) with basic health related knowledge were recruited and trained for 2 days specifically for this study. The training focused on general interviewing procedures, sample collection and storage. The training also focused on obtaining consent, maintaining neutrality, privacy and ethics in social research.

3.9 Pre-testing

The interview guides and questionnaire were pre-tested to ensure clarity and logical sequence of questions. The tool was appropriately corrected. The outcomes from this pre-testing exercise were used to improve on the data collection tools.

3.10 Laboratory methods

Stool and rectal swabs from 113 study patients (72 of them from Kisenyi and other 51 from Komamboga Health Centres) were collected and sent to Central Public Health Laboratories for

bacterial culture and sensitivity. Cary- Blair's transport medium was used for sample transportation. However, 19 study patients were not able to provide stool samples.

Samples were cultured on MacConkey, Sorbitol MacConkey, Xylose Lysine Deoycholate (XLD) agar plates and Selenite F broth (sub- cultured on XLD agar after 8-12 hours).

Colonies were further processed by using suitable biochemical tests. Suspected Salmonella species and Shigella species were further confirmed by using serological test. All *E. coli* isolates were also tested by O157:H7 antisera.

3.11 Antimicrobial susceptibility testing

Antibiotic susceptibility test was performed on all the pathogenic isolates. E coli ATCC25922 were used as a control strain.

The Bauer-Kirby disk diffusion method was performed as recommended by the Clinical and Laboratory Standards Institute. A standard inoculum adjusted to 0.5 McFarland was inoculated by swabbing onto Muller-Hinton agar. Susceptibility testing of all isolates was done using the single disc diffusion technique against Ampicillin (10µg); Chloramphenicol (30µg); Nalidixic acid (30µg); Sulphamethoxazole (25µg); Ciprofloxacin (5µg) and Tetracycline (30µg).

3. 12 Data handling and data analysis

The data was entered in Microsoft excel version 2007 and imported in SPSS ver.16.0 for statistical analysis, frequencies and percentages were computed for all variables. Differences in proportions were assessed by Chi-square test. P-value <0.05 were considered statistically significant.

3.13 Ethical consideration

Before the commencement of the study ethical clearance was obtained from International Health Sciences University. In addition to that permission from the study sites was obtained. Written informed consent was obtained from each caregiver of the children enrolled in the study.

CHAPTER FOUR
ANALYSIS OF RESEARCH FINDINGS

4.0 Introduction

The study enrolled 113 children aged 5years and below attending Komamboga HCIV and Kisenyi HCIV from January to April, 2014. Most of the study participants were from Kisenyi HCIV (59.3%, n= 67) with the remainder form Komamboga HCIV (40.7%, n= 46).

4.1 Demographic characteristics of the respondents

As shown in table 1 below, more females (58.4%) were recruited than males (41.6%).The ratio of females to males being 1.4**:**1.

All the participants were in the range of 3-59 months. The higher rate of diarrhoea was in the age group of 3-29 months with (76.1%) among which age group 10-29 months had 45.1% and 31% in the age group of less than10 months. The least rate of 3.5% was found in the age group 40-49 months.

Table 1: Socio-demographic Characteristics of Study Participants

		Age in months					
		>10 months	10-29 months	30-39 months	40-49 months	50-59 months	**Total**
Gender	Female	19	30	10	2	5	66
	Male	16	21	4	2	4	47
Total		35	51	14	4	9	113

One hundred and thirteen specimens were collected from children less than 5 years old complaining of diarrhoea .The overall prevalence of Enteric bacteria among diarrhoeic samples was 23.9%. No isolates were obtained in 86 samples. The pathogenic organisms isolated were *Escherichia coli* 11 (9.7%), *Shigella flexneri* 7(6.2%), *Salmonella typhi* 1(0.9%), *Salmonella*

paratyphi 4(3.5%), *Shigella boydii* 1(0.9%), *Shigella dysentriae* 2(1.8%) and *Enterobacter* species 1 (0.9%).

Of the total enteric bacterial positive cases, *Escherichia coli* were found to be the highest constituting 40.7%, followed by *Shigella flexneri* 25.9%, *Salmonella paratyphi* 14.8% and *Shigella dysentriae* 7.4%. *Shigella boydii* and *Enterobacter* species were the least each constituting 3.7% of the pathogens respectively.

Table 2: A distribution table showing bacterial culture results.

	Age in months					**Frequency**	**Percent (%)**
	≥10 months	**10-29 months**	**30-39 months**	**40-49 months**	**50-59 months**		
Non -significant growth	26	35	13	4	8	86	76.1
E.coli 0157:H7	3	7	1	0	0	11	9.7
Shigella flexneri	3	3	0	0	1	7	6.2
Shigella dysenteriae	1	1	0	0	0	2	1.8
Salmonella typhi	0	1	0	0	0	1	0.9
Shigella boydii	0	1	0	0	0	1	0.9
Enterobacter ssp	1	0	0	0	0	1	0.9
Salmonella paratyphi	1	3	0	0	0	4	3.5
Total	**35**	**41**	**14**	**4**	**9**	**113**	**100**

The prevalence rate was highest in the age group of 10-29 months (59%), followed by age group less than10 months (33%). The least infection (4%) was found in the age group 30-39 months (4%) and 50-59 months (4%) respectively.

There were no detectable cases of bacterial infection among the age group 40-49 months in this study as depicted in figure 1.

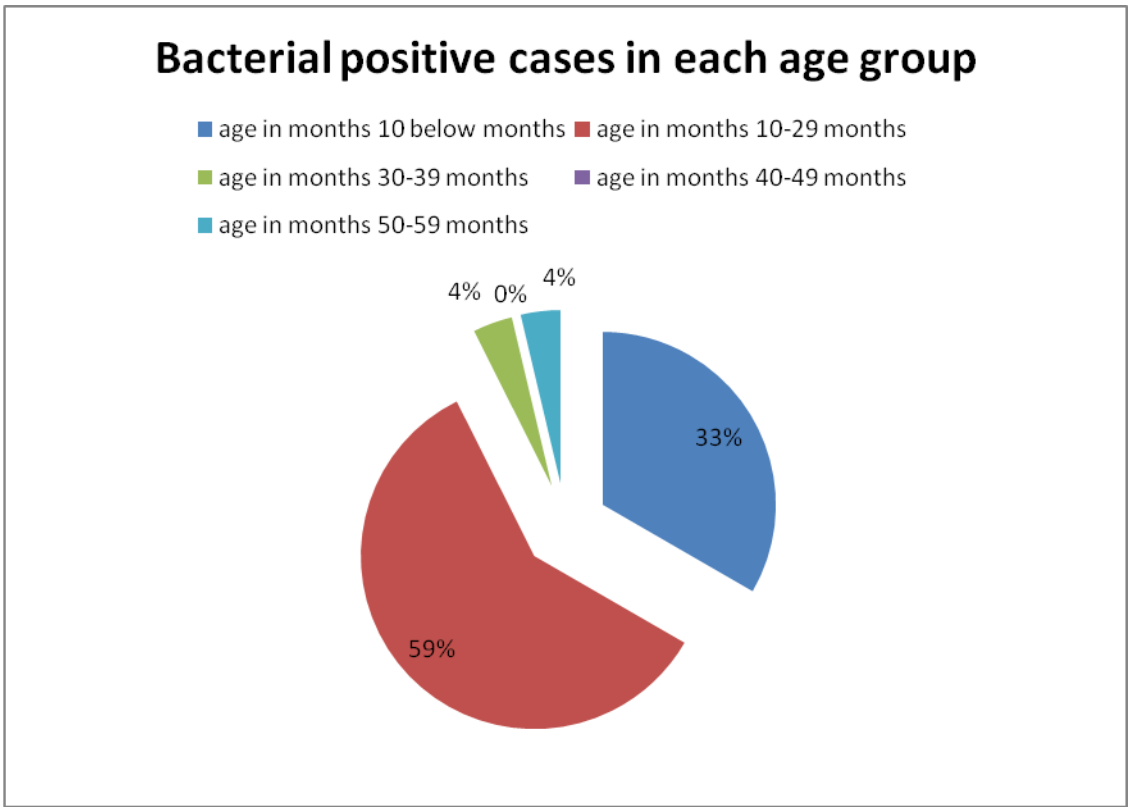

Figure 1: A pie-chart showing bacterial positive cases in each age group.

Among the *Escherichia coli*, ciprofloxacin showed 8 (72.7%) sensitivity whereas 9 (100%) isolates were resistant to Chloramphenicol, Nalidixic Acid, Sulphamethoxazole, and Ampicillin while 8 (81.8%) were resistant to Tetracycline.

Among the *Shigella flexneri*, 7 (100 %) isolates were susceptible to Ciprofloxacin followed by 4(57.1%) isolates susceptible to Chloramphenicol and 1 (14.3%) isolate was susceptible to Nalidixic Acid and Ampicillin. However, 7(100%) of the isolates were resistant to tetracycline and Sulphamethoxazole.

Ciprofloxacin and Chloramphenicol were the most effective antibiotics for *Salmonella typhi, Salmonella paratyphi, and Enterobacter* species with 100% efficacy.

Table 3: A frequency distribution table showing antimicrobial susceptibility of the isolates

Isolates	**C (30µg)**	**T (30µg)**	**CIP (05µg)**	**NA (30µg)**	**SXT (25µg)**	**AMP (10µg)**
E. coli 0157:H7	0	1	8	0	0	0
Shigella flexneri	4	0	7	1	0	1
Shigella dysenteriae	0	0	1	0	1	0
Shigella boydii	0	0	1	0	1	0
Salmonella typhi	2	0	2	0	0	2
Salmonella paratyphi	3	0	3	2	0	0
Enterobacter ssp	1	0	1	1	1	0
No of isolates susceptible	**10 (37 %)**	**1 (3.7%)**	**23 (85.2%)**	**4 (14.8%)**	**3 (11.1%)**	**3 (11.1%)**

Key: C- Chloramphenicol, T- Tetracycline, NA- Nalidixic Acid, SXT- Sulphamethoxazole, AMP- Ampicillin, CIP- Ciprofloxacin

Among the isolates, 88.9 % were resistance to Ampicillin and Sulphamethoxazole, 96.3% were resistant to Tetracycline 85.2% were resistant to Nalidixic Acid, 63 % were resistant to Chloramphenicol and Ciprofloxacin showed the least with 14.8% of the isolates being resistant (Figure 2).

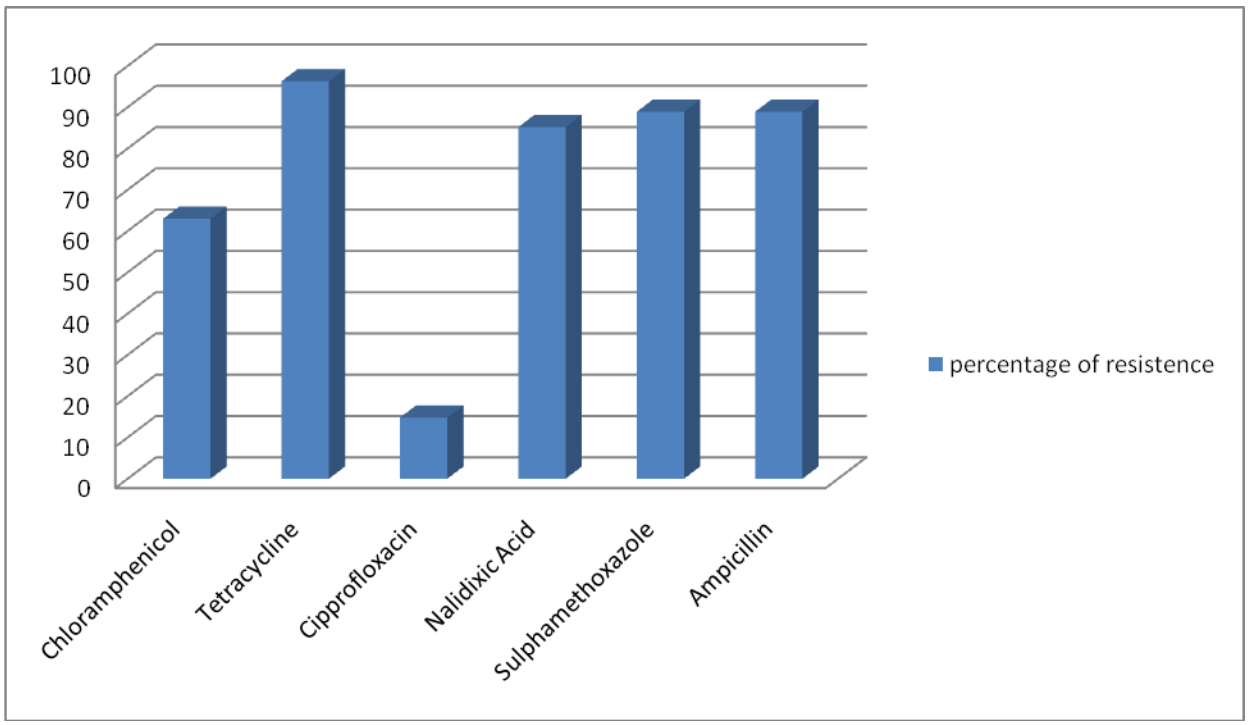

Figure 2: Antimicrobial resistance pattern of pathogenic isolates.

In the bivariate analysis, most of the exposure variables examined relating to lack of sanitary practices were associated significantly with diarrhoea. Several factors associated with hand washing were statistically significant, with odds ratios ranging from 4-6. Additionally exposure within the home relating to breast feeding, food preparation and water treatment were statistically significant with odds ratios ranging from 7-51.

Five variables were found to be associated independently with diarrhoeal illness in the final multivariate model. Two were related to hand washing; child did not wash hands before eating OR=5.5(1.8-18.7, p=0.005) and after visiting the toilet OR=5.9(2.3-15.5, p=0.001).Three factors were related to the exposure within the homes; child drunk un treated water from a water source OR= 10.0 (1.8-57.1, p=0.009). Child did not exclusively breastfed OR= 7.6 (1.5-39.1, p=0.020)., child eat food cooked the previous day OR= 23.8 (32-77.1, p=0.002) as depicted in table 4

Table 4: Final Model analysis of Risk factors associated with diarrheal diseases among the study participants in Kisenyi and Komamboga Health Centres, Kampala district

Factors	**AOR**	**95%CI**	***p*-value**
Hand washing			
Child did not wash hands before eating	5.5	1.8-18.7	0.005
Child did not wash hands with soap after visiting toilet	5.9	2.3-15.5	0.001
Exposure			
Child drunk untreated water	10.0	1.8-57.1	0.009
Child not exclusively breastfeed	7.6	1.5-39.1	0.020
Child eat food cooked the previous day	23.8	3.2-77.1	0.002
Key: % - percent, AOR- Adjusted odds ratio, LL - lower limit, UL- upper limit, CI- confidence interval			

CHAPTER FIVE

5.0 Discussion

The study was aimed at determining the prevalence of bacterial enteric pathogens among children under five in Komamboga and Kisenyi Health Centres, Kampala district.

The study showed that 23.9% of the cases had diarrhoeal disease caused by bacterial isolates. A similar study from Tanzania showed that 33% of isolates were of bacterial origin (Moyo, *et al*., 2011). This could be partly due to fact that the majority of the children could have used antibiotic prior to visiting the Health Centres. Another study in Kenya by Onyango (2010) found that prevalence of diarrhoea due to enteric bacterial in children 6-36 months was 16.7%.

The prevalence rate was highest in the age group of 3-29 months, implying that children aged between 3 months and 29 months were more susceptible to diarrhoeal than those above 29 months. The findings of the 2006 Uganda Demographic Health Survey (UDHS, 2006) showed that, young children ages 6 – 23 months were more prone to diarrhoea than children in the other age groups. There were no variations in the prevalence of diarrhoea by child's sex.

The pathogenic organisms found among these children with diarrhea were *Escherichia coli* 0157:H7 constitute the highest proportion of pathogens 40.7%, followed by *Shigella flexneri* constituting 24.9%, *Salmonella paratyphi* 10.8% and *Shigella dysentriae* 7.4%.The findings in this study are similar study done in Kericho and Kisumu hospitals in Kenya, showing that most of the isolates were *Escherichia coli* and *Salmonella* spices (Segecha Shirley, 2013). *Escherichia coli* 0157:H7 has been recognised by the World Health Organisation (WHO, 2008) to be one of the major causes of diarrhoea.

The antimicrobial susceptibility profile shows that high rates of resistance were recorded for first line drugs that are commonly prescribed in Uganda.Higher resistance was observed in Tetracycline, Ampicillin, Sulphamethoxazole and Nalidixic Acid (96.3%,88.9%, 88.9 %,85.2%) respectively. This corresponds with a study done in Kenya, which found a high level of isolates to be resistant to the antibiotics prescribed most commonly in Kenya, such as Amoxicillin, Ampicillin, and Tetracycline (Kim, *et al.;*2003). The mushrooming of clinics and pharmacies in Kampala with no control over the prescription and use of antibiotics, buying drugs over the counter and under dose/over dosage may explain the antibiotic resistance observed (Byarugaba, 2004).Similarly, previous studies conducted in developing countries have established that, in

some locations, antibiotics can be purchased from private hospitals, pharmacies, and patent medicine stalls without prescription, even when the practice is not legal (Sack,*et al.,* 1997).
Fortunately, the result of the antibiogram showed that the isolates were highly susceptible to the Ciprofloxacin. However, the use of Ciprofloxacin is not recommended in young children.
In this study, we found poor hand washing practices to be a strong risk factor for diarrhoea ($p=$ value 0.005). This is in agreement with a study carried out in Ethiopia which established that hand washing with soap is another important barrier to transmission (Teklemariam, *et al*., 2000) and has been cited as being one of the most cost effective public health interventions. A number of studies have also shown that hand washing with soap can reduce the incidence of diarrhoea caused by over 40 % (Jamison, *et al*., 1993).Non-exclusive breast feeding was associated significantly with diarrhoeal illness ($p=0.020$). The predisposing factors that enhance spread and increase the risk of diarrhoea in young children include failure to breast feeding exclusively for the first 4 – 6 months of life (Sule, *et al.,* 2011). The risk of developing diarrhoea is greater in non-breast fed infants than those exclusively breast fed. Breast feeding until at least one year of age reduces incidence and severity of diarrhoea disease by providing protective antibodies. (Abdullahi, *et al.,* 2010). In this study, most of the children with diarrhoea illness had eaten food cooked the previous day. The observation raises a question on food storage practices in this community.

5.1 Study Limitations

The sample size was not large enough to estimate the prevalence of the conditions of interest with adequate precision.

Campylobacter species which is one of the most common bacterial causes of diarrheal illness was not investigated due to limited diagnostic techniques.

Conclusion

The findings reveal that bacterial pathogens play an important aetiological role in acute diarrhoea amongst children under the age of five years in our environment. The pathogenic organisms found among these children with diarrhoea were *Escherichia coli* was, *Shigella flexneri*, *Salmonella typhi*, *Salmonella paratyphi* ,*Shigella boydii* ,*Shigella dysentriae* and *Enterobacter* species.

The organisms showed remarkable sensitivity to Ciprofloxacin while displaying limited sensitivity to the more commonly used antibiotics. Higher resistance was observed in Ampicillin, Sulphamethoxazole, Nalidixic Acid and Tetracycline. It was established that children who did not wash hands before eating and after visiting the toilet, children drinking un treated water and also children who did not breast feed exclusively significantly had enteric bacteria causing diarrhoea.

Recommendations

From the study above, the recommendations below are suggested;

1. Further studies including parasitic, fungal, bacterial and viral surveillance with more advanced facilities and more numbers of antimicrobial agents should be conducted.

2. This study highlights the need to explore further the impact of fluoroquinolones on child diarrhoea and effectively regulate the use of antibiotics in the management of diarrhoea while promoting diarrhoea prevention initiatives in the community.

3. There is need to scale up the use of ORS in the management of diarrhoeal, both at home and in health facilities, as well as efforts to improve personal and domestic hygiene .This should include conducting health education campaigns to promote appropriate hand washing using soap.

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APPENDICES

APPENDIX 1: CONSENT FORM

I am a final year Medical Laboratory Sciences student of International Health Sciences University. I am here to conduct a study on prevalence of bacterial enteric pathogens among children less than five years presenting with diarrhoea. The study is trying to find out the most common causative agents and to understand the important factors associated with diarrhoea morbidity among children less than five so that we could employ proper measures toward its prevention. Since the child is too young to decide on his/her own, I would like to interview you, and ask you for your permission to collect stool sample from your child.

I have few questions about diarrhoea and related issues. Your answers will be written and then used for analysis. All information you provide will be handled as confidential and your individual answers will not be known, except the interviewer and the coordinator of this study. The results will be used only to improve strategies for prevention of diarrhoea, one of the most common diseases among children in the community.

We will need at least 20 minutes to discuss and record the information. You can withdraw from the interview at any stage without any consequence if you do not wish to continue.

Will you participate in this study? Yes [ ] No [ ]

Do you have any question?

Thank you.

Date: …../……./2014.

Interviewee's signature: ……

APPENDIX 2: QUESTIONNAIRE

I. DEMOGRAPHIC AND SOCIO-ECONOMIC INFORMATION

1. Identification number: ……..

2. Address: ……

3. Your age: ……years. The child's age: …….

4. The child's sex (Put √ in the applicable box) [ ] Male [ ] Female

5. Occupation: Mother [ ] Father [ ] 1. Peasant

2. Employed

3. Self-employed

6. How many people are living in this house?.....

II. CLINICAL DATA

1. Hospitalized on: ……. /……. / 2014

2. Weight: …….kg. Height: …….cm.

3. Temperature: ……. º C.

4. Number of days with diarrhoea…….... days.

5. Stool frequency per day:.....

6. Is there blood in stool? Yes [ ] No [ ]

7. Has the child vomited? Yes [ ] No [ ]

If yes, state vomiting frequency per day:

8. Patient's dehydration status: [ ]

1. None
2. Mild
3. Moderate
4. Severe

9. Did the child contact to any diarrheal patient for the last 7 days? Yes [ ] No [ ]

10. Did the child eat any food sold by street vendors for the last 7 days? Yes [ ] No [ ]

If yes, state what food the child ate ……

III. SANITATION AND RUBBISH DIPOSAL

1. Do you have a latrine? Yes [ ] No [ ]

If no, how do you defecate yourself? [ ] 1. Directly excrete on the ground

2. Other: ……

If yes, is it in use? Yes in use [ ] Not in use [ ]

2. Is it private of public? Private [ ] Public [ ]

3. Type of the latrine used [ ] 1. Modern toilet

2. Dry latrine

4. How often is the latrine cleaned? [ ] 1. Every time it is spoiled

2. Every day

3. Not cleaned

5. Are your children able to use the latrine on their own? Yes [ ] No [ ]

6. If no in question 5, how do you dispose of the faeces? [ ]

1. Buried
2. Put in the latrine
3. Thrown away in open surrounding
4. Other: ……

7. Where do you dispose of household garbage? [ ] 1. Rubbish pit

2. Open surrounding

3. Other: ……..

IV. HYGIENE PRACTICES AND OTHER DOMESTIC BEHAVIORS

1. Does your child feed on his/her own? Yes [ ] No [ ]
2. Do you often wash the child's hands before eating? Yes [ ] No [ ]
3. If yes, how do you treat the child's hands before eating any food? [ ]

1. Washing by water only
2. Washing by water with soap
3. Others:……

V. WATER RELATED PRACTICES

1. From what sources do you get your drinking water? [ ] 1. Running water
   2. River
   3. Pond
   4. Well
   5. Rain-water
   6. Other:…….

2. What kind of utensils do you use for storing water? [ ] 1. Storage containers without lid
   2. Storage containers with lid

3. Do you always clean/empty the storage container before replacing with fresh water?
   Yes [ ] No [ ]

4. What type of water does your family use for drinking? [ ] 1. Boiled
   2. Filtered
   3. Other: …….
   4. Untreated

VI. BREASTFEEDING STATUS

1. Do you breastfeed your child? Yes [ ] No [ ]

2. If yes, have you exclusively breastfed the child in the first six month of his/her life?
   Yes [ ] No [ ]

3. If the child less than 6 months old, Have you exclusively been breastfeeding the child to date?
   Yes [ ] No [ ]

If no, how long by now have you introduced other foods to the child? ……

Date: ……. /… …. /2014

Interviewers signature…….

APPENDIX 3: INTRODUCTORY LETTER

IHSU INTERNATIONAL HEALTH SCIENCES UNIVERSITY

PART OF INTERNATIONAL MEDICAL GROUP

Office of the Dean, Allied Health Sciences

Kampala, 29th September 2014

CENTRAL PUBLIC HEALTH LABORATORIES MINISTRY OF HEALTH 08 OCT 2014 Received US3 Laboratory

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.....

.....

Dear Sir/Madam,

RE: ASSISTANCE FOR RESEARCH

Greetings from International Health Sciences University.

This is to introduce to you **Nandala Micheal Wanzila**, Reg. No. **2011-BMLS-PT-011** who is a student of our University. As part of the requirements for the award of a Bachelors of Medical Laboratory Science of our University, the student is required to carry out research in partial fulfillment of his award.

His topic of research is: **Prevalence of Bacterial Enteric Pathogens among Children under Five Years in Kampala District.**

This therefore is to kindly request you to render the student assistance as may be necessary for his research.

I, and indeed the entire University are grateful in advance for all assistance that will be accorded to our student.

International Health Sciences University P.O Box 7782, Kampala 08 SEP 2014

Sincerely Yours,

Okiria John Charles

Senior Lecturer / Dean, School of Allied Health Sciences

APPENDIX 4: ACCEPTANCE LETTER

TEL:
FAX: 256-041-345108
IN ANY CORRESPONDENCE ON
THIS SUBJECT PLEASE QUOTE .....

THE REPUBLIC OF UGANDA

CENTRAL PUBLIC HEALTH
LABORATORIES,
MINISTRY OF HEALTH,
P.O. Box 7272,
KAMPALA – UGANDA

18th January 2014

Nandala Michael Wanzila
Faculty of Allied Health,
International Health Sciences University,
Kampala.

Re: Approval to utilize CPHL Laboratory facilities for your academic research.

In reference to your request letter dated 10th January 2014 seeking permission to undertake your undergraduate research activities at this facility entitled **"Prevalence of bacterial enteric pathogens among children under five years in Kampala district"**, the research committee sat and reviewed your application.

I am happy to inform you that the committee resolved that you undertake the study using our laboratory facilities for the length of time as indicated in your application. The department will provide necessary reagents and consumables for your work and expects a copy of your report upon completion.

Wish you luck.

Atek Kagirita
Head of Department
CC: Aisu Stephen (Head CPHL)