PREVALENCE OF ENTERIC BACTERIA ASSOCIATED WITH DIARRHOEA IN CHILDREN LESS THAN FIVE YEARS OF AGE; AND THEIR SENSITIVITY TO ANTIBIOTICS IN UNGUJA ISLAND-ZANZIBAR

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A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR DEGREE OF MASTER OF SCIENCE (BIOLOGY) M.Sc OF THE OPEN UNIVERSITY OF TANZANIA

2015

CERTIFICATION

The undersigned certifies that they have read and hereby recommend for acceptance by The Open University of Tanzania the Thesis titled "*Prevalence of Enteric Bacteria Associated with Diarrhoea in Children less than Five Years of Age; and their Sensitivity to Antibiotics in Unguja Island- Zanzibar"* in fulfillment of the requirements for the degree of Master of Science (Biology) of The Open University of Tanzania.

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Date

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DECLARATION

I, **Muhiddin H. Omar**, do hereby declare that this Thesis is my own original work and has not been presented, and will not be presented, to any other university for a similar or any other degree award.

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Signature

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Date

DEDICATION

This piece of work is dedicated to my beloved wife Bishara A. Mohd, my son Ahmad and to daughters Ilham and Thamrat.

ACKNOWLEGEMENT

I would like to thank Professor E.S.P Kigadye (supervisor) for his encouragement and advice on how to develop this thesis whose experience, constructive criticism, generous support and encouragement gave me energy, strength and self-confidence to finish this study. Doctor Ali Rabia, for his idea and recommendation for title of this study. The director and all staff of the Pathology laboratory at Mnazi Mmoja Hospital for their participation in this study. All patients, who decided to participate and answered the questionnaire in this study. All of the staff in the Department of microbiology at Mnazi Mmoja Hospital as well as my friends and, for their technical support. I would like to extend my gratitude to Ministry of Health Zanzibar, Health research Unity, National Statistics Department, Zanzibar Health Information and System, All Districts Health officers provide all data and information which used in this research. Thanks to Mr. Abdullah who worked hard for me in data analysis assistance. Million of thanks to my lovely wife and sons for their patience and encouragement during the whole time of this study.

ABSTRACT

The study aimed to determine the prevalence of enteric pathogenic bacteria associated with diarrhoea in children less than five years of age and their sensitivity to antibiotics in Unguja Island- Zanzibar. The prevalence of pathogenic enteric bacteria were isolated and indentified by convectional method and Antimicrobial resistance by Kirbeur method while Qustionnaire were used to identify the associated factors for bacteria diarrhoea and antibiotic resistance. The common pathogenic enteric bacteria were *Shigella* compare to *Salmonella*, Pathogenic *E.coli* and *Vibrio parahaemolyticus*. Prevalence by spatial of enteric bacteria was high in urban than rural areas and rainy seasons reported high prevalence of enteric bacteria compare to dry seasons. The children at the age between 7months to 2 years old were at high risk to be infected with enteric pathogenic bacteria than any other age groups. The pathogenic enteric bacteria were more resistance to sulfamethoxazole/ Trimethoprim, Erythromycin, tetracycline and Ciproflaxin compare to Gentamycine, and Chloromphenicol and Ampicilline. Poor hygienic and sanitary practices by mothers and poor immunity of children were among of the associated factors for diarrhoea in children and lack of awareness to antibiotics resistance to community was among of the factors associated with microbial antibiotic resistance in Zanzibar. The *Shigella* was predominant enteric pathogenic bacteria caused diarrhoea to less than five years children. Isolated enteric pathogenic bacteria were more resistant to common used antibiotics for treatment of diarrhoaea to less than five years children in Zanzibar. Improvements of hygienic and sanitary practices to community particularly in rainy seasons could reduce the diarrhoea incidence. Zanzibar standard treatment guideline should be reviewed.

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LIST OF ABBREVIATIONS AND ACRONYMS

Amp	Ampicillin
API	analytical profile index
APW	Alkaline peptone water
CDC	Center for Disease Control
Ch	Chloromphenicol
CIN	cefoperzone deoxycholate, cefsulodin-Irgasan novobiocin
CIP	Ciproflaxin
DNA	Deoxyribonucleic Acid
E.coli	*Escherichia Coli*
Eager	Entero-aggregative *E.coli* (Eager),
EIEC	Entero-invasive *E.coli*
EPEC	*E.Enteropathogenic Escherichia coli*
Ery	Erythromycin
ETC	Electron Transport Chain
ETEC	Enterotoxigenic E.coli
GDP	Growth Domestic Product
Gen	Gentamycin
H_2S	Hydrogen sulfide
HAS	Health Surveillance Assistants
HEA	Helton enteric agar
HUS	Haemolyticus-uramic syndrome
KIA	Kligler's Iron Agar):

KOH	Potassium hydroxide
MIC	Minimum Inhibitory Concentration
MIO	Motility Indole test
MOH	Ministry of Health
MR	Methyl red
ORS	Oral Rehydration Salt
*S. enteric*a	*Salmonella enterica*
SMCA	MacConkey agar with Sorbitol
TCBS	Thiosulfate citrate bile salt
Tet	Tetracycline
TMX	Sulfamethoxazole/Trimethoprim
TSI	Triple Iron Sugar
UNICEF	United Nation International Children and Emergency Fund
V.parahaemolyticus	*Vibrio parahaemolyticus*
VP	Voges – Proskauer
VTEC	Verocytoxin- producing *E.coli*
VTEC	Verocytoxin- producing *E.coli*
WHO	World Health Organization
XLD	Xylose Lysine decarboxylase

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background to the Problem

Diarrhoea is an important cause of morbidity and mortality among children in developing countries. Every year there are approximately 1.5 billion diarrhoeic episodes worldwide and 4million deaths in children less than five years of age (mostly from 6 months to 12 years) caused by this disease (Vargas *et al.,* 2004).

Worldwide, the most common pathogens responsible for acute diarrhoea are bacteria *Campylobacter jejuni*, *Escherichia coli*, *Salmonella* spp, *Vibrio cholerae*, *Yersinia enterocolitica* and *Acromonas* spp), Enteroparasite such as *Giardia* spp, *Cryptosporidium* spp and *Entamoebahistolytica* and *Balatidium coli* as well as viruses such as Adenovirus, Norwalk virus and Rotavirus (Stephan *et al.,* 2009**).** Salmonellosis, Shigellosis, Yersiniosis and Campylobacter infection cause potentially serious diarrhoea disease (CDC April 10, 2009).

The common route of infection by these pathogens is through ingestion of food and drinking contaminated by feaces. The common infected food includes raw egg, raw meat, poultry, sea foods, poorly cooked food, fresh vegetable and tomatoes. The common symptoms for these infection is diarrhea, vomiting, nausea, abdominal pain and bloody or mucus stool, convulsion (seizure) as well as skin rash specific to shigellosis, rice watery diarrhoea (cholera) and foul smell stool (campylobacter infection) (Pandey *et al.,* 2001).

Environmental factors influence the distribution, diversity, and incidence of the disease. Diarrhoea as other human specific illness is linked to change of season, local weather pattern and other environmental factors. These factors facilitate the spread of diseases since they have interaction between disease agents and vectors, which have particular environments that are optimal for growth, survival as well as transport (Kosek *et al.,* 2003).

Zanzibar like other parts in the world has experienced seasonal epidemics of acute diarrhoea such as cholera and dysentery usually during the rainy seasons, when many cases are reported in both primary health centers and central hospitals. Severe outbreaks of cholera occurred both on Unguja and Pemba islands in 1997/98 and in 2004, with the number of reported cases ranging from 520 to 650 cases per outbreak. (Zanzibar Health Information System Unity 2012). In Zanzibar, cholera outbreaks are typically linked to the seasonal rains that occur from March to June and in October/November.

According to epidemiological study on diarrhoea conducted in Zanzibar, cholera was recognized by 88% reported at the peri-urban site compare to dysentery. Dirty environment was the most prominent perceived cause, followed by unsafe drinking water and germ-carrying flies (Chaetti *et al.,* 2010).

The enteric pathogens that have been frequently isolated are *Escherichia coli, Salmonella* spp and *Vibro spp (*Fauzia, 2013). Other pathogens however are not routinely isolated and identified, this is because some healthcare facilities do not carry out further medical laboratory examination in culturing stool samples to

identify other kinds of pathogens due to lack of the necessary equipment. Due to this reason the data of common isolated enteric pathogens from stool of under five years of age in Zanzibar are not available.

A severe infection of diarrhoea in children is highly associated with risk factors which include poor environment sanitation and hygiene, inadequate water supplies, poverty and malnutrition (Rebecca*et al*., 2012). Some risk factors vary with age and the weaning practices of the children; bottle-feeding is highly associated with diarrhea to children with age between 1 to 6 months (Woldemicael *et al*., 2001). The major practices of mothers or caregivers related to bottle- feeding may contaminate the food as well as affect the nutritional status and lead malnutrition to child. Malnutrition lowers the body immunity which associated with diarrhoea (Sally et al., 20069. In general the morbidity in diarrhoea is lowest in exclusive breast- fed children and it is higher in partially breast- feed children, and in fully weaned children (Molbak*et al*., 2000).

Diarrhoel diseases is the second leading cause of the death in children under five years old, and is responsible for killing around 760,000 children every year (WHO,2013). Globally, there are nearly 1.7 billion cases of diarrhoea disease every years in less than five years old, diarrhea can last several days and can leave the body without the water and salts that are necessary for survival (WHO, 2013).

The prevalence of diarrhea among children under five year in Tanzania in 2012 was at 15%, Unguja 13.7%, Pemba 13.6% and Dar es Salaam 7.4%. Estimated deaths due to diarrhoea in Tanzania were 23,900 in 2004 and in 2012 23,900 annual (WHO

2004). Diarrhoea morbidity ranked second after malaria and third among cause child mortality (National Bureaus of Statistic Tanzania, 2012). In Zanzibar diarrhea (including dysentery and cholera) is the leading cause of morbidity and mortality in children (Zanzibar Health Information system Unity, 2013).

There is a limited data regard to prevalence of enteric pathogenic bacteria in Zanzibar and many surveys conducted focused only epidemiology of cholera and few diarrhoea cases rather than prevalence of molecular epidemiology of particular pathogens. Antimicrobial resistance among enteric Gram negative bacteria is becoming a global public health concern with rapid increase in multidrug resistant organisms (Kosek *et al.*, 2003). There are concerns from parents that their children use two or more antibiotics at different time but could not get relief. Also some complain that their children are given ineffective antibiotics and the types of antibiotic prescribed to them for similar case of diarrhoea, differ between one physician and another. This information also was verified by the Food and Drugs Board registrar (2013). The outcome to such inconsistence and provision of improper antibiotics to treat diarrhoea may favor the resistance of bacteria to antibiotics.

The aim of this study was to determine spatial and temporal prevalence of enteropathogenic bacteria causing diarrhoea among children less than five years old in Zanzibar; and their susceptibility to antibiotics. The information emanating from this study will be invaluable to the general public health practitioners, planning and policy makers in prevention of diarrhea diseases in Zanzibar. Moreover no study of seasonal and regional prevalence of enteropathogens causing diarrhoea to children has ever been conducted in Unguja Island- Zanzibar before.

1.2 Statement of Problem

In Zanzibar epidemiological data related to diarrhea shows that the prevalence of diarrhoea incidence is high during the long rainy season (March -May) (Zanzibar Epidemiological Unity, 2013). Data from Mnazi Mmoja Hospital shows that diarrhoea is the leading course of mortality in less than five years children in Zanzibar. Most children suffering from diarrhoea cases are from rural and peri-urban areas where there is overcrowding, poor housing settlements and where availability of safe water is a problem. Unfortunately most of these areas have not been researched thoroughly.

In addition currently there is no study focused on the prevalence of common pathogens associated with diarrhoea in Zanzibar. Antibiotic resistance is a global problem and the provision of effective antimicrobial drugs to diarrhoeal patient is still a challenge to our health care providers (Mellon *et al*., 2009). According to Zanzibar treatment guideline, the first line choice of antibiotics recommended for treatment of diarrhoea in children are erythromycin and sulfamethoxazole/ trimethoprim. The availability and continuous use of these antibiotics to treat diarrhea probably favours bacterial resistance and may pose the challenge to physicians in drugs prescription.

Currently no study has been carried to evaluate their effectiveness in Zanzibar, however the studies on antibiotics sensitivity carried out in Morogoro Tanzania and Kenya showed most of the diarrhoeal pathogens were resistant to erythromycin and trimethoprim antibiotics (Jackson *et al*., 2009: Vargas *et al*., 2004) Definitely Zanzibar may have a similar problem, because similar antibiotics are still being used

for treatment of diarrhea, in both public and private hospitals for a number of years. Therefore antibiotic sensitivity test to enteric pathogenic bacteria is important to address the situation in study area.

1.3 Objectives

1.3.1 General Objective

To determine the spatial-temporal prevalence of pathogenic enteric bacteria, in children less than five years of age and their sensitivity to antibiotics in three districts of Unguja Island- Zanzibar.

1.3.2 Specific Objectives

To identify the common pathogenic enteric bacteria, affecting children less than five years of age in three districts of Unguja Island- Zanzibar.

To determine frequency of occurrence of pathogenic enteric bacteria associated with diarrhoea in children less than five years of age in Unguja Island- Zanzibar.

To establish factors responsible for the increasing diarrhoea incidence in children less than five years of age in the study area.

To carry out laboratory antibiotic sensitivity test of isolated bacteria in the study area.

1.4 Significance of the Study

There is paucity of information on identifying seasonal and spatial prevalence of enteric pathogens associate with diarrhoea in Zanzibar. Definitely this study provides useful and valuable information about etiology of bacterial diarrhea in Zanzibar. Also it provides more information to public health stakeholders in their effort to

control the diarrheal diseases as well as decision making and guide planning in health care service provision.

Moreover determination of antimicrobial susceptibility pattern not only -could help to formulate a successfully treatment plan for diarrhea for individual patient but also the development of public policy for population at risk in Zanzibar. The data of antimicrobial susceptibility pattern will help to in reviewing the regular treatment of diarrheoa as well as improvement of the treatment of diarrhoea and therefore reducing mortality and morbidity among children hence improving public health to population of Zanzibar. Furthermore proper treatment will reduce needless use of antibiotics hence reduce rate of antimicrobial resistance among enteric pathogenic bacteria.

1.5 Hypothesis

Shigella and Salmonella are the common pathogens enteric bacteria isolated in Unguja island- Zanzibar.

There is a relationship between occurrence of pathogenic enteric bacteria and season.

Lack of knowledge to diarrhoea and poor hygienic practices, favour-increasing rate of gastroenteritis in children less than five years of age in the study area.

Erythromycin, Sulfamethoxale/Trimethoprin and tetracycline are not effective antimicrobials to treat enteric bacteria in children.

1.6 The Scope of the Research

The research involved investigation of enteric bacteria causing diarrhoea in a selected sample of less than five years children attending in selected primary health care centres for study.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of Terms

Diarrhoea is characterized by an increased frequency and volume, and decreased consistency of stool from the normal. It must be remembered that frequency of passing stool varies with age and is higher in infants. Dysentery is defined as the passage of blood and mucous in stools. Persistent diarrhoea occurs when the duration of symptoms exceeds seven days and chronic diarrhoea when it lasts more than 14 days (Vargas *et al.*, 2004).

Prevalence of a disease: Is the total number of existing cases respective to the entire population. In community statistic, it is usually represented as the percentage of the population having a particular disease at any given time (Talaro *et al*., 1996).

Antibiotic resistance: Is the natural ability of microorganisms to withstand the effect of drugs that are lethal to most members of its species or is the ability of a bacterium or other microorganism to survive and reproduce in the presence of antibiotic dose that were previously thought effective against them(Modern Medical Dictionary, 2002).

Susceptibility to antibiotics is the quality or state of being susceptible or the state of being predisposed or lacking the ability to resist something (www.medical dictionary/org). The antimicrobial susceptibility testing is to predict the in vivo success or failure of antibiotic therapy. Test is performed in vitro, and measures the

growth response of an isolated organism to particular drug. This helps to provide clinical information for selecting appropriate antibiotic for your patient. The data from the test will be interpreted as susceptible, resistance or intermediate.

Intermediate: Include isolated with antimicrobial minimum inhibitory concentration (MICs) that approach usually attainable blood tissue level and for which response rates may be lower than for susceptible isolates (Bruce, 2007).

2.2 Types of Diarrhea

According to World Health Organization (2013), diarrhoea could be classified into four types, based on clinical symptoms to less than five years children, each reflecting different pathogenesis; watery diarrhea, dysentery, prolonged diarrhea and chronic diarrhea.

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. It is accompanied by flatulence, malaise and abdominal pain, nausea, vomiting, fever also may be present. The common causes of acute watery diarrhea are viral, bacterial and parasitic infections. In general the pathogenic bacteria which are most important includes; *Shigellae, Pathogenic E.coli, Vibrio spp, Camplobacter jejuni, and Salmonella spp* (Blanaca *et al*., 2007).

Dysentery: May be defined as diarrhea containing blood and mucus in feaces. The illness also includes abdominal cramps, fever, and rectal pain. The most important cause of bloody diarrhoea is *shigella*. The *Shigella* can cause septicemia (blood

poisoning), rectal prolapsed and haemolyticus-uramic syndrome (HUS) which affect the kidney and blood clotting system (WHO, 2005).

Persistent diarrhoea: Is defined as diarrhoea episodes of presumed infectious etiology that have an unusually long duration and last at least 14 days. About 10 percent of diarrhoea in children from developing countries become persistence, especially among those less than three years and more so among infants. This diarrhoea cause substantial weight loss in most patients and it may be responsible for about one third to half of all diarrhoea related deaths. The pathogens responsible for persistent diarrhoea are Entropathogenic *E.coli* (EPEC), EnteroAggregassive E.coli (EAggC) and Cryptosporidium (Molbak *et al*., 2000, Veskar*et al*., 2002).

Chronic diarrhoea: This term refers to diarrhea which is recurrent or long lasting due to mainly noninfectious causes. This may be caused by gastrointestinal diseases, or may be secondary to systematic disease. In addition chronic diarrhoea could because by enteritis, food intolerance, medication and irritable bowel syndrome etc. (www.uspharmacist.com).

2.3 The main Causative Agents of Diarrhoea

Though some diarrhoea is due to errors of metabolism, chemical irritation or organic disturbance, the vast majorities are caused by infectious pathogens (Woldemicael *et al*., 2000).

Bacterial infections: Diarrhea caused by enteric bacterial infections is the most prevalent worldwide, especial in tropical and developing countries where it cause

serious problem among older children and adults as well as in infants and young children. The causative microorganisms are very diverse they include; *E. coli, Salmonella, Shigella, Camplobacter, Yersinia, Vibrio spp and Clostridium difficile* (WHO/UNICEF, 2009).

Viral infections. Rotavirus is one of the most common causes of severe diarrhea. Also other virus may be important causes of diarrhoeal diseases in human, these include; Norwalk *virus, Norwalk-like viruses, adenoviruses, calici viruses and* astroviruses (Nguyen et al., 2004).

Parasites: Parasites can enter the body through food or water in the digestive system. Parasites that cause diarrhoea include *Giardia lambia, Entamoebahistolytica and Cryptosporidium (G*racey, 2004).

Food intolerances: Some people are unable to digest some components of food, such as lactose sugar found in milk, or gluten found in wheat (Tumwine *et al.*, 2002).

Reaction to medicine: Some kinds of antibiotics such as sulphonamide and laxative may cause diarrhoea (Warren et *al*., 2003).

2.4 Transmission Routes of Causative Agents (Pathogens)

Infectious diarrhoea is acquired by fecal-oral transmission that includes consumption of contaminated food or water, person-to-person contact, or direct contact with fecal matter. With regard to water–borne diarrhoea, transmission patterns occur when domestic water storage facilities or/and water sources for human consumption are contaminated (Jensen *et al*., 2004). There are four transmission routes that the major

infectious agents use to reach human host, namely human to human via the environment, human to human multiplying in the environment, human to animal to human via the environment, and animal to human via the environment. In situation where feacal contamination of the domestic environment is high, the majority of cases of endemic disease probably occur either by human-to-human transmission, or from the human-to-human transmission, or from the human-to-human transmission of the pathogenic agent, which have multiplied in the environment (Curtis *et al*., 2000).

In study area the common routes of infection of theses enteric bacteria to under five years children are through poor person and environmental hygienic practices involves poor food handling, lack or poor knowledge of hands washing by children and parents as well poor waste disposal of children feace.

2.5 Common Enteric Isolated Bacterial Pathogens Associated with Diarrhea

2.5.1 Salmonella

Salmonella is a bacterium that causes one of the most common enteric (intestinal) infections known as salmonellosis. (Miller *et al*., 2005) It has long been said that, in 1885, pioneering American veterinary scientist, Daniel E. Salmon, discovered the first strain of *Salmonella* (Millwer *et al*., 2005). Actually, Theobald Smith, research-assistant to Dr. Salmon, discovered the first strain of *Salmonella* known as *Salmonella* cholerae (Kass *et al*., 1987) but, being the person in charge, Dr. Salmon received credit for the discovery (Kass *et al*., 1987). In any case, today the number of known strains of the bacteria totals over two thousand (Behravesh *et al*., 2008).

The term *Salmonella* refers to a group or family of bacteria that variously cause illness in humans. *Salmonella* serotype typhimurium and *Salmonella* serotype Enteritidis are the most common (CDC, 2007) *Salmonella* infections can have a broad range of illness, from no symptoms to severe illness. The most common clinical presentation is acute gastroenteritis. Symptoms include diarrhea and abdominal cramps, often accompanied by fever of 100°F to 102°F (38°C to 39°C) (Behravesh *et al.,* 2008). Other symptoms may include bloody diarrhoea, vomiting, headache and body aches.

The incubation period, or the time from ingestion of the bacteria until the symptoms start, is generally 6 to 72 hours; however, there is evidence that in some situations the incubation can be longer than 10 days (Medus *et al.,* 2006). People with salmonellosis usually recover without treatment within 3 to 7 days. Nonetheless, the bacteria will continue to be present in the intestinal tract and stool for weeks after recovery of symptoms—on average, 1 month in adults and longer in children (Behravesh *et al.,* 2008).

S. typhi and *S.paratyphi* generally cause a bacteremic illness—*Salmonella* found in the blood—of long duration. This illness is called enteric, typhoid, or paratyphoid fever. (Miller *et al*., 2005) Symptoms start gradually, and include fever, headache, malaise, lethargy, and abdominal pain. In children, it can present as a non-specific fever. The incubation period for *S. typhi* is usually 8 to 14 days, but it can range from 3 to 60 days. (Behravesh *et al.,* 2008 and Miller *et al*., 2005). For *S. paratyphi* infections, the incubation period is similar to that of non-typhoidal *Salmonella*, 1 to 10 days (Medus *et al*., 2006).

In 2009, over 40,000 cases of *Salmonella* (13.6 cases per 100,000 persons) were reported to the Centers for Disease Control and Prevention (CDC, 2010) by public health laboratories across the nation, representing a decrease of approximately 15% from the previous year, but a 4.2% increase since 1996. Overall, the incidence of *Salmonella* in the United States has not significantly changed since 1996. (CDC, 2009).

Only a small proportion of all *Salmonella* infections are diagnosed and reported to health departments (Tauxe *et al.*, 1997). It is estimated that for every reported case, there are approximately 38.6 undiagnosed infections (Tauxe *et al.*, 1997). The CDC estimates that 1.4 million cases, 15,000 hospitalizations, and 400 deaths are caused by *Salmonella* infections in the U.S. every year (Voetsch *et al*., 2004).

Salmonella can be grouped into more than 2,400 serotypes (Miller *et al*., 2005) .The two most common serotypes are *S. typhimurium* and *S. enteritidis*. *S. typhi*, the serotype that causes typhoid fever (Behravesh *et al*., 2008).But, globally, typhoid fever continues to be a significant problem, with an estimated 12-33 million cases occurring annually. Moreover, outbreaks in developing countries have a high death rate, especially when caused by strains of the bacterium that are resistant to antibiotic treatment (Miller *et al*., 2005).

Salmonella are found in the intestinal tract of wild and domesticated animals and humans. Some serotypes of *Salmonella*, such as *S. typhi* and *S. paratyphi* are only found in humans (Miller *et al*., 2005). For ease of discussion, it is generally useful to

group *Salmonella*e into two broad categories: typhoidal, which includes *S. typhi* and *S. paratyphi*, and non-typhoidal, which includes all other.

The USDA Economic Research Service (ERS) published its first comprehensive cost estimates for sixteen food borne bacterial pathogens in 1989 (Kass *et al*., 1987). Five years later, it was estimated that the medical costs and productivity losses that *Salmonella* infections caused each year ran from $1.188 billion to over $11.588 billion, based on an estimate of 1.92 million cases and between 960-1,920 deaths (CAST, 1994).

Salmonella serotyping still serves as the predominately used surveillance tool for detection of outbreaks and corresponding sources, to monitor trends over time, and attribute different food and animal reservoirs to human infections. Despite this, there is today only limited knowledge of the global distribution of *Salmonella* serovars in humans. In the last decade, some countries have collected annual prevalence data on serovar distribution among humans, but very few publications have summarized the global distribution of the serovars responsible for human infections and further analyzed the data (Herikstad *et al.*, 2002; Galanis *et al.*, 2006).

In approximately 5% of non-typhoidal infections, patients develop bacteremia (Varma *et al*., 2005). In a small proportion of those cases, the bacteria can cause a focal infection, where it becomes localized in a tissue and causes an abscess, arthritis, endocarditis, or other severe illness. Infants, the elderly and immune-compromised persons are at greater risk for bacteremia or invasive disease (Buzby *et al*., 1996). Additionally, infection caused by antimicrobial-resistant non-typhoidal

Salmonella serotypes appears to be more likely to cause bloodstream infections (Jone *et al*., 2008).

Overall, approximately 20% of cases each year require hospitalization, 5% of cases have an invasive infection, and one-half of 1% die. Infections in infants and in people 65 years of age or older, are much more likely to require hospitalization or result in death (Varma *et al*., 2005). There is some evidence that *Salmonella* infections increase the risk of developing digestive disorders, including irritable bowel syndrome (Mearin *et al*., 2005).

Although most persons that become ill with diarrhoea caused by *Salmonella* recover without any further problems, a small number of persons develop a complication often referred to as Reactive Arthritis. The terminology used to describe this type of complication has changed over time. The term "Reiter's Syndrome" was used for many years, but has now fallen into disfavor. The precise proportion of persons that develop reactive arthritis following a *Salmonella* infection is unknown, with estimates ranging from 2 to 15 % (Townes *et al.,* 2010). Symptoms of reactive arthritis include inflammation (swelling, redness, heat, and pain) of the joints, the genitourinary tract (reproductive and urinary organs), or the eyes.

One study showed that on average, symptoms developed 18 days after infection. A small proportion of those persons (15%) had sought medical care for their symptoms, and two thirds of persons with reactive arthritis were still experiencing symptoms 6 months later (Townes *et al*., 208). Although most cases recover within a few months,

some continue to experience complications for years. Treatment focuses on relieving the symptoms.

In general, safe cooking and preparation of food can kill existing *Salmonella* bacteria and prevent it from spreading. Additionally, safe choices at the grocery store can greatly reduce the risk of *Salmonella*. (USDA, 2004) Always wash your hands before you start preparing food, cook poultry until it reaches an internal temperature of 165 ºF. Cook beef and pork until they reach 160ºF. High quality steaks (not needle or blade tenderized) can be safely cooked to 145ºF.Cook eggs until they reach 160ºF or until the yoke is solid. Pasteurized eggs are available in some grocery stores (USDA, 2004).

Do not eat or drink foods containing raw eggs. Examples include homemade eggnog, hollandaise sauce, and undercooked French toast. Never drink raw (unpasteurized) milk. Avoid using the microwave for cooking raw foods of animal origin. Microwave-cooked foods do not reach a uniform internal temperature, resulting in undercooked areas and survival of Salmonella. If you are served undercooked meat, poultry, or eggs in a restaurant don't hesitate to send your food back to the kitchen for further cooking. Avoid cross-contamination. That means that you should never allow foods that will not be cooked (like salads) to come into contact with raw foods of animal origin (e.g., on dirty countertops, kitchen sinks, or cutting boards) (CDC, 11). Wash hands, kitchen work surfaces, and utensils with soap and water immediately after they have been in contact with raw foods of animal origin. Wash hands with soap after handling reptiles, amphibians or birds, or after contact with pet

feces. Infants and persons with compromised immune systems should have no direct or indirect contact with such pets (CDC 2012).

2.5.2 Shigella

Shigella is a species of enteric bacteria that causes disease in humans and other primates (DuPont *et al*., 2000). The disease caused by the ingestion of *Shigella* bacteria is referred to as shigellosis, which is most typically associated with diarrhea and other gastrointestinal symptoms. (CDC 2009) "*Shigella* infection is the third most common cause of bacterial gastroenteritis in the United States, after *Salmonella* infection and *Campylobacter* infection and ahead of *E. coli* O157 infection" (Gupta *et al*., 2004).

The global burden of shigellosis has been estimated at 165 million cases per year, of which 163 million are in developing countries (Kotloff *et al*., 1999). More than one million deaths occur in the developing world yearly due to *Shigella* infection (Krilov *et al*., 1999). By one estimate, *Shigella* infections are responsible for 300,000 illnesses and 600 deaths per year (Lee *et al*., 1991).

In general, *Shigella* is one of the most communicable and severe forms of the bacterial-induced diarrheas (Gomez *et al*., 2002). No group of individuals is immune to shigellosis, but certain individuals are at increased risk. Small children acquire *Shigella* at the highest rate, and (Krilov LR. *et al*., 1999).

Shigella is easily spread person-to-person because of its relatively tiny (compared to other bacteria) infectious dose (Kotloff *et al*., 1999). Infection can occur after

ingestion of fewer than 100 bacteria (DuPont et *al*., 1989). Another reason *Shigella* so easily cause infection is because the bacteria thrive in the human intestine and are commonly spread both by person-to-person contact and through the contamination of food (CDC 2009 and Keusch *et al*., 1996).

The several types of *Shigella* bacteria have been named after the lead workers who discovered each one (Hale *et al*., 1999) The first bacterium to be discovered, *Shigella dysentariae*, was named after Kiyoshi Shiga, a Japanese scientist who discovered it in 1896 while investigating a large epidemic of dysentery in Japan (Trofa *et al*., 1999) .The bacterium was also referred to more generally as the dysentery bacillus (the term "bacillus" referring to a genus of Gram-positive, rod-shaped bacteria of which *Shigella* is a member) (Trofa *et al*., 1999).

In a summary published annually, the CDC provides an overview of the classification of various types (species) of *Shigella* bacteria, as follows:
There are 4 major subgroups of Shigella, designated A, B, C and D, and 44 recognized serotypes. Subgroups A, B, C and D have historically been treated as species: subgroup A for Shigella *dysenteriae; subgroup B for* Shigella flexneri*; subgroup C for* Shigella *boydii and subgroup D for* Shigella *sonnei*. These subgroups and serotypes are differentiated from one another by their biochemical traits (ability to ferment D-mannitol) and antigenic properties. The most recently recognized serotype belongs to subgroup C (*S. boydii*) (CDC, 2007).

S. sonnei, also known as Group D *Shigella*, accounts for over two-thirds of shigellosis in the United States. *Shigella flexneri*, or group B *Shigella*, accounts for

almost all the rest (Gupta *et al*., 2004) more specifically, according to one recent study, "From 1989 to 2002, *S. flexneri* accounted for 18.4% of *Shigella* isolates submitted to CDC (Baer *et al.*, 1999). From 1973 to 1999, only 49 *S. flexneri*-associated outbreaks of food borne disease were reported" (Reller *et al*., 2006). In contrast, in developing countries, *S. flexneri* is the most predominant cause of shigellosis, but *S. dysenteriae* type 1 is still a frequent cause of epidemic throughout the developing world (Trofa *et al*., 1999).

The CDC estimates that 450,000 total cases of shigellosis occur in the U.S. every year (Haley *et al*., 2010). Shigellosis is also characterized by seasonality, with the largest percentage of reported cases occurring between July and October, and the smallest proportion occurring in January, February, and March. Sporadic (or non-outbreak) infections account for the majority of cases and, in general, the exact means by which persons are infected (risk factors) are not yet well documented or understood *(Gupta et al., 2004).* As previously noted, *Shigella* species are transmitted by the fecal-oral route, and most infections are transmitted from person to person, reflecting the low infectious dose. As also noted, as few as ten *Shigella* bacteria can result in clinical infection (DuPont *et al*., 1989).

The risk of transmission and infection with shigellosis, increases with poor hand hygiene, ingestion of contaminated food or water, inadequate sanitation and toileting, overcrowding, and sexual contact. (*Shigella* bacteria are present in the stools of infected persons while they are sick and for up to a week or two afterwards. It is estimated that up 80% of all infection is the result of person-to-person transmission (Mohlet *et al*., 1995).

Several studies have demonstrated an increased frequency of shigellosis cases in young adult men residing in urban settings who have little, if any, exposure to these traditionally recognized risk groups. Although some of these studies indicated that sex between men could be a risk-factor, most of these studies occurred before the HIV epidemic (Baer *et al.,* 1999).

2.5.3 *Esherichia Coli (E.Coli)*

Escherichia coli (or *E. coli*) are the most prevalent infecting organism in the family of gram-negative bacteria known as enterobacteriaceae (Eistntein *et al*., 2000). *E. coli* bacteria were discovered in the human colon in 1885 by German bacteriologist Theodor Escherich (Feng *et al*., 2002). Dr. Escherich also showed that certain strains of the bacterium were responsible for infant diarrhoea and gastroenteritis, an important public health discovery. Although *E. coli* bacteria were initially called Bacterium coli, the name was later changed to *Escherichia coli* to honor its discoverer (Feng *et al*., 2002).

E. coli is often referred to as the best or most-studied free-living organism (Jame et al., 2000) More than 700 serotypes of *E. coli* have been identified (Eisntein *et al*.,2000). The “O” and “H” antigens on the bacteria and their flagella distinguish the different serotypes (Griffin *et al*., 1999). It is important to remember that most kinds of *E. coli* bacteria do not cause disease in humans (Feng *et al*., 2002). Indeed, some *E. coli* are beneficial, while some cause infections other than gastrointestinal infections, such as urinary tract infections (Eisntein *et al*., 2000).

The *E. coli* that are responsible for the numerous reports of contaminated foods and beverages are those that produce Shiga toxin, so called because the toxin is virtually

identical to that produced by *Shigella* dysenteria type 1 (Griffin *et al*., 1991). The best-known and also most notorious *E. coli* bacteria that produce Shiga toxin is *E. coli* O157:H79 (Griffin *et al*., 1991). Shiga toxin–producing *E. coli* (STEC) causes approximately 100,000 illnesses, 3,000 hospitalizations, and 90 deaths annually in the United States (Mead *et al*., 1998). Most reported STEC infections in the United States are caused by *E. coli* O157:H7, with an estimated 73,000 cases occurring each year (Mead *et al*., 1998) .A study published in 2005 estimated the annual cost of *E. coli* O157:H7 illnesses to be $405 million (in 2003 dollars), which included $370 million for premature deaths, $30 million for medical care, and $5 million for lost productivity (Frenzen *et al*., 2005).

2.5.4 E. Coli O157:H7

E. coli O157:H7 is one of thousands of serotypes of *Escherichia coli*. The testing done to distinguish *E. coli* O157:H7 from its other *E. coli* counterparts is called stereotyping (Bell *et al*., 1994). Pulsed-field gel electrophoresis (PFGE), sometimes also referred to as genetic fingerprinting, is used to compare *E. coli* O157:H7 isolates to determine if the strains are distinguishable (Jame *et al*., 2000:Bala et al., 2001). A technique called multilocus variable number of tandem repeats analysis (MLVA) is used to determine precise classification when it is difficult to differentiate between isolates with indistinguishable or very similar PFGE patterns (Kouno *et al*., 2001).

E. coli O157:H7 was first recognized as a pathogen in 1982 during an investigation into an outbreak of hemorrhagic colitis associated with consumption of hamburgers from a fast food chain restaurant (Riley *et al*., 1983). Retrospective examination of more than three thousand *E. coli* cultures obtained between 1973 and 1982 found

only one isolate with serotype O157:H7, and that was a case in 1975(Griffin *et al*., 1991). In the ten years that followed, there were approximately thirty outbreaks recorded in the United States (Feng *et al*., 1995). This number is likely misleading, however, because *E. coli* O157:H7 infections did not become a reportable disease in any state until 1987, when Washington became the first state to mandate its reporting to public health authorities (Ostroff *et al*., 1989). Consequently, an outbreak would not be detected if it was not large enough to prompt investigation (Rangel *et al*., 2005).

E. coli O157:H7's ability to induce injury in humans is a result of its ability to produce numerous virulence factors, most notably Shiga toxin (Stx), which is one of the most potent toxins known to man. (Griffin *et al*., 1991, Johnnes *et al*., 2011, Suh *et al*., 1998). Shiga toxin has multiple variants (e.g., Stx1, Stx2, Stx2c), and acts like the plant toxin by inhibiting protein synthesis in endothelial and other cells (Sandvig *et al*., 2002). Endothelial cells line the interior surface of blood vessels, and are known to be extremely sensitive to *E. coli* O157:H7, which is cytotoxigenic to these cells (Sandving *et al*., 2002).

In addition to Shiga toxin, *E. coli* O157:H7 produces numerous other putative virulence factors, including proteins which aid in the attachment and colonization of the bacteria in the intestinal wall and which can lyses red blood cells and liberate iron to help support *E. coli* metabolism (Welinder *et al.*, 2005).

E. coli O157:H7 evolved from enteropathogenic *E. coli* serotype O55:H7, a cause of non-bloody diarrhea, through the sequential acquisition of phage-encoded Stx2, a

large virulence plasmid, and additional chromosomal mutations (Kaper *et al*., 2008, Wick *et al.,* 2005). The rate of genetic mutation indicates that the common ancestor of current *E. coli* O157:H7 like, existed some 20,000 years ago (Zhang *et al*., 2006). *E. coli* O157:H7 is a relentlessly evolving organism, constantly mutating and acquiring new characteristics, including virulence factors that make the emergence of more dangerous variants a constant threat (Robin et al., 2005). The prospect of emerging pathogens as a significant public health threat has been emphasized by the CDC for some time (Tauxe *et al*., 1997).

Although foods of bovine origin are the most common cause of both outbreaks and sporadic cases of *E. coli* O157:H7 infections, outbreaks of illnesses have been linked to a wide variety of food items. For example, produce has been the source of substantial numbers of outbreak-related *E. coli* O157:H7 infections since at least 1991(Rangel *et al*., 2005). Outbreaks have been linked to alfalfa, clover and radish sprouts, lettuce, and spinach (Elder *et al*., 2000). Other vehicles for outbreaks include unpasteurized juices, yogurt, dried salami, mayonnaise, raw milk, game meats, hazelnuts, and raw cookie dough (Breuer *et al*., 2001).

E. coli O157:H7 bacteria and other pathogenic *E. coli* mostly live in the intestines of cattle, but *E. coli* bacteria have also been found in the intestines of chickens, deer, sheep, and pigs(Eistein *et al*., 2000, Elder *et al*., 2002). A 2003 study on the prevalence of *E. coli* O157:H7 in livestock at 29 county and three large state agricultural fairs in the United States found that *E. coli* O157:H7 could be isolated from 13.8% of beef cattle, 5.9% of dairy cattle, 3.6% of pigs, 5.2% of sheep, and 2.8% of goats (Keen *et al*., 2003). Over 7% of pest fly pools also tested positive for

E. coli O157:H7 (Keen *et al*., 2003). Shiga toxin-producing *E. coli* does not make the animals that carry it ill (Eisntein et al., 2000) .The animals are merely the reservoir for the bacteria (Elder *et al*., 2000).

According to a study published in 2011, an estimated 93,094 illnesses are due to domestically acquired *E. coli* O157:H7 each year in the United States (Scallar *et al*., 2011). Estimates of food borne-acquired O157:H7 cases result in 2,138 hospitalizations and 20 deaths annually (Scallar *et al*., 2011).

What makes *E. coli* O157:H7 remarkably dangerous is its very low infectious dose, and how relatively difficult it is to kill these bacteria (Griffin *et al*., 1991). "*E. coli* O157:H7 in ground beef that is only slightly undercooked can result in infection" (Griffin *et al*., 1991). As few as 20 organisms may be sufficient to infect a person and, as a result, possibly kill them (Griffin *et al*., 1994). And unlike generic *E. coli*, the O157:H7 serotype multiplies at temperatures up to 44° Fahrenheit, survives freezing and thawing, is heat-resistant, grows at temperatures up to 111 F, resists drying, and can survive exposure to acidic environments (Juneja *et al*., 1997). And, finally, to make it even more of a threat, *E. coli* O157:H7 bacteria are easily transmitted by person-to-person contact (Rangel *et al*., 2005).

Beef and dairy cattle are known reservoirs of *E. coli* O157:H7 and non-O157 Shiga toxin-producing strains of *E. coli* (Hussein *et al*,. 2005). In reviews of STEC occurrence in cattle worldwide, the prevalence of non-O157 STEC ranged from 4.6 to 55.9% in feedlot cattle, 4.7 to 44.8% in grazing cattle, and 0.4 to 74% in dairy cattle feces. The prevalence in beef cattle going to slaughter ranged from 2.1 to

70.1%. While most dairy cattle-associated food borne disease outbreaks are linked to milk products, dairy cattle still represent a potential source of contamination of beef products when they are sent to slaughter at the end of their useful production life (termed “cull” or “spent” dairy cows); this “dairy beef” is often ground and sold as hamburger (Hussein *et al*., 2005).

The high prevalence of *E. coli* O157 and non-O157 STEC in some cattle populations combined with the lack of effective on-farm control strategies to reduce carriage, represents a significant risk of contamination of the food supply and the environment. Non-O157 STEC is also harbored in other ruminants, including swine (Frantamia *et al*., 2007).

Numerous Shiga toxin-producing *E. coli* serotypes known to cause human illness are of bovine origin, thus putting the beef supply at-risk. Both *E. coli* O157:H7 and non-O157 STEC may colonize the gastrointestinal tract of cattle, and potentially contaminate beef carcasses during processing. Although not as well studied, the risk factors for contamination of beef products from cattle colonized with non-O157 STEC are probably the same or very similar to *E. coli* O157:H7. For example, cattle hides contaminated with *E. coli* O157:H7 during slaughter and processing are a known risk factor for subsequent *E. coli* O157:H7 contamination of beef products. One study showed that the prevalence of non-O157 STEC (56.6%) on hides is nearly as high as that found for *E. coli* O157:H7 (60.6%)(Hussein *et al*., 2003).

A review of published reports from over three decades found that non-O157 STEC were more prevalent in beef products compared with *E. coli* O157 (Barkocy *et*

al.,2003) . In this study, the prevalence of non-O157 STEC ranged from 1.7 to 58% in packing plants, from 3 to 62.5% in supermarkets, and an average of 3% in fast food restaurants. In a recent survey of retail ground beef products in the United States, 23 (1.9%) of 1,216 samples were contaminated with non-O157 STEC. (Samadpour *et al*., 2009) In another study, researchers found a 10 to 30% prevalence of non-O157 STEC in imported and domestic boneless beef trim used for ground beef (Rasko *et al*., 2011).

E. coli O157:H7 bacteria and other pathogenic *E. coli* are believed to mostly live in the intestines of cattle, but these bacteria have also been found in the intestines of chickens, deer, sheep, and pigs (Elder *et al*., 2000). A 2003 study on the prevalence of *E. coli* O157:H7 in livestock at 29 county and three large state agricultural fairs in the United States found that *E. coli* O157:H7 could be isolated from 13.8% of beef cattle, 5.9% of dairy cattle, 3.6% of pigs, 5.2% of sheep, and 2.8% of goats (Keen *et al*., 2003). Over seven percent of pest fly pools also tested positive for *E. coli* O157:H7. Shiga toxin-producing *E. coli* does not make the animals that carry it ill, the animals are merely the reservoir for the bacteria (Elder *et al*., 2000).

There is a paucity of information on the vehicles of transmission for human non-O157 STEC infections, but contaminated raw dairy products, produce, and water have been implicated in the United States (CDC, 2008). A review of non-O157 STEC in Connecticut showed that exposures, including ground beef, were similar in both non-O157 STEC and *E. coli* O157:H7 cases, suggesting that the routes of transmission are similar (CDC 2007). Considering the relatively high prevalence of both *E. coli* O157:H7 and non-O157 STEC in cattle populations and their products, it

is not surprising that ground beef and other beef products could be a common food vehicle.

Non-O157 STEC outbreaks attributed to ground beef and its sausage products have been documented outside the United States including Argentina, Australia, Germany, and Italy. These beef-related outbreaks involved 8 STEC serogroups (O1, O2, O15, O25, O75, O86, O111, and O160). HUS cases were reported in five of the six outbreaks, mostly striking children and the elderly. *E. coli* O157:H7 and other Shiga toxin-producing E. coli (STEC) infections can lead to a severe, life-threatening complication called the hemolytic uremic syndrome (HUS) (Rangel *et al*., 2005).

HUS accounts for the majority of the acute deaths and chronic injuries caused by the bacteria (Jame *et al*., 2000). HUS occurs in 2-7% of victims, primarily children, with onset five to ten days after diarrhea begins. "*E. coli* serotype O157:H7 infection has been recognized as the most common cause of HUS in the United States, with 6% of patients developing HUS within 2 to 14 days of onset of diarrhea." (Garga *et al*., 2003, Safdar *et al*., 2002). And it is the most common cause of renal failure in children (Su *et al*., 1995).

Approximately half of the children who suffer HUS require dialysis, and at least 5% of those who survive have long term renal impairment. The same number suffers severe brain damage (Siegler *et al*., 2003). While somewhat rare, serious injury to the pancreas, resulting in death or the development of diabetes also occurs. There is no cure or effective treatment for HUS. And, tragically, children with HUS too often die, with a mortality rate of five to ten percent (Su *et al*., 1995).

Once Shiga toxins attach to receptors on the inside surface of blood vessel cells (endothelial cells), a chemical cascade begins that results in the formation of tiny thrombi (blood clots) within these vessels. Some organs seem more susceptible, perhaps due to the presence of increased numbers of receptors, and include the kidney, pancreas, and brain. Consequently, organ injury is primarily a function of receptor location and density (CDC, 2009).

Once they move into the interior of the cell (cytoplasm), Shiga toxins shut down protein machinery, causing cellular injury or death. This cellular injury activates blood platelets too, and the resulting "coagulation cascade" causes the formation of clots in the very small vessels of the kidney, leading to acute kidney failure (Siegler *et al*., 1995). The red blood cells are either directly destroyed by Shiga toxin (hemolytic destruction), or are damaged as cells attempt to pass through partially obstructed micro-vessels (Brooker *et al*., 2005). Blood platelets become trapped in the tiny blood clots, or they are damaged and destroyed by the spleen (Siegler *et al*., 2003).

By definition, when fully expressed, HUS presents with the triad of hemolytic anemia (destruction of red blood cells), thrombocytopenia (low platelet count), and renal failure (loss of kidney function)(Garg *et al*., 2003). Although recognized in the medical community since at least the mid-1950s, HUS first captured the public's widespread attention in 1993 following a large *E. coli* outbreak in Washington State that was linked to the consumption of contaminated hamburgers served at a fast-food chain (Bell *et al*., 1994) Over 500 cases of *E. coli* were reported; 151 were

hospitalized (31%), 45 persons (mostly children) developed HUS (9%), and three died (Bell *et al*., 1994).

Of those who survive HUS, at least five percent will suffer end stage renal disease (ESRD) with the resultant need for dialysis or transplantation (Robitaille *et al*., 1997). But, "because renal failure can progress slowly over decades, the eventual incidence of ESRD cannot yet be determined" (Siegler *et al*., 1995). Other long-term problems include the risk for hypertension, proteinuria (abnormal amounts of protein in the urine that can portend a decline in renal function), and reduced kidney filtration rate (Siegler *et al*.,1995). Since the longest available follow-up studies of HUS victims are 25 years, an accurate lifetime prognosis is not really available and remains controversial (Robitaille *et al*., 2006).

IBS is a chronic disorder characterized by alternating bouts of constipation and diarrhea, both of which are generally accompanied by abdominal cramping and pain. (Hungin *et al*., 2005) Suffering an *E. coli* O157:H7 infection has been linked to the development of post-infectious irritable bowel syndrome (IBS).

In most infected individuals, symptoms of a Shiga toxin-producing *E. coli* infection last about a week and resolve without any long-term problems (Eisntein *et al*., 2000). Antibiotics do not improve the illness, and some medical researchers believe that these medications can increase the risk of developing HUS (Wong *et al*., 2012.)Therefore, apart from supportive care, such as close attention to hydration and nutrition, there is no specific therapy to halt *E. coli* symptoms (Tarr *et al*., 1995). The recent finding that *E. coli* O157:H7 initially speeds up blood coagulation may lead to

future medical therapies that could forestall the most serious consequences. Most individuals who do not develop HUS recover within two weeks (Tarr *et al*., 1995).

2.5.5 Vibrio Parahaemolyticus

Vibrio parahaemolyticus, of the Vibriomaceae family, is a gram- negative, halophilic, non-spore forming, curved rod-shaped bacterium that is 0.5 - 0.8 μm in width and 1.4 - 2.4 μm in length(Nair *et al*., 2007). It is an oxidase-positive facultative anaerobe that can ferment glucose without gas production (Yeung *et al*., 2004). It has a polar flagellum which enables its high motility in liquid media, and its lateral flagella allow it to migrate across semi-solid surfaces by swarming (Drake *et al*., 2007) Virulent strains isolated from patients have been shown to produce thermos table direct hemolysin (TDH), and/or TDH-related hemolysin, which is a characteristic that is not observed in other non-pathogenic/non-virulent strains found in the environment. TDH-producing isolates are known as Kanagawa positive and can be identified using β-hemolysis on a Wagatsuma blood agar (Butt *et al.,* 2004) *Vibrio parahaemolyticus* is the most common non cholera *Vibrio* species reported to cause infection(Baker *et al.,* 2008).

Infections usually present in one of three major clinical syndromes: 60-80% of infections cause gastroenteritis, 34% wound infections, and 5% septicaemia (Butt *et al*., 2004). The most common presentation is gastroenteritis, with symptoms including diarrhea (sometimes bloody and watery) with abdominal cramps, nausea, vomiting, headache, chills, and low-grade fever. Infection is usually self-limiting and of moderate severity, lasting approximately 3 days in immune competent patients, and can be treated with oral rehydration alone (Su *et al*., 2007).

Worldwide - widely distributed in inshore marine waters, and has been found in seawater, sediments, and is a part of the natural flora of bivalve shellfish (Yeung *et al*., 2004). The bacteria are most prevalent during warm summer seasons. Food-borne outbreaks have been caused by *V. parahaemolyticus* (serotype O3:K6 strain has increasing prominence) in Chile, France, Japan, Korea, Laos, Mozambique, Peru, Russia, Spain, Taiwan, United States, and especially in far east countries such as India, Bangladesh, and Thailand, where raw seafood consumption is high (Nair *et al.*, 2000).

The first recorded outbreak was in Japan in 1950, where there were 272 cases and 20 deaths after the consumption of semi-dried juvenile sardines. Outbreaks have also occurred in the United States, such as an outbreak in 1971 due to consumption of contaminated crab-meat, where the bacteria are estimated to be responsible for 5000 illnesses annually. Humans, finfish, seafood such as codfish, sardines, mackerel, flounder, clams, octopus, shrimp, crab, lobster, crawfish, scallops, and oysters(Drake *et al*.,2007). Infection can occur upon ingestion of 10^{10}organisms (Lee *et al*., 2004).

Primary mode of transmission is through the ingestion of raw, undercooked, or contaminated shellfish (such as oysters, clams, and mussels). Cooked crustaceans (such as crab, lobster, and shrimp) can still harbour the bacteria if it has not been properly cooked/heated, or if recontamination occurred by coming in contact with uncooked seafood (Yeung et al., 2004) Exposure of open wounds to contaminated seawater, shellfish, or finfish can cause infections and septicemia (Butt *et al.*, 2004). Usually at 15 hours after infection, with a range of 4 - 96 hours, Infection cannot be transmitted from person-to-person (Su *et al*., 2007).

V. parahaemolyticus can survive in shellfish during warm seasons, and are naturally part of the flora of bivalve shell fish (Yeung *et al.,* 2007). Susceptibility has been shown for a range of antibiotics such as doxycycline, or ciprofloxacin, tetracycline, ceftriaxone, chloramphenicol, imipenem, ofloxacin, nitrofurantoin, meropenem, oxytetracycline, fluoroquinolones, third generation cephalosporins, and aminoglycoside (Baker et al. 2008).

Extremely sensitive to heat as cells are no longer detectable at 48 - 50 C after 5 minutes; therefore proper cooking of shellfish products can effectively inactivate the bacteria (Laboratory Safety Manual, 1993). Reduction of the bacteria in seafood can be achieved by cold storage at 3 °C for 7 days, freezing, and low temperature pasteurization; viable cells can be completely inactivated at -18 °C or -24 °C for 15-28 weeks (Su *et al.,* 2007). High hydrostatic pressure can also destroy bacterial cells in food without affecting the nature of the food, and irradiation using Cobalt-60 gamma at 0.75 kGy has been shown to reduce bacteria to undetectable levels.

Salinity is crucial for its survival and growth, with multiplication observed at 0.5 - 10%, the optimal level being around 1 - 3 % (Yeung *et al*., 20040. The bacteria can survive through the winter season in marine sediments, and will resume multiplication when temperature rises to at least 15 °C, and the bacteria is also highly resistant to metal ions (up to 300 mM) (Yeung *et al*., 2004) *V. parahaemolyticus* can enter a viable but non-culturable state in the presence of extreme conditions such as after 12 days of food-starvation or temperature stress around 4 °C. Administer appropriate antibiotic therapy. Oral rehydration in infected patients with mild gastroenteritis symptoms, and intravenous fluid and electrolyte replacement should

be administered in severe cases (Yeung *et al.*, 2004). Antibiotics should be used for patients with wound infections or septicaemia (Butt *et al*., 2004).

2.6 Prevalence of Enteric Pathogenic Bacteria Associated with Childhood Diarrhoea in some Africa Countries

Due to the lacking of diarrhoea surveillance programs in Zanzibar and Tanzania in general as other most the Africa countries, information regard to the prevalence of the enteric pathogenic bacteria in stool sample is limited. Many studies done in Zanzibar focused on epidemiology of diarrhea particularly cholera outbreak without focused on magnitude of causative pathogens of particular kind of diarrhea. However the unpublished study done in Zanzibar (hamad *at al*., 2010) reported highly prevalence of shigellosis to about 70% among the diarrheal patients.

The different studies in developing countries reported the high prevalence of Diarrheic *E.coli* as common enteric pathogen associated with less than five years children diarrhoea. According to some studies in Tanzania, diarrheic *E.coli* was the most common enteric pathogenic bacteria isolated from stools less than five years age (Moyo *et al*., 2011, Martha *et al*., 2014). In Kenya the diarrhoeic Enteroaggregative *E.coli* reported to be dominant, among enteric pathogenic bacteria (Clara et *al*., 2007, Sang *et al*., 2012, Shirley *et al*,. 2013 and Zhi *et al*., 1999, Rebbeca *et al*., 2012). In Nigeria as well, the study by (Ifeany *et al*., 1999) and in Dakar (Sushmita *et al*., 2013).

Highest prevalence of shigellosis also reported in various studies elsewhere. According to study of Gascon *et al*., 2000, Mnah *et al*., 1984, Jafar *et al*., 2004, Urio

et al., 2001. In Tanzania reported that *shigella* species were the only enteropathogens related with less than five years children. Highest incidences of shigellosis, reported by Njuguni *et al*., 2013. Study from other developing countries reported similar trend (Lorenz *et al*., 2006) in Bangladesh, Tesfaye *et al*., in Ethiopia, Remon *et al*., 2004 in Egypt and in Nepal Ansari *et al*., 2012.

Prevalence of Salmonella and its association to less than five years children diarrhoea was reported (Sharmilla *et al*., 2011) in India, (Buchana *et al*., 1998) in Colombia (Elamreen *et al*., 2008). Study in Libya reported highest prevalence of Salmonella as the major causative agents of childhood diarrhoea (Amal *et al*.,2011).The study in Mozambique(Inacio *et al*,.2007) reported *Salmonella* and *Shigella* and *E.coli* species as the common entero pathogenic bacteria associated with diarrhoea. Salmonella are frequently cause and invasive bacteremia and tend to be fatal in less than 1% of those infected. However the new strain of invasive non-typhoid salmonella is fatal in up to 45% of the cases in Africa and every 4 people infected with strain has been died (Christine et al 2012).

Zeddy *et al*., 2012 reported *Salmonella typhimurium* as was the predominant to about 75% cases among bacteremia in Kenya and *Salmonella enteritis* made up only 4.8% cases (Madigam *et al*., 2009).Study by Brooks *et al*., 2003 in Western Kenya *Diarrhoeagenic E.coli* indentified as the common enteric pathogen associated with diarrhoea to about 34% in under five years old. Also Vargas *et al*., 2004, Ifakara Tanzania reported increased proportion of *Dirrhoeagenic E.coli* by 34.6%. International collaboration study of travelers diarrhoea in Kenya, India and Jamaica

(Zhi et al., 2001) reported *Salmonella, Shigella* and *E.coli* as the predominant pathogens associated with childhood diarrhoea.

2.7 Antibiotic Resistance

Microorganisms are termed drugs resistance when they are no longer inhibited by antimicrobial which were previously sensitive. Drug resistance is genetic where microbes develops or acquire a gene that code for method of inactivating or escaping the antimicrobial. Resistance is selected for the environment where antimicrobial are present in high concentration such as in hospitals. Microbial resistance develops through acquisition of resistance that involves drugs inactivation, decreasing drugs uptake, decrease receptors sites and metabolic pathway attacked by the drugs. Moreover widespread indiscriminate prescribing of antibiotics favors resistance to all common drugs (David et al., 2007).

2.8 The Mechanism of Antibiotic Resistance

An antibiotic is a drug that kills or stops the growth of bacteria. Antibacterial are one class of "antimicrobials", a larger group, which also includes anti-viral, antifungal, and anti-parasitic drugs. They are relatively harmless to the host, and therefore can be used to treat infection. The term originally described only those compounds derived from living organisms, but is now applied also to synthetic antimicrobials, such as the sulfonamides (Bessard *et al.,* 1993).

In treatment of infectious diseases, the antibiotics are drugs that target microbes without harming the host. Antibiotics are not effective in viral, fungal and parasitic infections, and individual antibiotics vary widely in their effectiveness towards

various types of bacteria. Some specific antibiotics target either gram-negative or gram-positive bacteria; others are more wide-spectrum antibiotics. The effectiveness of individual antibiotics varies with the local of the infection and the ability of the antibiotic to reach this site. There are many ways to classify antibiotics. Chemical structure can be used to classify them. Another classification is by their mechanism of action. Antibiotics can also be classified by the organisms against which they are effective; and by the type of infection in which they are useful, which depends on the sensitivities of the organisms (Bessard *et al.*, 1983). Increased use of antibiotics has caused the development of bacterial resistance to antibiotics. Antibiotic resistance can be defined as the ability of a microorganism to destroy or be unaffected by an antibiotic, or prevent it from entering the microorganism. Antibiotic resistance is a consequence of evolution via natural selection (Brown *et al.*, 1996).

The antibiotic action is an environmental pressure; those bacteria which have a mutation allowing them to survive will live on to reproduce. They will then pass this trait to their offspring, which will be a fully resistant population. Antibiotic resistance develops through mutation or plasmid exchange between bacteria of the same species. If a bacterium carries several resistance genes, it is called multi resistance (http: www.worldhistory. com).

Other factors contributing to resistance include incorrect diagnosis, unnecessary prescriptions, improper use of antibiotics by patients, and the use of antibiotics as livestock food additives for growth promotion. Resistance to antibiotics is currently a major concern in treating infectious diseases. Antibiotics use and antibiotic resistance are clearly connected. Bacteria have developed mechanisms of resistance

to all classes of antibiotics available for systemic use in human beings. Resistance can be either inherent or acquired. Inherent resistance is a result of the normal genetic, structural, or physiologic state of a microorganism. This resistance is predictable and therefore recognized once the identity of the microorganism is known. Acquired resistance is when the organism has been able to either develop resistance by spontaneous mutation or has acquired a resistance mechanism from an external source. Acquired resistance can occur by acquiring resistance genes from other organisms (Rosman *et al*., 1996).

Acquired bacterial resistance is common in isolates from healthy persons and from patients with community-acquired infections in developing countries, where the need for antibiotics is driven by the high incidence of infectious diseases 6. Among isolates of commensally enteric pathogens, diarrhoea, and respiratory resistance is increasing, particularly to the first-line, inexpensive, broad-spectrum antibiotics. Furthermore, the introduction of newer drugs has been followed relatively quickly by the emergence and dissemination of resistant strain (Rosman et al., 1996).

In general, antibiotic resistance results in gene action, bacteria acquire genes conferring resistance in any of three ways: (1) in spontaneous DNA mutation; (2) in a form of microbial sex called transformation, one bacterium may take up DNA from other bacterium; and (3) DNA acquired from a small circle of DNA called a plasmid, that can transfer from one type of bacterium to another. A single plasmid can encode different resistances to antibiotics (Alangaden *et al.*, 1997).

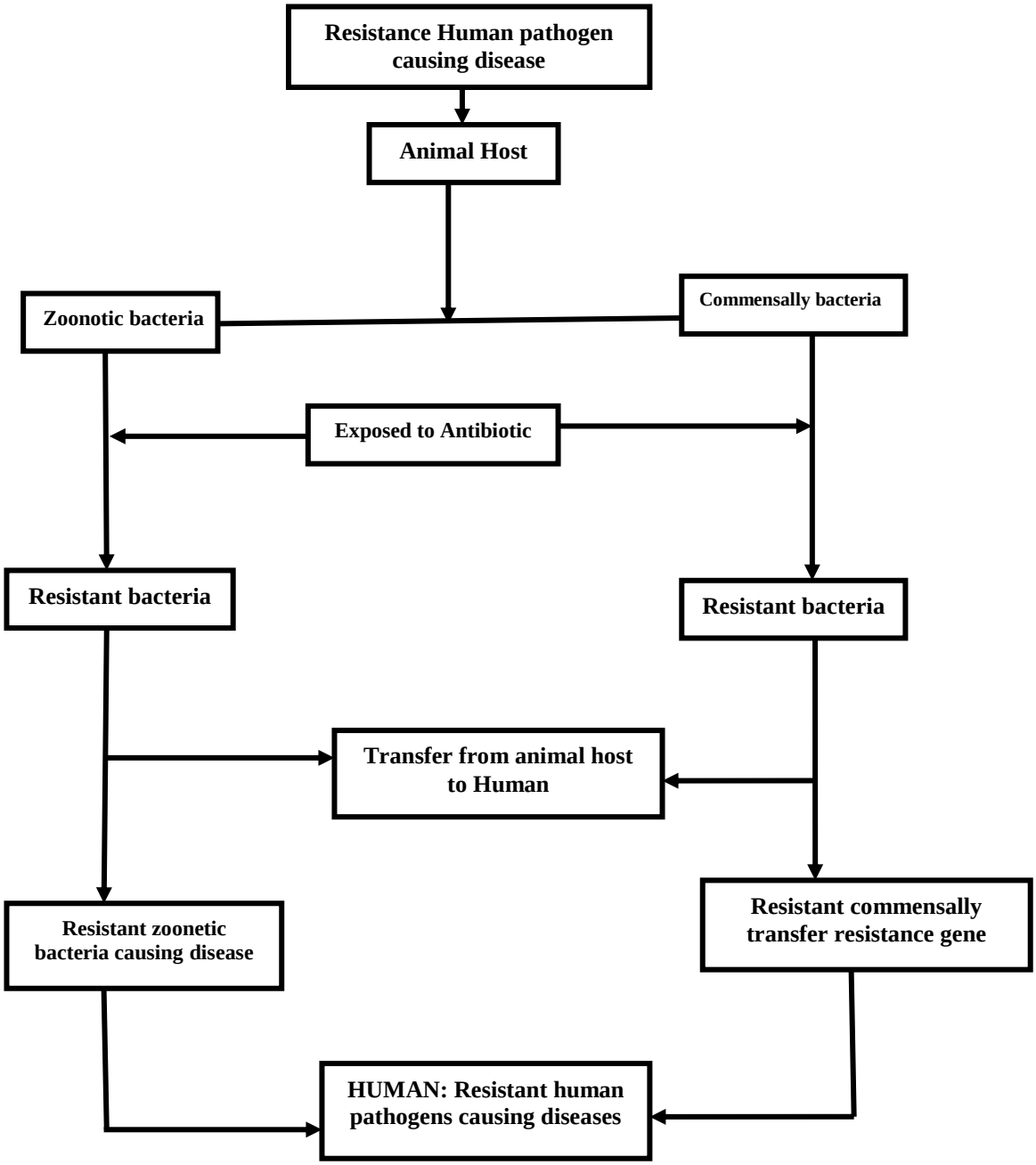

Figure 2.1: Spread of Antibiotics Resistance Pathogenic Bacteria from Animals to Human

Source: (Barza *et al.,* 2002)

2.9 The Potential Risk Factors of Antibiotic Resistance

Antibiotic resistance has become a serious problem in both the developed and developing countries. One side effect of misusing antibiotics is the development of antibiotic resistance by the infecting organisms, similar to the development of pesticide resistance in insects.

2.9.1 Misuse of Antibiotics by Physicians in Clinical Practice

As inappropriate use increases the risk for selection and dissemination of antibiotic-resistant bacteria, one would expect that drugs more commonly affected by bacterial resistance in the developing countries are generally inexpensive and popular broad-spectrum agents (Murray *et al.*, 2001). Unnecessary prescription of antibiotics seen in industrialized nations has also been documented in many developing countries, particularly in cases of acute infantile diarrhoea and viral respiratory infections (Murray *et al*., 1985). Clinical misuse of antibiotics may be more common among private practitioners who charge higher fees; the demand for antibiotics seen in private patients is higher and more drugs are available in private clinics than in public hospitals (Guymon *et al*., 1994).

Health workers in many developing countries have almost no access to objective health information (Basu *et al*., 2008) Pharmaceutical company representatives typically outnumber practitioners and often adversely influence their prescription habits, as reflected by sales. Drugs labels and package inserts often fail to provide accurate information (Kumar *et al.*, 1996) Antibiotic use in clinical practice alone cannot explain the high frequency of resistant organisms in developing countries. However, excessive clinical use is at least partially responsible for the escalating rates of resistance, especially in hospital settings (Reynold *et al*., 2009).

2.9.2 Misuse of Antibiotics by Unskilled Practitioners

Common forms of antibiotic misuse include taking an inappropriate antibiotic, in particular the use of antibiotic agents for viral infections like the common cold, and

failure to take the entire prescribed course of the antibiotic, usually because (Hossain *et al.*, 1982) the patient feels better before the infecting organism is completely eradicated. In addition to treatment failure, these practices can result in antibiotic resistance. In many developing countries, well-trained health personnel are scarce and cannot serve the entire population, especially in rural areas. Community health workers and others with minimal training treat minor ailments. The qualifications and training of community health workers, as well as the quality of care they provide, vary from country to country. Unskilled personnel are less aware of the deleterious effects of inappropriate antibiotic use (Thamlikitkul *et al.*, 1988).

2.9.3 Misuse of Antibiotics by the Public

In most of the developing countries, antibiotics can be purchased without prescription, even when the practice is illegal. In many African, Asian, and Latin American countries, antibiotics are readily available on demand from hospital, pharmacies, patent medicine stalls and hawkers. In rural Bangladesh, for Example, 92% of drugs consumed for 1 month by more than 2,000 study participants came from local pharmacies; but only 8% of them had been prescribed by physicians (Hossain *et al.,* 1982). People are encouraged to buy from unofficial distributors because drugs often are not available in government hospitals. Drug vendors usually have little or no knowledge of the required dosage regimen, indications or contraindications (Goel *et al.,* 1996). Antibiotic use in developing countries is underestimated. The quantity of drugs distributed within a country is calculated under the assumption that each person purchases a complete regimen. However, medication can be purchased in small quotas from roadside stalls, and distribution of

locally produced or counterfeit antibiotics is not recorded. The motives for self-medication and antibiotics overuse by laypersons are similar to those for clinical abuse by health professionals; to cut costs and act expeditiously to treat confirmed or suspected bacterial infection (Simpson *et al*., 2007).

For example 50% to 80% of Bangladeshi patients infected with shigellosis admitted that they had taken at least one antibiotic in the 15 days before going to hospital (Shahid *et al*., 1985). Common cultural beliefs about antibiotics include the notions that there is a pill for every symptom; antibiotics are presumed to heal many diseases, including dyspepsia and headaches. Injections are seemed as more powerful than pills. The misuse of antibiotics frequently becomes integrated into the local culture to prevent diarrhea after eating suspected contaminated foods or to prevent sexually transmitted diseases (Hakk *et al.,* 1988). Another cause of antibiotic abuse and selection for resistant bacteria is poor patient compliance. Firstly, physician-patient interactions are often inadequate. In Mexico, poor patient-physician communication was partially responsible for the non-compliance of patients with antibiotic regimens (Reyes *et al.,* 1997).

Secondly, because patients often travel long distances and incur large expenses for medical care, they are unlikely to return for follow-up visits. In addition, the patient may be unable to read medicine labels. Finally, because many drugs are expensive, indigent patients purchase incomplete regimens whenever possible and discontinue treatment when symptoms disappear but before the pathogen is eliminated (Lansang *et al*., 1990.

2.9.4 Poor Quality of Antibiotics

Beside the risk for therapeutic failure, degradation products or adulterants in poor quality antibiotics can produce sub-inhibitory concentrations in vivo, which increase the selection of resistant strains. Drugs that do not comply with minimum standards are illegal in all countries. In many cases, therapeutic failure is the only indication of substandard drugs as laboratories to detect substandard drugs are uncommon, and when they exist, health workers, distributors and consumers are often unaware of them (Taylor *et al*., 1995).

2.9.5 Dissemination of Resistant Organisms

Residents of developing countries often carry antibiotic-resistant faecal commensally organisms (Calva *et al*., 1996). Visitors to developing countries passively acquire antibiotic-resistant *Escherichia coli (E.coli)*, even if they are not taking prophylactic antibiotics; this suggests that they encountered a reservoir of antibiotics-resistant strains during travel. Several factors, such as urban migration with overcrowding and improper sewage disposal, encourage the exchange of antibiotic-resistant organisms between people and the exchange of resistance genes among bacteria, thereby increasing the prevalence of resistant strains (Lamikanra *et al*., 1998).

Most residents of developing countries have no sanitary facilities for sewage disposal. Also pipe-borne water, often scarce in developing countries, is not always potable. The development of sanitation and other facilities is not always proportionate to the rapid rise in urban population. As urban migration continues, overcrowding increases and bad hygienic conditions are increasing the probability of

the spread of antibiotic-resistant and commensally pathogens. Potable water disposal should reduce infections and the need for antibiotics and the subsequent development of antibiotic resistance (Levin *et al.,* 1997). Because tropical conditions encourage the survival of bacteria, more pathogens and commensally organisms are found in tropical environments than in temperate climates. The warm and humid tropical climate and the low levels of health care, hygiene and sanitation contribute to a relatively high prevalence of infectious disease in developing countries. Infection control practices in many hospitals in developing countries are rudimentary and often compromised by economic shortfalls and opposing traditional values. Due to improper disposal of hospital waste, the resistant organisms may be disseminated to the outside community. Untreated hospital waste in Uganda was often dumped into public sewers or thrown into rubbish heaps ravaged by scavengers (Okello *et al*., 1997).

2.9.6 Inadequate Surveillances Antibiotics Resistance

Information from routine sensitivity testing of bacterial isolates and surveillance of antibiotics resistance, which provide information on resistance trends, including emerging antibiotic resistance, is essential for clinical practice and for rational policies against antibiotic resistance. The antibiotic susceptibility pattern of bacterial isolates in many developing countries is unknown, and Testing cannot be done readily because equipment, personnel and consumables are scarce and expensive (Brown *et al*., 1996). In most of the clinical infections, no clinical specimens are cultured. So if possible, a community-based antibiotic resistant surveillance data might be useful for medical doctors when they prescribed a medicine in the areas

where they could not perform specific antibiotic (Hakk *et al*., 1988) susceptibility tests. For example, it was demonstrated that resistance among respiratory pathogens was infrequent in parts of Ethiopia. This information would help local Ethiopian health workers to treat such infections with inexpensive, broad-spectrum antibiotics (Reyes *et al*., 1997).

2.10 Diarrhea Occurence in Under Five Children in Zanzibar

All types of diarrhea as mentioned in section above are present in the population of Zanzibar. Zanzibar as other developing countries where diarrhoea is still a public health concern and is the first leading cause of mortality and morbidity to children less than five years old children each year. Several cases of diarrhoea have been reported; dozens of children have been hospitalized. In Zanzibar water supply, sanitation, hygiene and poor urbanization yet look a challenge and therefore diarrhoea still a big public health problem.

Zanzibar has been regularly affected by cholera outbreak since 1978, severe outbreak occurred both Unguja and Pemba Islands in 1997/1998 and in 2004 with number of reported ranging from 520 to 650 cases per outbreak. Most of these outbreak index cases were observed among mobile fisherman travelling between Island and Tanzania Main land (WHO 2006). According to WHO in 1999 alone, about 650 cases of cholera were reported in the country, which resulted in about 100 deaths? The figure 1.3 indicates the distribution of the cases of cholera with different years from 1999 to 2006.

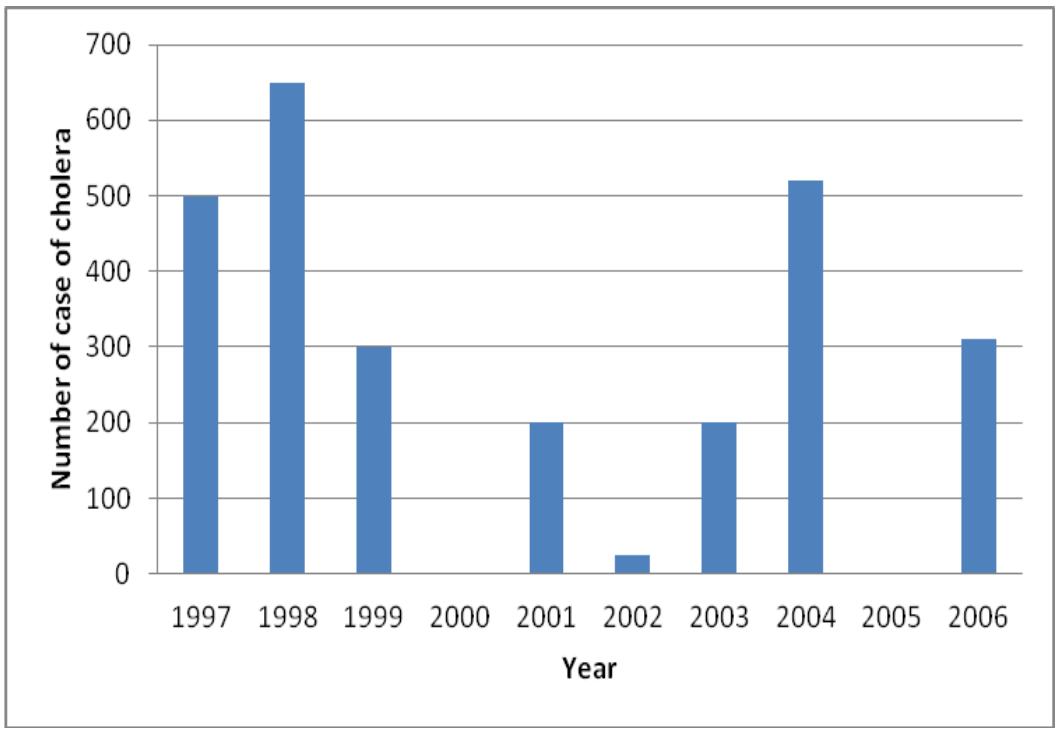

Figure 2.2: Number of Cases of Cholera in Zanzibar
Source: World Health Organization (2006)

The hospital data from Mnazi Mmoja referral hospital showed the magnitude of diarrhoea morbidity or mortality among the under-five children in the country; in the year 2013 at pediatric ward at Mmazi Mmoja Hospital only to report 423 cases of the under-five children were taken to hospital in response to a recent attack of diarrhoea. Urban show high number of cases than rural areas (Zanzibar Health Information system unit 2013).

The strategies and effort to control diarrhoea have been taken both towards preventing new cases and deaths from dehydration in children. The Oral Rehydration Salts (ORS) is the recommended treatment for diarrhea and currently available countrywide. Moreover the vaccine against measles and cholera has been promoted to children under five years old in Zanzibar (Zanzibar Health information

system 2013). According to Ministry of Health about 70% of fewer than five years children have been vaccinated against cholera and measles. Table (2.1) illustrates the number of diarrheic cases to under five years age reported in three Districts of Unguja Island, while the urban is the leading district with high incidence of reported diarrhoea case in 2013.

Table 2.1: The Number of Diarrhea Cases Reported in Selected Districts for Survey

Districts	Reported Diarrhea Cases in Three Selected Districts between 2012-2014
Urban	**56925**
South	**6305**
North A	**11754**

Source: (Zanzibar Health Information System Unity 2013)

2.11 Overview of the Situation of Blood Diarrhea Cases in Zanzibar by District

Blood diarrhea cases have been reported in less than five years children in all districts of Zanzibar with 27 weeks where several were admitted in district hospitals. The incidence of blood diarrhea was reported in Micheweni district in Pemba Island to 11 cases followed by urban and central in Unguja Island to 8 and 3 cases respectively. Figure (2) shows the distribution of diarrhea cases reported in different health centers located in urban and rural area of Zanzibar. Pemba's' villages revealed high incidence of diarrhea cases followed by urban in Unguja (Zanzibar Health Information System Unity 2013).

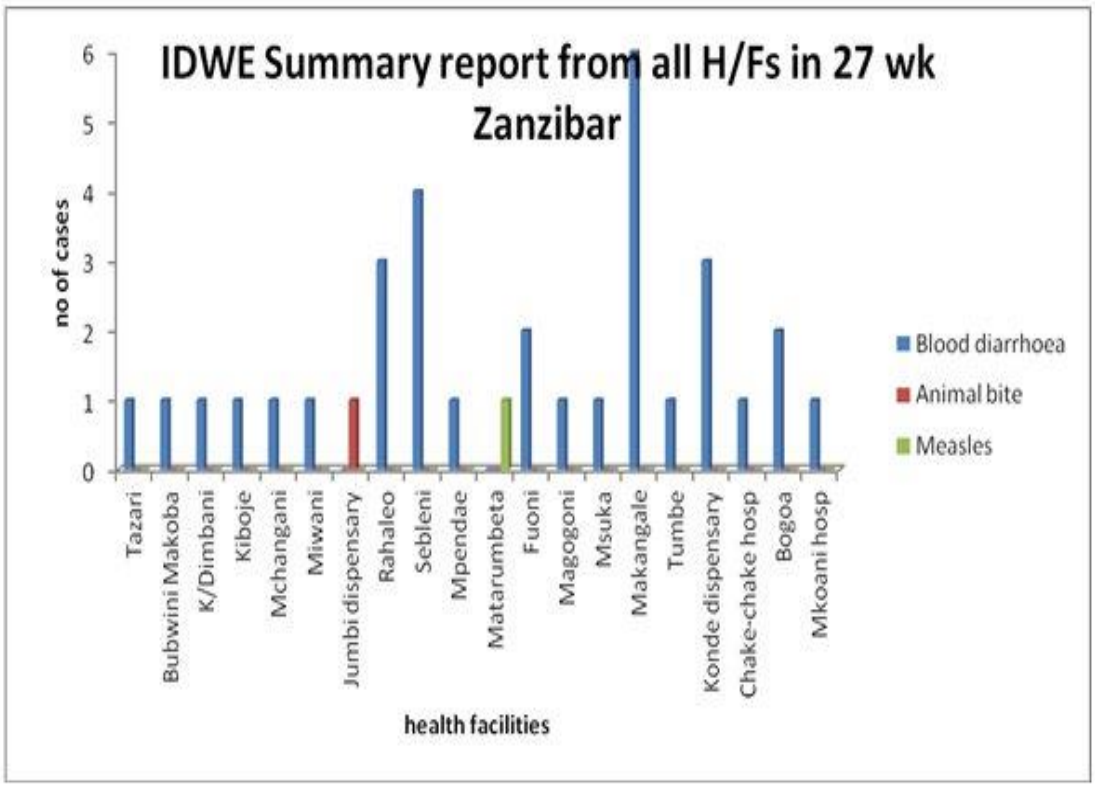

Figure 2.3: Number of Cases of Blood Diarrhea Reported In Zanzibar Villages
Source: Zanzibar national statistics department 2014)

2.12 Effect of Breast Feeding on the Reduction of Diarrhoea Mortality

The benefits of breastfeeding on infant and child morbidity and mortality are well documented, with observational studies dating back to the 1960s and 1970s (Victoria et *al*., 1987). Studies show that human milk glycans, which include oligosaccharides in their free and conjugated forms, are part of a natural immunological mechanism that accounts for the way in which human milk protects breastfed infants against diarrheal disease (Morrow *et al*., 2005). In addition, breastfeeding reduces exposure to contaminated fluids and foods, and contributes to ensuring adequate nutrition and thus non-specific immunity. Despite evidence supporting the positive and cost-effective health impacts of exclusive breastfeeding on child survival (Riordan et al., 1997) the practice in resource-poor areas of the world is low.

In Tanzania the women nutritional status is about 22%, Zanzibar is 33.6%. Provides a newborn with colostrums, a key supplement for the infant's immune system. , 49% of newborns are breastfed within the first hour of life, and 94% within the first day. 31% of newborns given food or liquid other than breast milk (pre lacteal feed), although this is not recommended, 97% of infants are ever breastfed Children who receive only breast milk and no other foods or liquids, even water, are considered exclusively breastfed. The early initiation of breastfeeding is important for a number of reasons. Early suckling benefits mothers because it stimulates breast milk production and releases a hormone that helps the uterus to contract and reduce postpartum blood loss. It also fosters bonding between mother and child (Tanzania Demographic Health Survey 2010).

However, WHO recommends that around the age of 6 months, children be given solid foods because by that age breast milk by itself is no longer sufficient to maintain a child's optimal growth. The nutritional status of young children is a comprehensive index that reflects the level and pace of household, community, and national development. Malnutrition is a direct result of insufficient food intake or repeated infectious diseases or a combination of both. It can result in increased risk to illness and death and can also result in a lower level of cognitive development. Children are breastfed for a median of 20.9 months.

However, children are exclusively breastfed for less than 2.4 months, 50% of children under 6 months are exclusively breastfed, and 37% are already receiving complementary foods, 42% of children are stunted (short for their age), 11% of

women are thin, while 22% are overweight or obese 59% of children age 6-59 months and 40% of women age 15-49 are classified as having anemia. 59% of households with tested salt had an adequate amount of iodine in the salt.

Given that diarrheal disease accounts for approximately 1.34 million deaths among children ages 0-59 months and continues to act as the second leading cause of death in this age group (Black *et al*., 2008), it is important to quantify the preventive effect of breastfeeding practices on diarrhea-specific morbidity and mortality. Very few individual studies have been designed to detect the effects of breastfeeding practices on diarrhea-specific morbidity and mortality for children 0-23 months of age in resource-limited setting area.

In 2001, a systematic review of sixteen independent studies conducted by the WHO attempted to resolve the "weanling's dilemma" in developing countries. The review, which assessed the effects of exclusive breastfeeding for 6 months versus 3-4 months with mixed breastfeeding thereafter, resulted in the recommendation to promote exclusive breastfeeding for the first 6 months of life (Riordan *et al*., 1997). More recently, the authors of the Lancet nutrition series published a random effects meta-analysis estimating the increased risk of diarrhea-specific morbidity and mortality among children younger than 2 years in relation to suboptimal breastfeeding practices (Kraner *et al*., 2002). Building upon previous reviews, this systematic review and meta-analyses use carefully developed and standardized methods to focus on the effects of breastfeeding practices as they relate to diarrhea incidence, prevalence, mortality and hospitalization among children 0-23 months of age.

Here we present a comprehensive systematic review and meta-analysis as evidence to be utilized by the Lives Saved Tool (LiST) to model the effect of breastfeeding practices on diarrhea-specific morbidity and mortality (Walker *et al*., 2010) In Tanzania the percentage the percentage of feeding practices by women illusted in the Figure 2.4.

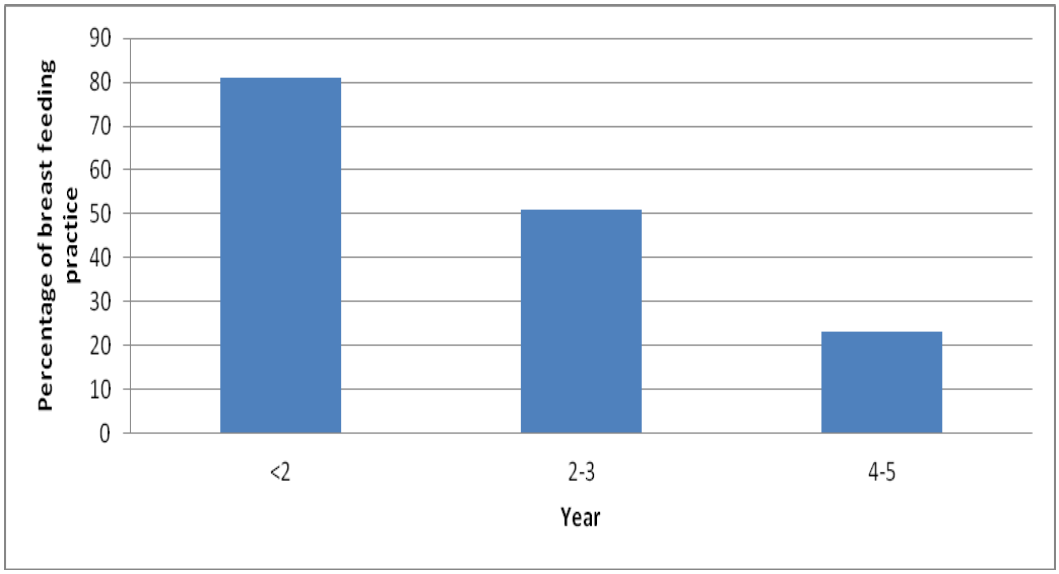

Figure 2.4: Percentage of Breast Feeding Practice by Women in Different Years

Source: National bureau of statistics and Tanzania demographic and health surveys (2005).

Table 2 2: Monthly Distribution of Diarrhoea Incidences Under Five in Relation to Breast Feeding and Weaning Practices During Study Period

Months	**Diarrhoea incidences**	
	Breast feeding children	**Weaning children**
0-6	34	2
7-12	68	9
13-24	46	48

Source: Zanzibar food and nutrition unity (2013-2014)

2.13 Community Awareness and Perception

Great effort and measures have been taken by the government and public stakeholders in control the infection and transmission of the diarrhoea both in Urban and remote areas. Among this measure is the provision of the vaccine against cholera to children and adults as well as provision of health education to both public and private mass media. Sometimes these measures have been faced with many constraints which include misconception and wrong perception from some people. For instance some people believe that vaccines provided by western countries, are harmful and aimed to lower their reproductive potential (Personal observation).

The campaign of having lavatory in each house has been established in each shehia of Zanzibar but yet many people continue to use shrubs as their toilet facilities, also there is random disposal of feaces in open space. Many people have tendency of not attending clinic during the early stage of infection, and many people use herbal and traditional medicine or even spiritual therapy for the reason that every disease is by God act. People attend clinic for chemotherapy when the condition of patients become worsen. (Annual report of epidemiology Ministry of Heath Zanzibar, 2010).

2.14 Risk factors for Diarrhoea

Demographical factors: Many studies have established that diarrhoea prevalence is higher in younger children (Karim *et al.,* 2001). The prevalence is higher for children 6 to 11 months of age and decrease in children in age from 3 to 5 year old, high rate of diarrhoea has been observed in boys than girls. Poor of immunity, malnutrition and lack of breast feeding to children are among of the factors increase incidences of diarrhoea (Molbak, 2000).

Social-economic factors: Some studies have shown that the association between socio-economic factors such as poor housing, crowded conditions and low income are among the factors that put people to be at risk of acquiring diarrhoea diseases (Elite, 2004).

Water related factors: As diarrhea is acquired via contaminated water and food, water related factors are very important determinant of diarrhoea occurrence. Increase distinctive from water sources, poor storage of drinking water, use unsafe water source (such as rivers, pools, dams lake, streams well and other surface water sources) have been found to be risk factors for diarrhea occurrence among children less than five years (Brooks *et al.,* 2003).

Sanitation factors: Sanitation obviously plays a key role in reducing the diarrhea mobility. Some sanitation factors such as indiscriminate or improper disposal of children's stool and household garbage, no existence of latrine or unhygienic toilet, sharing latrine and house without sewage system increase the risk factors for diarrhea in children (Brooks *et al*., 2003).

Hygienic practices: Some studies have revealed that children not washing hands before meals or after defecation, mothers not washing hands before feeding children or prepared foods, children eating with their hands rather than with spoon, unhygienic domestic places (Gorter *et al*., 2002), presence of animal inside the house, presence of flies inside the house were associated with risk factors (Curtis *et al*,. 2000).

Malnutrition: Diarrhoea is common in low income societies the children whose immune system has been weakened by malnutrition are more vulnerable to diarrhea. (Brown *et al* 2003).

Knowledge of diarrhoea: In Zanzibar the knowledge related to diarrhea is still a problem particularly in low income people who do not have radio, television and do not have tendency of reading newspaper. This is due to the fact that most of them are poor and depend on farming activities for their basic needs. Most of mothers lack knowledge about diarrhea (Personal communication).

Baby Weaning Practice

Breast milk is the best and safest food for young babies. Older babies need extra foods as well as breast milk. It is important that older babies are given extra foods as well as breast milk at the right age, and in sufficient amounts, to enable them to grow and stay healthy (America Public Health Association 2005). Too little food, given too late or inadequate food with too few nutrients may lead to poor growth and malnutrition. The malnourished child will get sick more often and will be less able to fight off illnesses such as diarrhoea. Weaning foods can, however, be very dangerous for babies if they are not hygienically prepared and they can be a major source of infection (UNICEF 2004).

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was done in Unguja Island part of Zanzibar with collaboration and support of Ministry of health (MOH). The study was conducted from 2013 to 2015. Zanzibar has been selected because currently there is no study ever conducted about prevalence of enteric bacteria and drugs sensitivity. Definitely this is the first study and provides a base line to other studies.

Figure 3.1: Map of Unguja Island

Source: (www.lonelyplanet.com/map/Africa /tanzania/Zanzibar). Zanzibar

Zanzibar is located about 35km off the cost of Dar es Salaam (commercial capital city of Tanzania), between 39° degree longitudinal and 6° degree latitude south of equator. Zanzibar consisting two sisters Islands named Zanzibar (Unguja) and Pemba. The island of Unguja is situated on the latitude 6° south and 39.5° east and separated from Mainland by shallow channel 37 kilometers across at it narrowest point of Zanzibar (Unguja) Island is 73 kilometer from Dar es Salaam on the Tanzania coast and 219 kilometer from Mombasa on the Kenya coast Unguja Island is about 86kilometer long and 39kilometers wide and has area of 3354 square kilometer. The capital of Zanzibar goes by the same name of Zanzibar town.

Pemba Island lies about 40 kilometers to the northeast of Unguja Island and situated on the latitude 5 south and longitude 40° east. Pemba has three major towns, Wete, Chake and Mkoani. It is about 68 kilometers long and 22.75 kilometer wide and has an area of 1537square kilometers. The combined area of the island is 4891 squire kilometers located about 35km off the cost of Dar s Salaam, between 39° longitudinal and 6° latitude south of equator (www.africamecca safari.com/ Zanzibar/guide location (www.lonelyplanet.com/map/Africa /tanzania/ Zanzibar), Zanzibar.

3.2 Health Sector

The health care system in Zanzibar is organized along a three-tiered pyramid. At the top of the pyramid is the Ministry of Health, which is the main national authority in the health sector and together with the district and shehia (community). At the central level, the Ministry of Health (MOH) is a national authority responsible for health care in the whole country. It also directly controls the activities of development

programs, central medical store and national hospitals and ministries unities and headed by Minister of Health. The country now is divided into 5 regions with 10 districts and 50 province, municipalities, and shehia which supported by central government. Each district has a hospital serving an average population of 100,000-150,000 directly coordinate by MOH.

In Figure 3.2 Health system in Zanzibar districts. In this level there is health center with public pharmacy and a district hospital serving a population about 100,000 to 150,000 persons in each district. The last level is shehia level with a community health center. Each community health center has 3 to 4 health staffs authorized by the Mnistry of Health (Zanzibar health information system unity 2013).

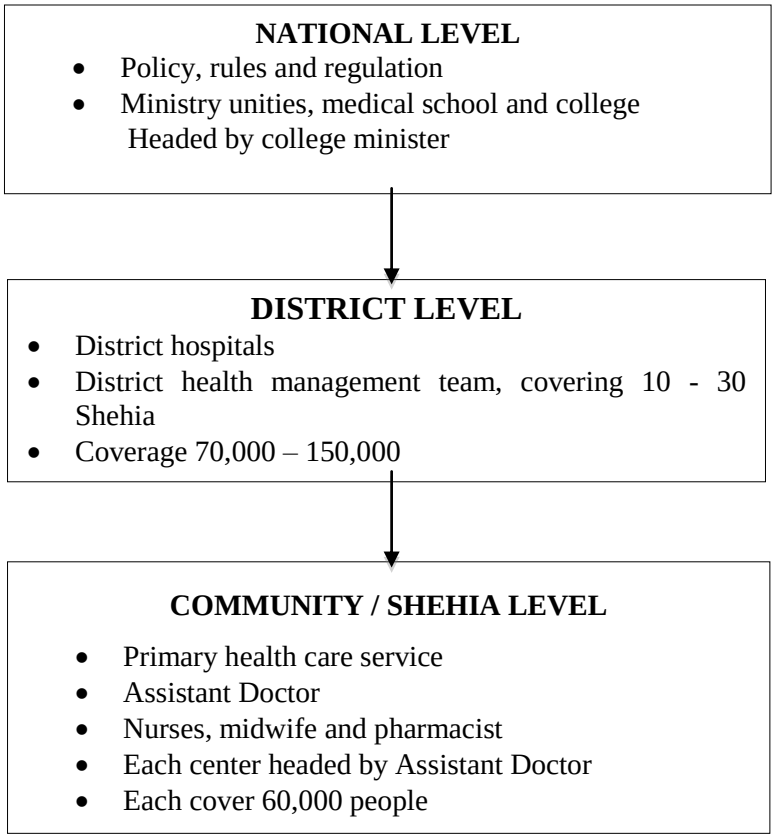

Figure 3.2: Health System in Zanzibar

Source: Ministry of Health Zanzibar

3.3 Study Population

Study population was included children less than five years of age who attend clinics only since were difficult to reach them those children with diarrhoea but don't attend clinic at their home in study period. Because children under five years are still young to express themselves, their parents were involved.

3.4 Study Design

A Cross section study was carried out in both quantitative and qualitative research designs and survey was carried out at in longitudinal (Kothari, 2004). Quantitative research design involved laboratory analysis of stool samples for determination of seasonal and spatial prevalence of enteric pathogens, as well as antibiotics susceptibility test of isolated pathogenic bacteria of selected population in Urban, North and South districts of Unguja Island. Qualitative research design was carried to establish the factors responsible for the increasing rate of diarrhoea in less than five years children and in identify factors for increasing resistance of bacteria to antibiotics in study area. Interview was employed to solicit information from selected cases.

Table 3.1: The Population of Children Less than Five Years in the 3 Districts to be investigated

District	Number of Children less than five years
Urban	143,044
South	13,293
North	21,784

Source: Zanzibar Health Information system 2013 and Zanzibar National Bureau of statistic (2013)

3.5 Sample Collection

Overall population of children less than five years age from Urban, North and South district were presented on (Table 3.1) and the children who were involved as cases in this study expected to be selected from 137,121 populations of all children less than five years in study area. The stool samples were collected from diarrhoea patients attend the heath centers in North district included areas shown in Figure 3.1, named Nungwi, Matemwe, Mkookotoni, Mangapwani and Tumbatu. South district included areas Bwejuu, Paje, Jambiani, Makunduchi and Kizimkazi while urban district was the whole areas of urban.

Sample size: The number of children stool samples to be collected, the prevalence of diarrhoea in children should be regarded which defined as a number of cases of diarrhoea reported at time to total population of children in selected districts. The formula for calculating sample size was available in (http://www.open epi.com/OEZ.3/Menu/open Epi .Menu.htp).

The prevalence was determined by regard the number of diarrhoea incidence in children under five years during a specified time period in study area, number includes both new cases and old cases. The size of the population of children under five years in which the diarrhoea occurred in study area.

The prevalence was then calculated by dividing the number of diarrhoea incidence during the specified time period by the size of the population of children less than five years of age, the result is expressed as a percentage. The total number of cases of

diarrhoea reported in Zanzibar between 2012 - 2014 were 74984 and total numbers of children were 107121. The prevalence was calculated as shown 74984/178121 = 0.7(70%). Therefore prevalence of diarrhoea was 0.7.

The number of stool samples to be collected obtained from the formula shown below

$$n = \frac{(Z_1 - \propto)^2 (P(1-P)}{D^2}$$

Where $Z_{1-\propto} = Z_{0.95} = 1.96$ (From normal distribution table. This value of 1.96 is standard for confidence level of 95%)

P Is the prevalence of diarrhoea = 0.7

D Is the degree of cumulative = 5% =0.05

$1-\propto = 95\%$ Is the confidence interval

From formula above n = $(1.96)^2 \times$ 0.7 (I-0.7)

$(0.05)^2$

n= 319

The number of sample collected in this study was 319. *(*www.tulane.edu/~ panda3/Survey/Chapter2/**epi_info_sample**_size.htmo*).*

3.6 Sampling of Health Facilities

The probability sampling technique was used. All Primary health Unity, Regional Hospitals were indentified in each district involved in study and the number of Healthcare centers were obtained from Ministry of Health of Zanzibar. Using simple random sampling, 16 Primary Healthcares Centers out of 37, 2 Regional Hospitals out of 2 as well as 1 Mother and Children Hospitals were selected from each district

giving a total of 19 Health care centers (Zanzibar Health Information system2013 and Zanzibar National Bureau of statistic 2013).

3.7 Sampling Technique

The children less than five year of age attending clinic in a particular district were selected. This selection was being conducted seasonal, during rainfall and dry season. Any diarrhoea case reported in selected health facilities and during study periods and meet the criteria of study were considered for sampling.

Inclusion Criteria of sample

(i) The watery, mucous, or bloody and fresh stool without dried were considered.

(ii) Diarrhoeic patients caused by enteric pathogen attend the selected hospital for seeking treatment or admitted from September 2013 and February 2014.

(iii) The stool samples have been collected from those patients who did not take antibiotics before attending the hospital

(iv) The patients who had diarrhoea 3 or more times a day

(v) All the patients that were less the five years old of age at the day of interview

Exclusion criteria of sample

(i) Any patient did not accept to participate in the interview and collection of stool sample from his/her child

(ii) Patient with diarrhoea caused by the food poisons and drug reaction admitted at the hospital or attend for medical seeking

(iii) Patients attend hospital and already have taken antibiotics.

3.8 Data Collection

3.8.1 Data Collection Tools

In this study the qualitative and quantitative data were collected. In qualitative study and structured interview was conducted using verbally administered questionnaire and observation schedule was to assess the general behavior of children and parents. Qualitative data were obtained from laboratory analysis of stool sample and testing of antibiotics sensitivity of isolated enteric bacteria. To avoid ambiguous answer, clear closed and opened questions in structured questionnaire was designed, and pre-tested to it was performed to test its relevant. It have the section of heath facilities, section of demographic, socioeconomic characteristics, section of knowledge of diarrhoea, by mother, a section on sanitation and rubbish disposal, a section of hygienic related practice, section of drinking water related practices, section of breast feeding and vaccination status of the children and laboratory examination result, regard to antibiotic resistance knowledge, awareness, prescription, and ways of drugs use, price, and follow medical instructions.

Pre-Testing of Questionnaire

The questionnaire was pre- tested on 20 mothers with children less than five years of age in one of selected hospital. This testing was to check the suitability and if the answer given were relevant to the questionnaire to avoid any unclear information that would arise from this or change was to be made.

3.8.2 Continuous Variables

All the continuous variables such as age were collapsed into groups and were therefore treated as categorical variables in statistical analyses; they were again not

transformed to assume the state of a normal distribution and therefore non-parametric methods were used in testing their association with diarrhoea occurrence.

3.8.2.1 Categorical Variables

All the variables that were pre-determined as categorical variables in the planning stage of the study and were pre-coded during the data collection phase remained as they were even during statistical analyses. The risk factors were organized into different categories and were put into computer. Finally the SPSS program was used to analyze the data. The general description of the data was done using frequencies and mean. The relationship between the independent variables and the dependent variable was done using the Chi-square test (x^2) with the statistical significance that was set at the level $p < 0.05$.

3.8.2.2 Dependent Variable

The study has two types of dependent variable namely diarrhoea and bacterial resistance. Acute diarrhoea defined as the three or more or loose, liquid or watery stool or at least one bloody stool with 24 hours. Persistence diarrhoea defined as diarrhoea begins acutely and at least 14 days.

3.8.2.3 Independent Variable

The independent variable in this study involved the risk factors for diarrhoea and bacterial resistance among children under five years' children. On risk factors for diarrhoea, demographical and socio-economic factor, knowledge of mother to diarrhoea, mother occupation, hygienic practices, breast feeding and vaccination and house hold. On risk for bacterial resistance involved mother knowledge to antibiotics

use, awareness to antibiotics resistance, follow the medical instruction on using drugs, completion of dose, price.

Demographic and socio-economic factors include age of children, level of education to mother/parent and occupation of parent/ mother. Mother/ parents were asked about their education level either primary, secondary, tertiary and none. On occupation were asked self-employee, public employee, and farmers. Knowledge of the parent to diarrhoea were asked if have knowledge, have little and or don't have. Sanitation and disposable of child waste mother were asked if the ways of dispose their child waste if the through open space, buried or put in latrines. About sanitation were asked if have latrine in the house or don't have. Hygienic practices mother were asked if wash hands their children before eating, by mother after going to toilet and after helping children defecation. Water related practice mother were asked accessibility of water either from private providers or public or about source of drinking water if they use bottled water, boiled water, tape water or open well water.

Breast feeding and vaccination mother were asked if their children were still being breastfed or weaned. In case of breast feed were asked if they have been exclusively breastfed to the day of the interview or not. About child vaccination were asked if children have been vaccinated against measles or not. Variable about risk factors for antibiotic resistance the knowledge also about antibiotic use, if they have or don't have, awareness to antibiotic resistance mother were asked if have know something or not about antibiotic resistance of bacteria. Antibiotic prescription if follow instruction from medical doctor on using antibiotic or not. Proper uses of antibiotic were asked if they complete doze or not and price of antibiotics if expensive or not.

3.8.3 Data Handling and Data Analysis

Continuous variables like age of child and mother, water access and latrine and the categorical variables like antibiotic prescription and bacteria presence etc. were entered in the pre-coded sections of the data collection tools. The data for interview were checked from data questionnaire for errors after each field day of data collection exercise and corrections were made where necessary and possible.

Both qualitative and quantities analysis data collection were completed were entered into a computer in the SPSS 16 .0 program for analysis. Two people entered the data into the computer program: one was spelling out the variable entries from the data collection tools and the other was typing them on the program. This helps to minimize data entry errors by one person doing both.

Uni- variate analysis

Uni-variable data were analysis using SPPS 16 version. Firstly each variable was described frequency distribution include data about prevalence of enteric bacteria and age related prevalence of diarrhoea.

Bi- variate analysis

Persons' chi-squire test used to give chi-squire value (X^2) has been used to determine association between each independent variable and diarrhoea among children less than five years of age in Zanzibar. This was involved association between diarrhoea with risk factors to diarrhoea and association between bacterial resistance and its risk factors.

3.8.4 Permission and Ethical Consideration

Ethical approval was obtained from the concerned authorities for sample collection which is the Ministry of Health. Informed consent obtained from the participating patient's guardians or parents. The study dealt with human and since involved collection of sample direct from human the consensus of participants was necessary. All the participants were asked for permission in the interview and since were voluntary issue they were not forced to participate. We dealt only to those accept to participant and those did not accept were left freely without any objection. All the participant were informed the purpose of the study and asked their permission to participate and stool samples collection. They were guarantee that the provided information will be confidential and would not be disclosed to anyone else.

3.8.5 Sample Transport

All stool samples were transported to the laboratory in the sterile containers, and transported in an ice box immediately after collection. Sample were labeled by necessary data including date, time of collection, sample type. Symptoms of each patient were recorded, included; fever, chills, presence of blood in stool, watery stool and shivering the quality of stool and the smell of the stool for each patient.

3.8.6 Materials

3.8.6.1 Media for Isolation and Enrichment

Alkaline peptone water, selenite F broth, Xylose Lysine decarboxylase (XLD) MacConkey agar with Sorbitol (SMCA), Helton enteric agar (HEA), Thiosulfate citrate bile salt (TCBS).

3.8.6.2 Media for Biochemical Identification

Motility Indole test (MIO), Lysine, Triple Iron Sugar (TSI), Urea, citrate, Voges – Proskauer (VP), Methyl red (MR), Manitol, phenylanine deaminase agar, Kovac's reagent, Mineral oil, Barrit's reagents A and Oxidase stick and oxidase test respectively.

3.8.6.3 Susceptibility Test Media and Reagent

Muller Helton Agar, (MHA), Normal saline of 0.85% and recommended antibiotics.

3.8.6.4 Gram's Stain Reagents

Crystal violet, Gram'siodine, ethanol (95%) and safranin and water and methyl violet.

3.8.6.5 Isolation Procedures of Enteropathogenic Bacteria *Salmonella and Shigella*

Pre-enrichment

Feaces were inoculated in Selenite-F broth, incubated at 37°C for 18. This was carried out before culture enrichment. This required the sample to be diluted in a non selective medium and then cultured in a special type of selective enrichment broth, such selenite F broth. The broth was then centrifuged to form a pellet that is made up of the cultured salmonella, thus allowing them to be isolated.

Cultural Enrichment

The growth from tube with selenite F broth was subculture on *Salmonella-Shigella* for *Salmonella* enrichment or XLD, feaces were inoculated, and the colonies

suspected of corresponding to enteropathogenic bacteria were identified by standard microbiogical methods agar (PHE, 2014).

Diarrheic *E.coli* (E.coli 0157H)

Stool sample was cultured in MacConkey Sorbital agar, E. coli O157:H7 rapidly ferments lactose and is indistinguishable from most other E. coli on traditional lactose-containing media. Inoculate stool specimens onto SMAC and incubate at 18-24 hours at 35-37C. Sorbitol-negative colonies were appeared colorless on SMAC (Potter *et al*., 2008).

Vibrio parahaemolyticus

Alkaline peptone water (APW) was recommended as an enrichment broth, and thiosulfate citrate bile salts sucrose (TCBS) agar was the selective agar medium of choice for isolating.

A. Enrichment in Alkaline Peptone Water

Vibrio parahaemolyticus grown very rapidly in APW and at 6 to 8 hours was present in greater numbers than non-*Vibrio* organisms. Enrichment in APW enhances the isolation of *V. parahaemolyticus* when few organisms were present.

Selective Plating Media

Thiosulfate citrate bile salts sucrose agar **(**TCBS) was the medium of choice for the isolation of *V. parahaemolyticus*since is widely used worldwide. Overnight growth (18 to 24 hours) of *V. parahaemolyticus*was produced large (2 to 4 mm in diameter), slightly flattened, green colonies (Yukiko*et al*., 2006).

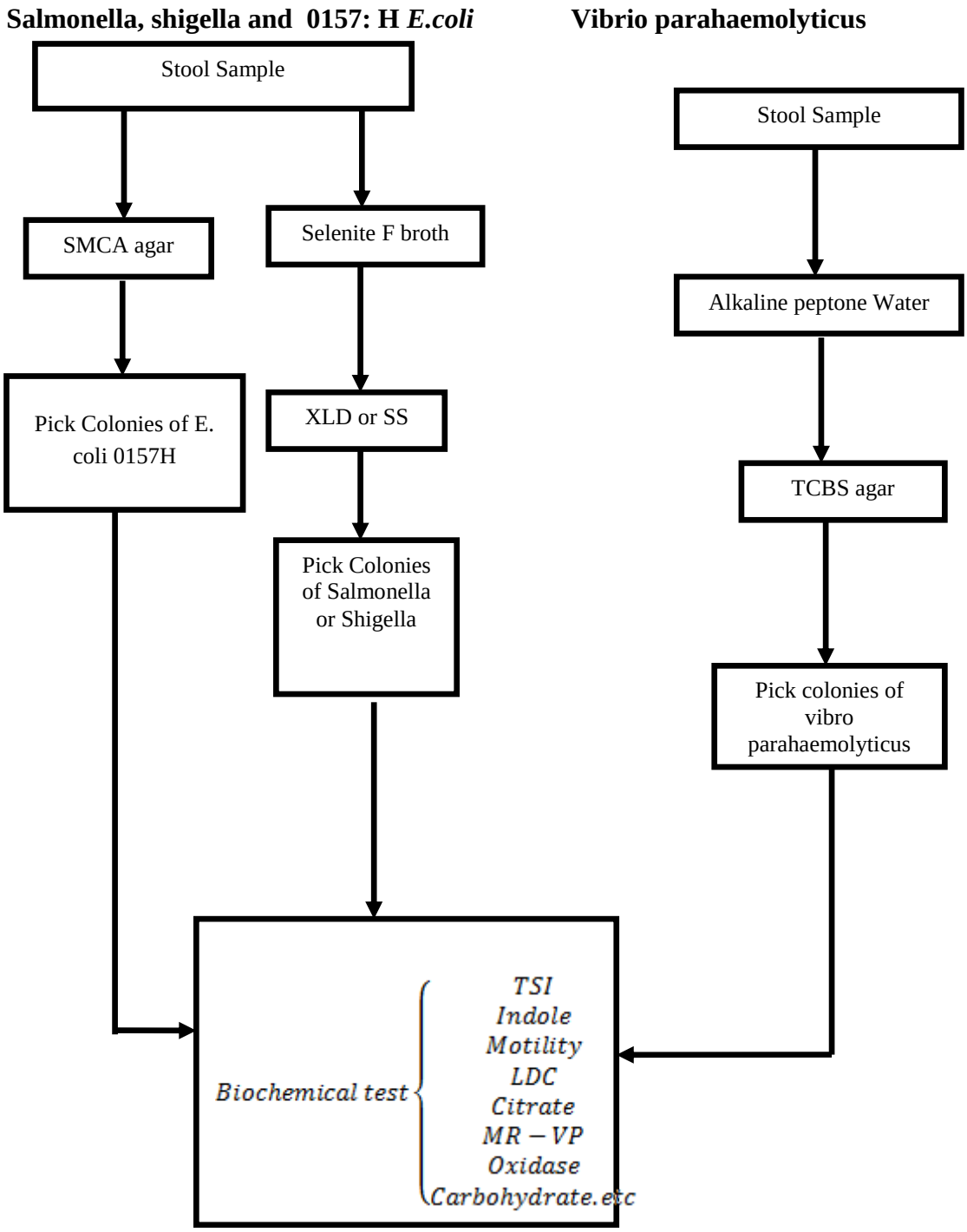

Figure 3.3: Summary of Procedures for Isolation of Salmonella, Shigella, and E.coli and Vibro Parahaemolyticus from Stool Specimen

Source: Issued by Microbiology pathology Laboratory Mnazi Mmoja hospital

Figure 3.4: Containers of collected Stool Sample

Source: Field Data (2013)

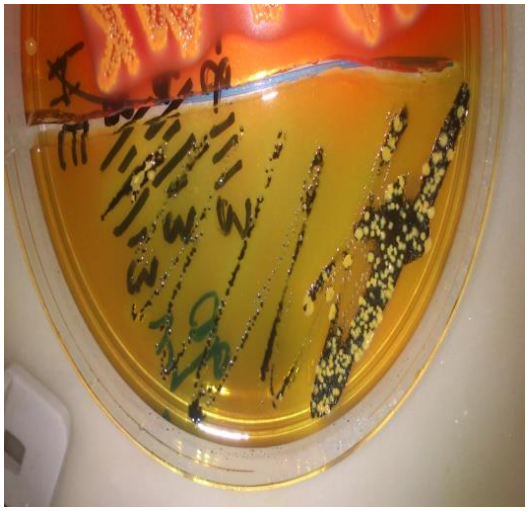

Figure 3.5: Colony of Isolated Salmonella in XLD

Source: Field Data (2013)

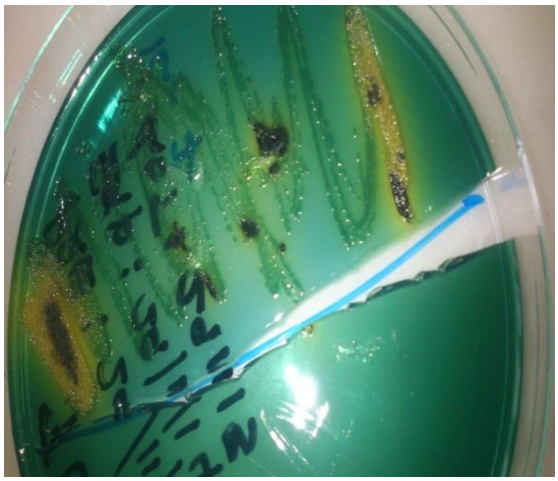

Figure 3.6: Colony of Vibrio parahaemolyticus in TCBS Media

Source: Field Data (2013)

Figure 3. 7: Colony of Shigella in XLD

Source: Field Data (2013)

3.8.6.6 Identification of Enteropathogenic Bacteria

3.8.6.6.1 Gram's Stain Technique

The Gram stain technique to identify bacteria based on their shape of cell wall involved the following steps. Heat fix: Light the prepare slide was passes (with the

bacteria mounted on it) through the interface between the blue flame and the yellow flame- 5 times. The slide was placed on a rack then flooded (cover completely) the entire slide with crystal violet, and allowed to stand the crystal violet stand for about 60 seconds. When the time has elapsed slide was washed for 5 seconds with the water bottle until blue-violet colour appeared when observed with the naked eye. The Slide was flooded with the iodine solution. Left to stand about a minute as well. When time has expired the slide was rinsed with water for 5 seconds a -The decolorizer, ethanol was added drop wise until the blue-violet color is no longer emitted from your specimen. As in the previous steps, rinsed with the water for 5 seconds. The final step involved applying the counter stain, saffranin by flooded slide with the dye as we did in steps 1 and 2 left to stand for about a minute to allow the bacteria to incorporate the saffranin. Gram positive cells were incorporated little or no counter stain and remained blue-violet in appearance. Gram negative bacteria, however, taken on a pink color and are easily distinguishable from the Gram positives. Again, rinsed with water for 5 seconds to remove any excess of dye. (http://www.microbelibrary.org/library/gram-stain/2886-gram-stain-protocols).

3.8.6.6.2 Biochemical Identification Methods

Motility Test (Motility Medium): Used to determine if a bacteria is motile

A needle was used to inoculate by making a single stab about two thirds down and then pulled the needle up the same path. - Incubated for 24-48 hours the tube appeared cloudy and usually the organism spread over the top of the media. Non-motile organism was grown along the streak line only; the media was not cloudy.

Carbohydrate Fermentation (Glucose, lactose, mannitol, maltose, sucrose)

Used to determine the ability of an organism to ferment a specific carbohydrate with or without the production of a gas. Phenol red is used as an indicator in the media. At a neutral pH, the media is red; at a pH of less than 7, the media is yellow. Fermentation of the carbohydrate produces acid, causing the media to change from red to yellow. The inverted tube in the broth, called a Durham tube, captures some of the gas the organism produces, allowing production to be seen (if it ferments, gas will be produced). Each media was inoculated with the organism, Incubated at the optimum temperature for 24-48 hours. Positive- Media turned yellow (fermentation has occurred) and gas produced, Negative- Media remains red (no fermentation) (Monica, 2000).

Methyl Red Test and Voges-Proskauer Test (MR-VP Broth)

Methyl Red Test: Used to determine the ability of an organism to produce mixed acid end products from glucose fermentations. Some organisms produce large amounts of various acids (lactic, acetic, succinic, formic) plus H2 and CO2. The large amounts of acids lower the pH to lower than 5.0. These organisms also produce great amounts of gas due to the presence of the enzyme formic hydrogen lyase.

Voges- Proskauer Test: Used to determine the ability of an organism to produce acetoin; 2, 3 butanediol; and ethanol which causes less lowering of the pH than the methyl red positive organisms. VP test detects the presence of acetoin, which is a precursor to 2, 3 butanediol. Two MR-VP broths were inoculated with organism, Incubated at optimal temperature for 3-5 days, 3-4 drops of Methyl Red reagent to

one tube added. Red color developed indicated positive result and yellow colour negative results. 1 ml of culture was pipetted from the other MR-VP tube into a small screw cap test tube. To the extracted 1 ml of culture, 18 drops of Barritt's Solution A (alpha-naphthol) and 18 drops of Barritt's Solution B (KOH) were added. Then Agitated vigorously for 1-2 minutes and allowed to stand for 1-2 hours. For VP test, wine red (burgundy) color develop positive results and Brown color develop as negative results (Isenberg, H.D. 1992).

Oxidase Test (trypticase soy agar plate)

Used to determine the presence of oxidase enzyme in case of *Vibrio parahaemolyticus* only. Aerobic organisms obtain their energy by respiration, which is responsible for the oxidation of various substrates through the cytochrome oxidase systems (ETC). Obligate aerobes have this enzyme. Isolated organism streaked on the TSA plate, Incubate at optimum temperature for 24-48 hours, added several drops of oxidase test reagent directly to organism and left to stand for 10-15 minutes. Positive result shown by organisms change color to a dark red/ black while negative result no color changed.

Tryptophan Hydrolysis/ "Indole Test" (Tryptone Broth)

Used to determine the ability of an organism to split indole from the amino acid tryptophan using the enzyme tryptophanase. The media was incubated with organism for 24-48 hours. After incubation period it was added with 10-12 drops of Kovacs Reagent. Red layer formed on surface of the media indicated positive result and negative yellow layer formed on the surface of the media as negative result.

Urease Test (Urea Broth)

Used to determine the ability of an organism to split urea to form ammonia (an alkaline end product) by the action of the enzyme urease. Media also contains the pH indicator phenol red, which turns an intense pink at alkaline pH. Urea broth was inoculated with the organism then incubated at optimum temperature for 24-48 hours. Intense pink/red color showed positive no color changed showed negative.

Hydrogen Sulfide Production (Kligler's Iron Agar)

Used to determine the ability of an organism to produce H_2S (Hydrogen sulfide). The media also contains glucose, lactose, and phenol red as a pH indicator to show fermentation of these sugars. Gaps, cracks, or bubbles in the agar indicate gas production. The KIA was stab with straight wire inoculums of organism, Incubated at optimum temperature for 24-48 hours. Black precipitate along stab line was positive result while no precipitate.

Citrate Utilization (Simmons Citrate Agar)

Used to determine if an organism is capable of using citrate as the sole source of carbon with production of the enzyme citratase. The media contains sodium citrate as the carbon source, and ammonium salts as the nitrogen source, with bromothymol blue as the pH indicator. An organism that uses citrate breaks down the ammonium salts to ammonia, which creates an alkaline pH. Simmons citrate agar was stab and streaked slant with the organism. Incubated at the optimum temperature for 24 -48 hours.

Phenylalanine Deamination (Phenylalanine Agar)

Used to determine the ability of an organism to deaminate the amino acid phenylalanine resulting in the production of phenylpyruvic acid and ammonia. This reaction is catalyzed by the enzyme phenylalanase.

Phenylalanine agar slant was streaked with the organism and incubated at optimum temperature for 24-48 hours. 5-10 drops of 10% Ferric Chloride placed on the slant culture. A deep green color appeared within 1-5 minutes. Negative an amber color developed.

Lysine Decarboxylase Test (Lysine Decarboxylase Broth)

Used to determine the ability of an organism to decarboxylate the amino acid lysine, resulting in the production of alkaline end product, by producing the enzyme lysine decarboxylase. The media contains lysine, glucose (as a substance for fermentation), and the pH indicator Bromo Cresol Purple (purple at alkaline pH; yellow at acid pH). The enzyme requires an acidic pH (below 5) for activation.

If the organism is capable of glucose fermentation (check with carbohydrate broth if available), and has the enzyme lysine decarboxylase, the following events occur. Microbe ferments glucose, producing low pH; indicator turns yellow. Broth was inoculated with the organism using a wire loop. Incubated at optimum temperature for 24- 48 hours, positive- Purple ferments glucose and yellow negative. The suspected *Pseudomonas* isolates were inoculated into 10 ml nutrient broths and incubated at 42oC for 24 hours and observed for signs of growth (Hueh *et al*., 1998; Lynne *et al.*, 2007; Monica, 2000**)**.

This stain was used in staining technique, Methyl violet, Grams lodine, Ethyl alcohol 95% v/v, Safranin and Distilled water. The slide was covered and washed with iodine stain, methyl violet, crystal violet, ethyl alcohol of safranin and allowed to act for 2 minutes, washed than decolourized with alcohol or acetone followed with water for resin in water, than dried and finally was examined under oil immersion.

3.8.6.6.3 Biochemical Confirmation

API 20E Test: (BioMerieux France)

The analytical profile index (API) 20E strips (BioMerieux) was used as biochemical system of identification and confirmation of gram-negative rod bacteria. The API 20E strip consists of 20 micro tubes containing dehydrated substrates. These strips were inoculated with bacterial suspension, which reconstitutes the media. The strip was incubated for 18 to 24 hours at 37^0C, during incubation; metabolism produces changes that are either spontaneous or revealed by the addition of reagents. The standard was scored according to reading table and the identification was obtained by referring to the API20E catalogue or using identification software (Analytic Profile Index API 20Ebook 1999).

3.8.6.6.4 Antimicrobial Surveillance for the Bacteria Isolates

Antimicrobial susceptibility testing (AST) will be performed by growing the isolates in the presence of a given antibiotic. Disk diffusion method for routine susceptibility testing of bacterial isolates was used. Antibiotic-impregnated paper disks are placed on the surface of an agar plate which has been seeded with the isolate being tested. If the organism is susceptible to the antibiotic tested its growth was inhibited and a

zone of inhibition result around the antibiotic disk. The diameter of the zone of inhibition of growth is proportional to the MIC value. The zone size is measured (in mm); the value is compared to the interpretive criteria developed by the manufacturer. The isolate is assigned a sensitive, intermediate or resistant category for each antibiotic after comparison with the appropriate manufacturer table or guideline (Patrick *et al*., 2000& Rodney, 2006).

Table 3.2: The Criteria for Judgment of used Drugs in Sensitivity Test

	S	**I**	**R**
Antibiotics	**= or >**		**= or <**
Erythromycin	23	14 – 22	13
Ciproflaxin	21	15 – 20	15
Sulfamethoxazole/	19	16 – 18	15
Trametheprin	19	15 – 18	14
Tetracycline	15	9 – 12	8
Gentamycin	18	13 – 17	12
Ampicillin	17	12-8	6

Source: Patrick*et al*., 2000& Rodney (2006)

R stands for resistance which is the ability of a bacterium or other microorganism to survive and reproduce in the presence of antibiotic dose that were previously thought effective against them (Modern Medical Dictionary, 2002).

S stands for susceptibility which is the quality or state of being susceptible or the state of being predisposed or lacking the ability to resist something (www.medicaldictionary)

I stands for intermediate: Include isolated with antimicrobial minimum inhibitory concentration (MICs) that approach usually attainable blood tissue level and for which response rates may be lower than for susceptible isolates (Bruce, 2007).

3.8.7 Limitation

The limitation of this study was in failure to isolate some pathogenic enteric bacteria include *campylobacter* and *Yesinia* as proposed earlier in our study. This was due to lack of gas system incubator for isolation of Campylobacter. *Yersinia* was not isolated due to lack obtain selective media for isolation of *Yersinia* bacteria suppliers in Tanzania. There were bureaucracy of obtain important information from Ministry of Health.

CHAPTER FOUR

RESULTS

4.1 Prevalence of Enteric Pathogenic Bacteria Isolated In Unguja Island-Zanzibar

Stool samples from three selected study sites were collected between 4 September 2013 and 30 February 2014. All samples were collected from under-five children with diarrhea and were analyzed by biochemical microbial and antimicrobial susceptibility methods. In total three hundred and nineteen (319) samples from three sites were analyzed for presence of enteric pathogenic bacteria in selected study sites.

The results showed that one hundred and thirty 130 stool samples were positive for enteric pathogenic bacteria. Figure 4.1 shows distribution of proportion of 130 isolates and among these, Diarrhoeic *E.coli* comprised 17%, *Salmonella* 25%, *Shigella* 38.5% and *Vibrio parahaemolyticus* 19.23%. *Shigella* was more prevalent enteric bacterial pathogens isolated Figure 4.1.

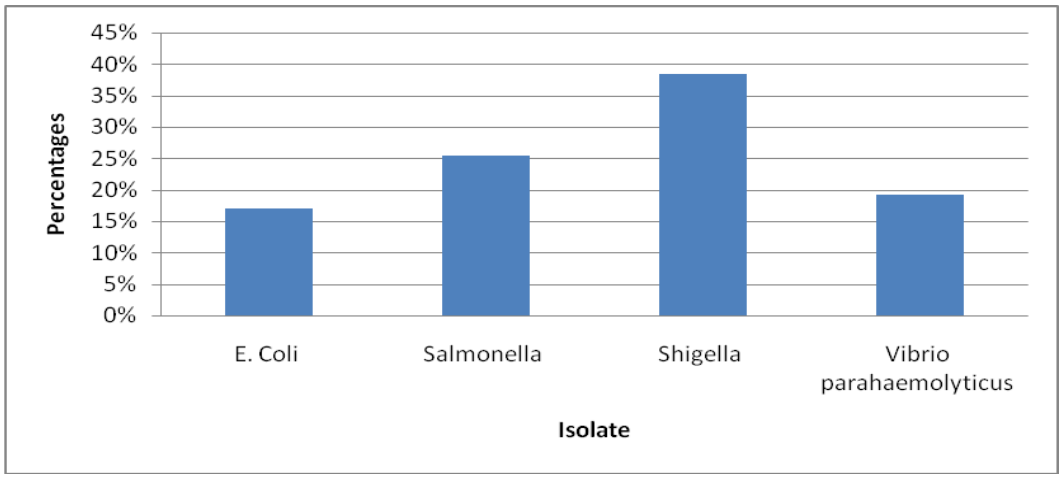

Figure 4.1: Prevalence of Enteric Bacteria Isolated in Under-Five Children in Unguja Island-Zanzibar

4.2 Spatial Prevalence of Pathogenic Enteric Bacteria Isolated In Under-Five Children in Unguja Island- Zanzibar

The spatial prevalence of pathogenic enteric bacteria isolated based on the three districts (Urban, South and North) is shown below.

4.2.1 Prevalence of Enteric Pathogenic Bacteria in Urban District of Unguja Island

Prevalence of isolates of enteric pathogenic bacteria in urban was 54% samples and out of these, 19.6% was Diarrheic *E.coli* 26% *Salmonella* 40% *Shigella* and 16% for *Vibrio parahaemolyticus* respectively (Figure 4.2).

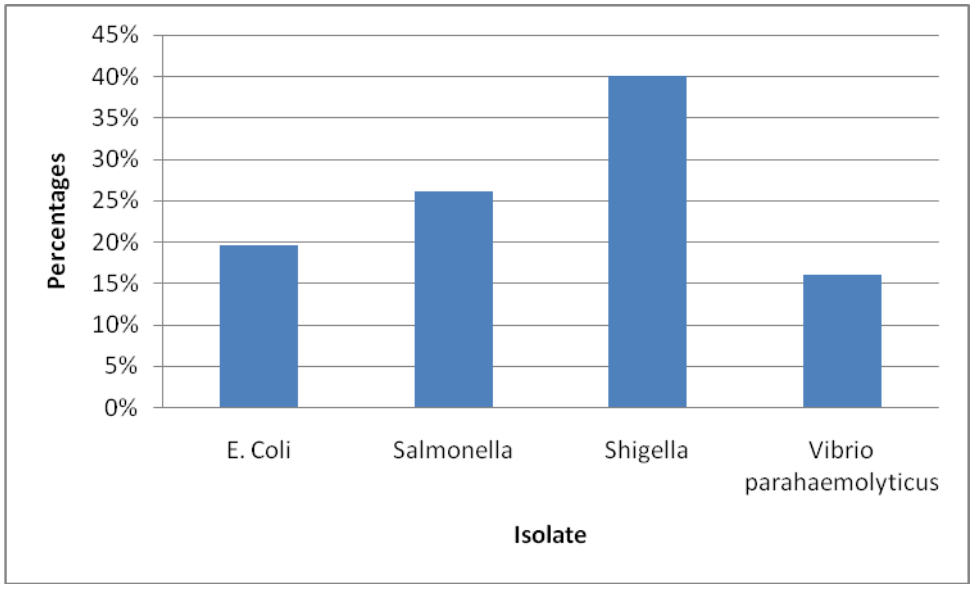

Figure 4.2(a): Prevalence of Enteric Pathogenic Bacteria Isolated in Urban District of Unguja Island

4.2.2 Prevalence of Pathogenic Enteric Bacteria in South District of Unguja Island

Figure 3.3 shows proportion of 38 isolates in South district whereas Diarrheic *E.coli* was 17%, *Salmonella* 24%, Shigella, 37% and 24% for *Vibrio parahaemolyticus*.

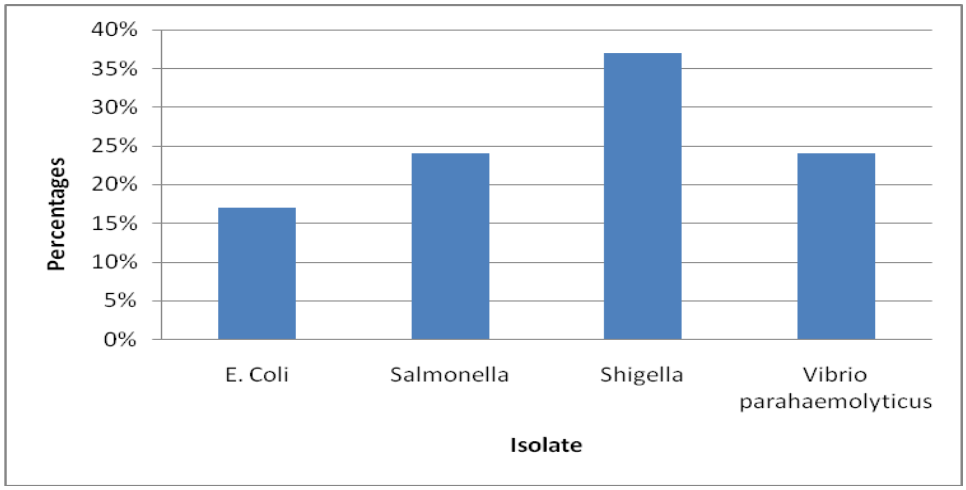

Figure 4.2(b): Prevalence of Isolates in South District of Unguja Island

4.2.3 Prevalence of Pathogenic Enteric Bacteria in North District of Unguja Island

The Prevalence of pathogenic enteric bacteria in North district is shows in Figure 4.2(c) whereas prevalence of *E.coli* was 14%, *Salmonella* 27.3% 36%, *Shigella* 36% and 22.7% for *Vibrio parahaemolyticus*. There were no significant difference in shigella and Salmonella prevalence compare to *Vibrio parahaemolyticus* and Diarrhoeic *E.coli* reported in all three districts at ($p>0.05$).

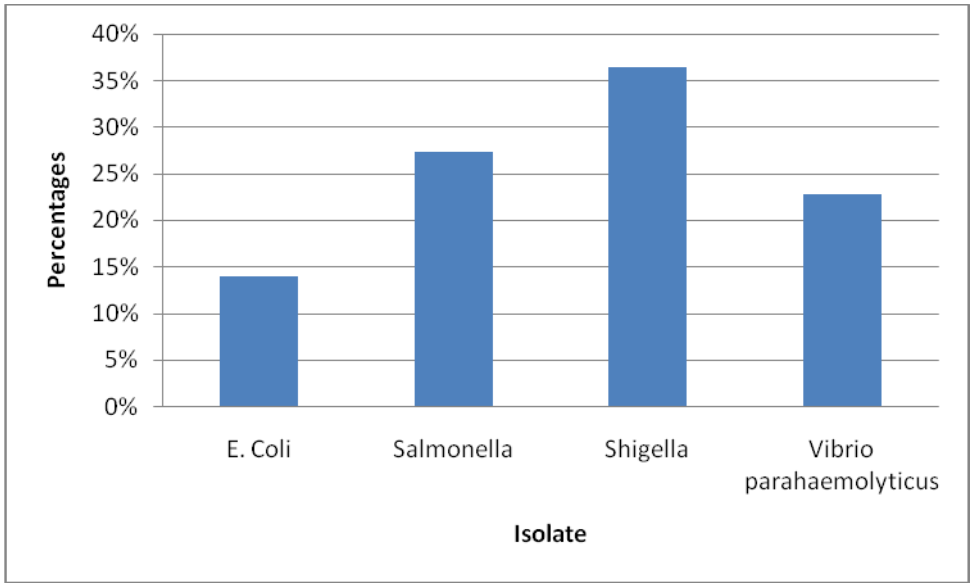

Figure 4.2(c): Prevalence of Isolates in North District of Unguja Island

4.3 Overall Spatial Prevalence of Enteric Pathogenic Bacteria

Figure 4.3 shows overall spatial prevalence of pathogenic enteric bacteria isolated in Zanzibar by district. The urban district showed high prevalence of four pathogenic bacteria isolated in 53.85%, South 29.3% and North 16.9%. There were a significant difference in the prevalence of the four types of isolated pathogenic bacteria (Cl 95%, df 3.017 p <0.05).

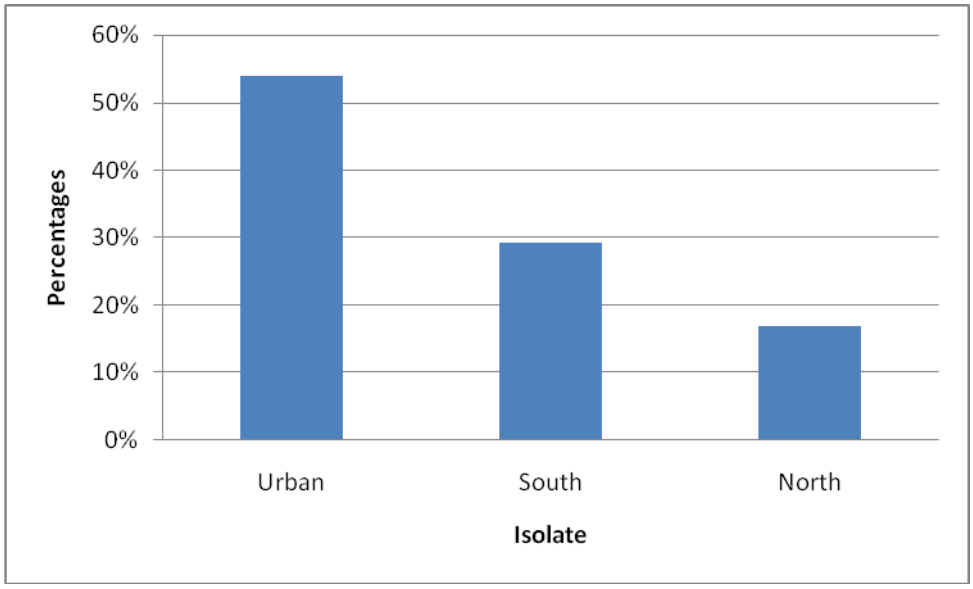

Figure 4.3: Overall Spatial Prevalence of Enteric Pathogenic Bacteria Isolated in Unguja Island- Zanzibar

4.4 Seasonal Prevalence of Enteric Bacteria Isolated in Unguja Island-Zanzibar

In order to investigate the seasonal prevalence of enteric pathogenic bacteria the study was conducted in both the rainy and dry seasons and the results revealed high prevalence of enteric bacteria to about 71.45% in rainy season to 28.55% in dry season as shown in Figure 4.4.

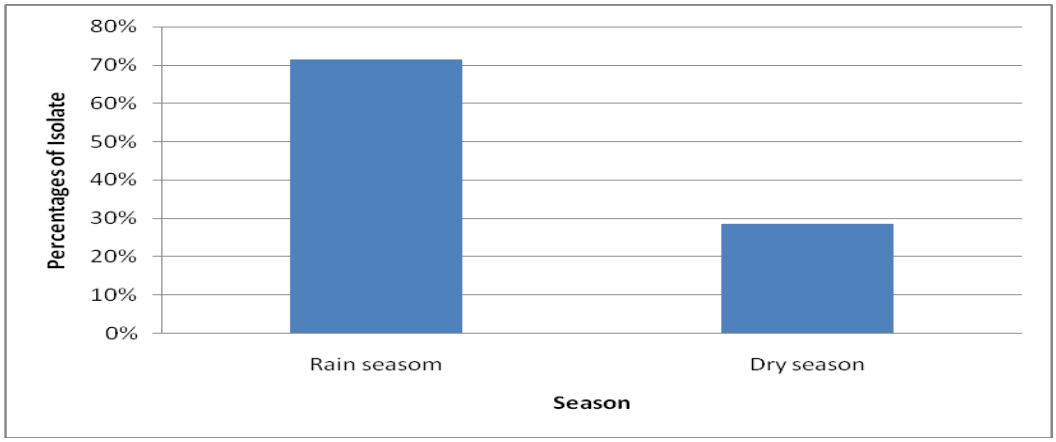

Figure 4.4: Overall Seasonal Prevalence of Enteric Pathogenic Bacteria Isolated in Unguja Island-Zanzibar

4.5 Prevalence by Age of Pathogenic Enteric Bacteria with Diarrhea of Under-Five Years Children

Bacteriological analysis showed that Diarrheic *E.coli, Salmonella* and *Shigella* were isolated in all age group while *Vibrio parahaemolyticus* were only isolated only in 13-24 and 49-60 months age groups and not isolated in 0-6 months age group. Figure 4.5 shows the overall distribution of diarrheal children by age. The present results reveal that diarrhea caused by bacteria is statistically associated with age and majority of cases occurring in children between 7months and 2 years of age group (x^2 14.659 df 5 ($p<0.05$) $p=0.12$).

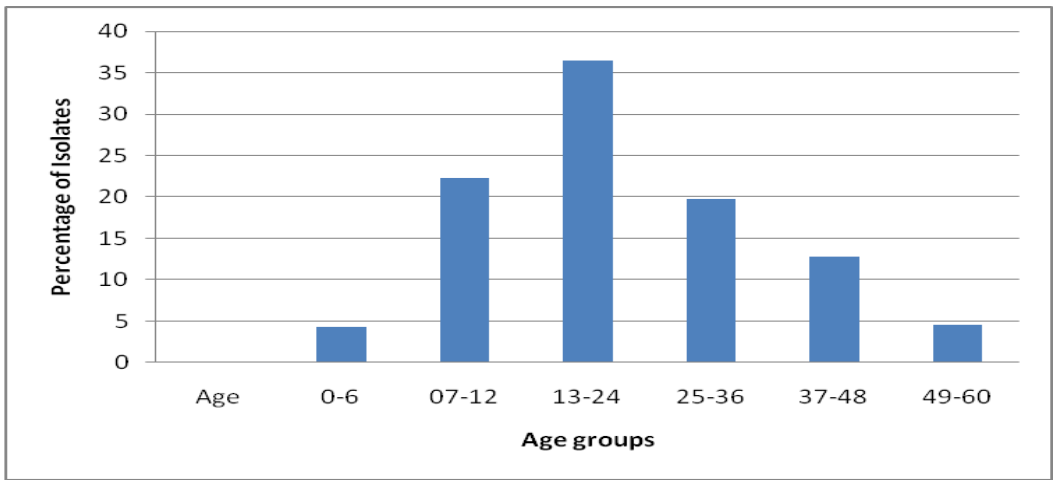

Figure 4.5: Overall Prevalence of Isolate within the Age Groups

The prevalence of enteric pathogenic bacteria by age groups was 4.3% at 0-6, 22.3% at 7-12, 36.44% at 13-24, 19.7% at 25-36, 12.8% at 37-48 and 4.8% 46-60 respectively. The results indicate that diarrhea in under five children is associated with age and noticed to decrease from 13-24 to 49-60 age categories. High prevalence was observed in the age between 13-21 in 36.4% Diarrheic *E.coli*, 27% *Salmonella* 34% and 36% *Vibrio parahaemolyticus* (Figure 4.5). The results in this study showed high prevalence of enteric pathogenic enteric bacteria at age group 13-24. This indicates that the children at this age group have a higher risk of getting diarrhea (Figure 4.6).

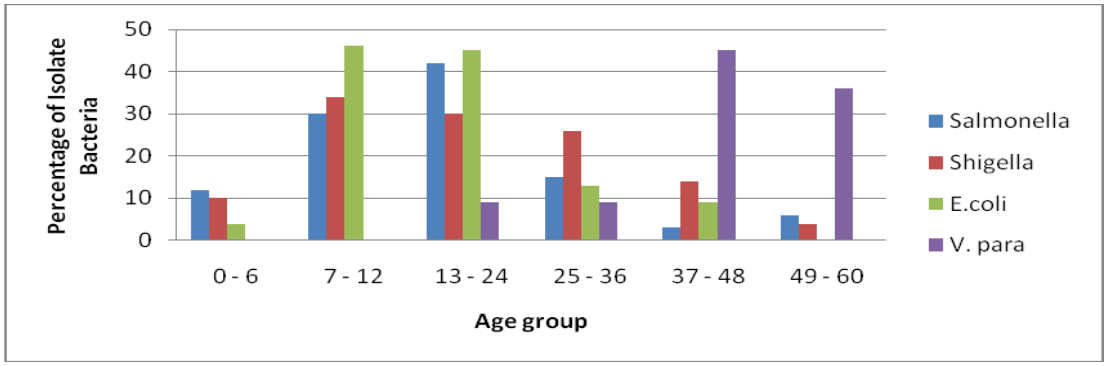

Figure 4.6: Prevalence of Individual Isolate within each Age Group

4.6 Antibiotics Sensitivity Results to Isolated Pathogenic Enteric Bacteria

Among 130 isolates tested for antibiotic sensitivity to common used antibiotics, 22 were Diarrheic *E.coli*, 33 *Salmonella*, 50 *Shigella*, and 25 *Vibrio parahaemolyticus*. All isolated bacteria showed high level of multidrug antimicrobial resistance to the common antimicrobial most frequently used to treat infection in Zanzibar.

4.6.1 Antibiotics Resistance Pattern of *Salmonella*

Salmonella showed resistance to 67% Sulfamethoxazole/Trimethoprim followed by 64% Erythromycin, 57% tetracycline and 46% Ampicillin, 15% Ciproflaxin,

Gentamycine 9.1% and chloromphenicol 9.1%. Highly resistance observed to Sulfamethoxazole/ Trimethoprim, Erythromycin, Tetracycline and Ampicillin and low resistance observed to Ciproflaxin, Gentamycine and Chloromphenicol respectively as illustrated in Figure 4.6(a).

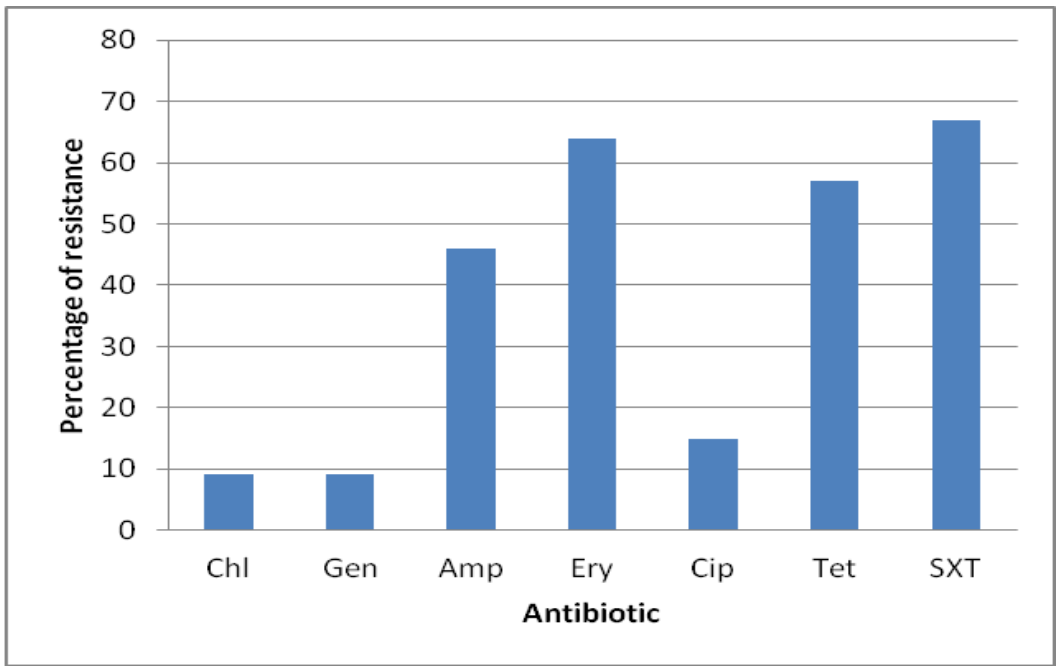

Figure 4.6 (a): Antibiotics Resistance Patterns of Salmonella

4.6.2 Antibiotics Resistance Patterns of Shigella

The results of resistance pattern to *Shigella* was 71% Sulfamethoxazole/ Trimethoprim, 74%, Tetracycline, 38% Ciproflaxin, 60%, Erythromycin, 67 % Ampicillin, 12 % Gentamycin and 56% Chloromphenicol. High resistance observed to Sulfamethoxazole/, Tetracycline, Erythromycine, Ampicilline and Chloromphenicol and low resistance to Ciproflaxin and Gentamycine respectively as shown in Figure 4.6(b).

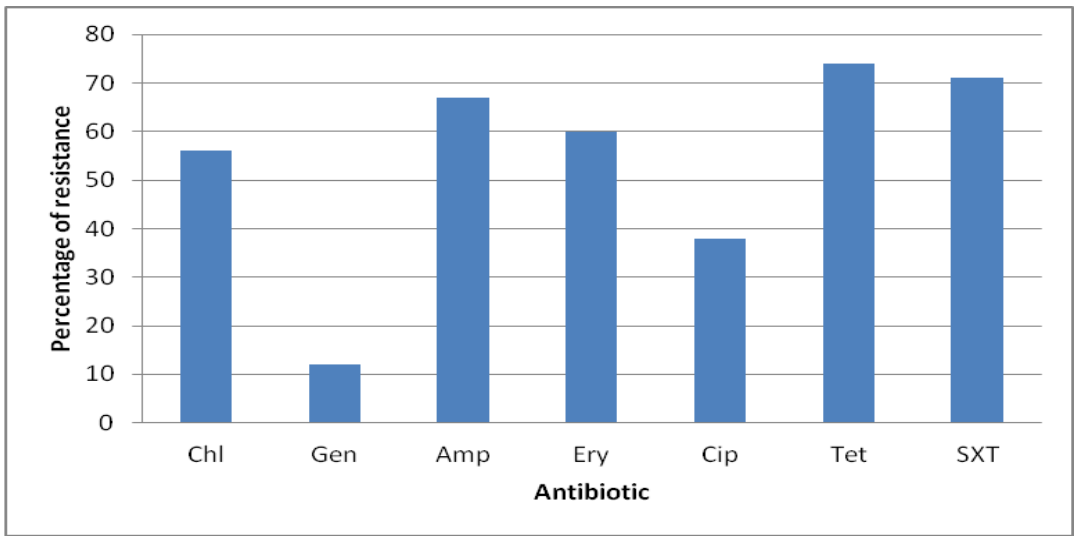

Figure 4.6(b) Antibiotics Resistance Pattern of Shigella

4.6.3 Antibiotics Resistance Patterns of Diarrheic E.coli

The resistance pattern of for Diarrheic *E.coli* was 68% Sulfamethoxazole/ Trimethoprism 77.3%, Tetracycline, 40.9% Ciproflaxin, 54.5% Erythromycin, 50% Ampicillin, 31.8% Gentamycine and 22.7% Chloromphenicol. Highly resistance was observed to Tetracycline, followed by Sulfamethoxazole/ trimethoprim, Erythromycin and Ampicilline. Low resistance observed to Gentamycine and Chloromphenicol and Ciproflaxine respectively as shown in (Figure 4.6(c).

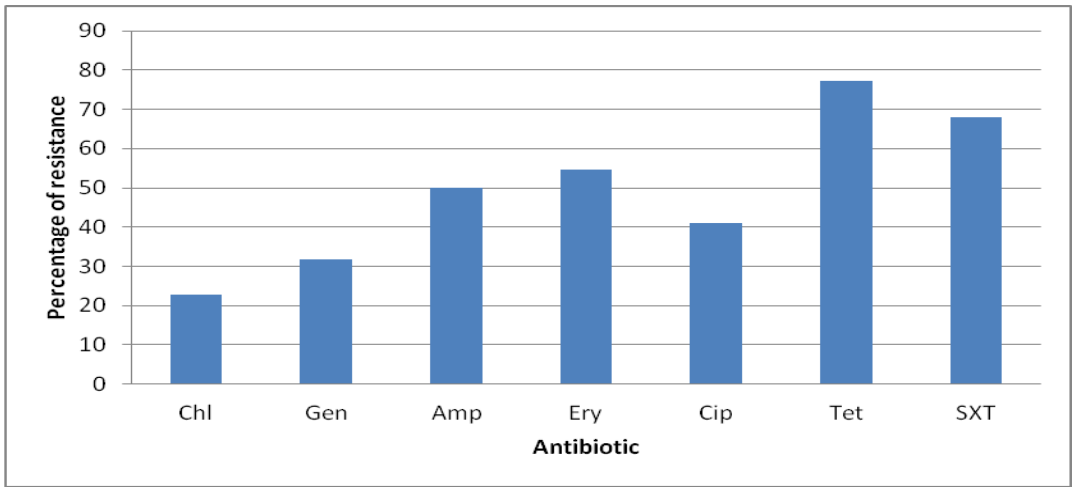

Figure 4.6(c) Antibiotics Resistance Patterns of Diarrhoic *E.Coli*

4.6.4 Antibiotics Resistance Patterns of *Vibrio parahaemolyticus*

The resistance pattern for *Vibrio parahaemolyticus* was 53% Trimethoprim/Sulfamethoxazole, 52% Tetracycline, 32% Ciproflaxin, 88% Erythromycin, 68% Ampicillin, 16% Gentamycin, and 24% Chloromphenicol . High resistance observed to Erythromycine, Ampicilline, Tetracycline and Trimethpprome/ Sulfamethoxazole .Low resistance observed to Gentamycine, Ciproflaxine and Chloromphenicol respectively as shown in 4.6(d).

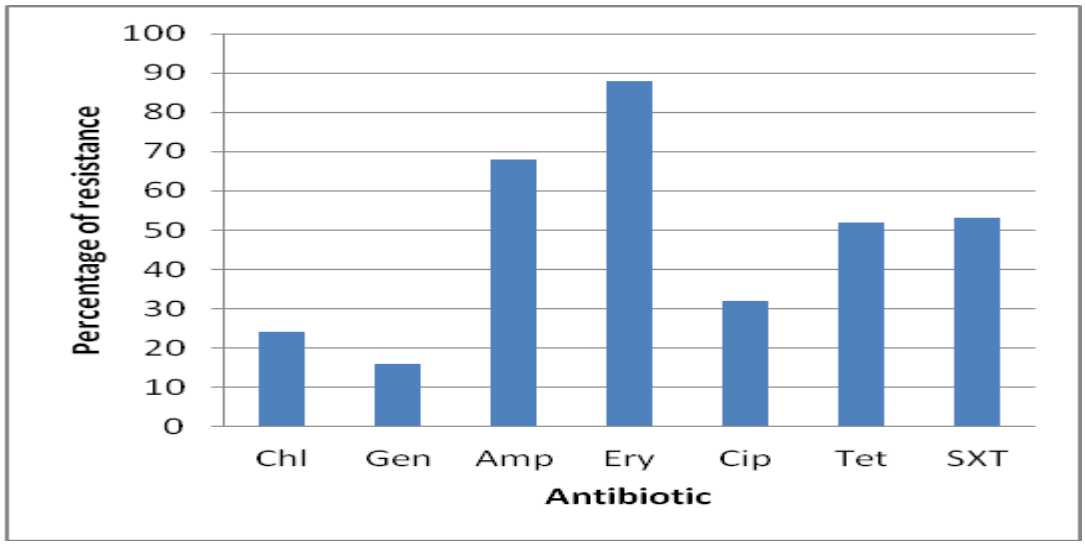

Figure 4.6(d): Antibiotics Resistance Patterns of *Vibrio pahaemolyticus*

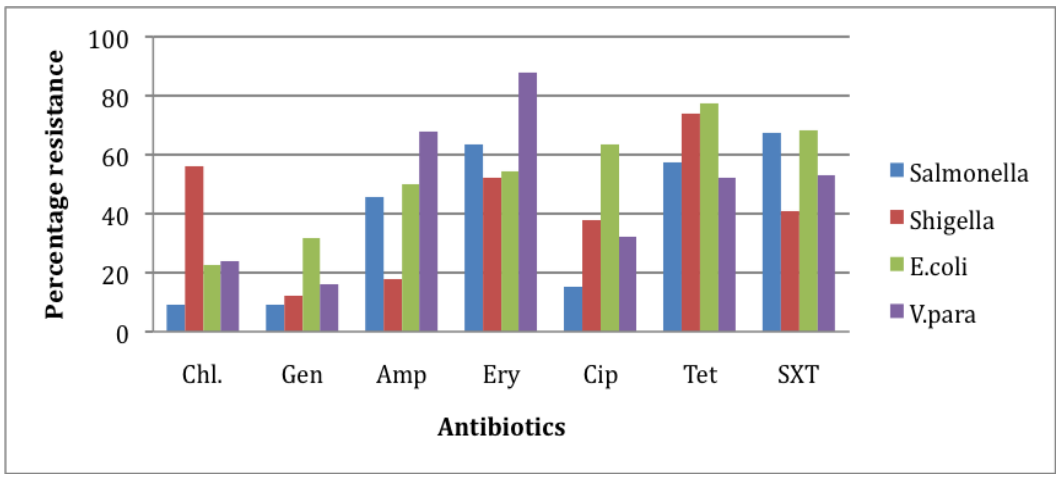

Figure 4.6(e): Proportion Antibiotic Resistance of each Bacteria

Source: Field Data (2013)

4.7 Risk Factors Associated With Bacterial Diarrhea in Under Five Years Age in Zanzibar

4.7.1 Mothers Education and Diarrhea

Among 319 mothers recruited into the study and about 33.3% mothers had children with bacterial diarrhoea cases and 66.7% had children with negative bacterial diarrhoea cases respond to have no education at all. Mothers with primary education had 42% of children with bacterial diarrhoea cases to 56.7 with non-bacterial diarrhoea cases. Mothers with secondary education had 42.9% children with bacterial diarrhoea cases to 57.1% children with non-bacterial diarrhoea cases. About 49 % mothers with secondary education had children with bacterial diarrhoea and 51% mothers with secondary education had non-bacterial diarrhoea while about 42.9% mothers with children had bacterial diarrhoea to 57.1% of mothers whose children had non-bacterial diarrhoea respond to have tertiary education. The statistic association between level of education and cause of bacterial diarrhea is not significant $p > 0.05 (p = 0.4)$ (Table 4.1).

4.7.2 Diarrhoea and Sources of Drinking Water

On the relation between the source of drinking water and diarrhoea, 40.6% of mothers whose children had bacterial diarrhoea and 59.4% mothers whose children had non-bacterial diarrhoea used tape water. 39.2% of mothers whose children had bacterial diarrhea to 60.8 mothers whose children had non bacterial diarrhoea used boiled water while mothers used open well water source had children with positive bacteria diarrhoea to about 43.2% to 56.9% mothers had children with negative bacterial diarrhoea. The proportion of source of drinking from open well was high

compare to tape water and boiled water, however there was no association between source of drinking water and prevalence of bacterial dairrhoea, and at p>0.05(p=0.37) (Table 4.1).

4.7.3 Disposal of Child Waste and Diarrhea

Disposal of child waste through dumping in open spaces was accounted for 34.1 % of mothers who put child waste in pit latrine had children with bacterial diarrhoea; and about 65.9% of mothers who put child waste in pit latrine, had children with non bacterial diarrhoea. 42.5% mothers buried child wastes in the ground had children with bacterial diarrhoea and 57.5% mothers buried child waste had non bacterial diarrhoea, and about 43.3% mothers dispose in open space had children with bacterial diarrhoea while 56.7% mothers dispose in open space had non bacterial diarrhoea children (P> 0.05 (p=0.366) (Table 4.1).

4.7.4 Feeding Status and Diarrhoea

When asked if mothers were practicing bottle feeding or exclusive breast feeding, it was found that 33.1% of mothers with positive bacterial diarrhoic cases to 66.9% mothers whose children shown negative bacteria diarrhoea cases practiced bottle feeding. In case of exclusive breast feeding, mothers whose children shown positive cases with bacterial diarrhoea were 42% to 58% mothers with children with negative cases of bacterial diarrhoea. This difference is statistically not significant at p>0.05(p=0.35). This indicates no positive association between feeding status and bacterial diarrhea (Table 4.1).

4.7.5 Washing Hands Before Feeding

About 70.1% mothers do not wash hands their children before feeding had bacterial diarrhoea children while about 29.9% had non-bacterial diarrhoea. About 32.9% Mothers wash hands of their children had bacterial diarrhoea to 67.1% mothers whose children had non-bacterial diarrhoea. The association between wash hand practices is significance at $p < 0.05 (p=0.02)$(Table 4.1).

4.7.6 Washing Hands after Helping Children Defecation and Diarrhea

The proportion of mother do not wash their hands after helping children defecate had 70.1% children with bacterial diarrhoea to 32.9% children with non bacteria diarrhea whose mothers wash hands after helping children defecation This relationship is statistical significant at $p<0.05 (P=0.020)$ (Table 4.1).

4.7.7 Washing Hands before Preparing Food and Diarrhoea

Mother washing hands before prepared children food accounted for 64.9.7% children with bacterial diarrhoea to 36.1% mothers with non-bacterial diarrhoea also wash hands. About 51% do not wash hands have children with bacterial diarrhoea to 49% mothers with non-bacterial diarrhoea children? The association is statistically significant at $p<0.05 (p=0.035)$ (Table 4.1).

4.7.8 Child Vaccination and Diarrhoea

A large proportion of children with diarrhea were not vaccinated, and bout child vaccination, 41.1% vaccinated had positive bacterial diarrhoea to 58.9% children with non-bacterial diarrhoea. Also 69.6% children with bacteria diarrhoea to about

39.4% children vaccinated with non bacterial diarrhoea. The children who were not vaccinated are at high risk of getting diarrhoea than those who are vaccinated P<0.05 (p=0.03) (Table 4.1).

Table 4.1: Risk Factors Associated with Diarrhoea

Risk Factor	Variable	% Positive cases with bacterial diarrhoea	%Negative cases with bacterial diarrhoea	P value
Level of Education	No Education	33.3	66.7	(X^2 2.916 and p>0.05,p=0.40)
	Primary Education	42.4	57.6	
	Secondary education	49	51	
	Tertiary Education	42.9	57.1	
Source of Water	Tap water	40.6	59.4	$X^2$0.174 and p>0.05,(p=0.37)
	Open wells	43.2	56.9	
	Boiling water	39.2	60.8	
Disposal of child waste.	Pit latrines	34.1	65.9	X^2 2.009 and p>0.05, (p=0.37)
	Buried	42.5	57.5	
	Open disposal	43.3	56.7	
Feeding	Bottle feeding	43.1	56.9	X^2 0.398 and p>0.05, (p=0.35)
	Exclusive Breast feeding	42	58	
Hand washing before feeding	Do not wash hands	70.1	29.9	X^2 0.398 and p<0.05,(p=0.02)
	Wash hands	32.9	67.1	
Hand washing after defecation	Do not wash hands	77.7	22.3	X^2 0.658 and p<0.05, (p=0.02)
	Wash hands	41.5	58.5	
Mother washing hand before preparing child food	Do not wash hands	6.9	36.1	X^2 0.36 and p<0.05,(p=0.04)
	Wash hands	51	49.	
Measles Vaccination	Vaccinated	41.1	58.9	X^2 0.07 and p<0,05(p=0.03)
	Do not vaccinated	69.6	31.4	

Source: Field Data (2014)

4.8 Factors Associated with Antibiotic Resistance to Bacteria

Mothers whose children had diarrhoea were interviewed about antibiotic use before hospitalization, awareness to antibiotic resistance, knowledge to antibiotics use, price of antibiotics, following physician instruction and dose incompletion. The response from them was summarized and tabulated (Table 4.2).

4.8.2 Association between Antibiotic use before Hospitalization and Resistance

The results shows that the percentage of mother who gave their children antibiotics without prescription was 25.4% and those did not give their children antibiotic were 74.6%. The statistical analysis showed that there is significant association between resistance and misuse of antibiotics (**$X^{2\,=}$ 7.136, p>0.05) p=0.01** (Table 4.2).

4.8.3 Association between Parents Awareness to Antibiotic Resistance

The percentage of mother having awareness about antibiotic resistance was that 32.9% were aware and 67.1% were not aware. This indicates that awareness to antibiotics resistance was low among mothers. The association between awareness and antibiotics use is statistically significant (**X^2 8.36 p<0.05) p=0.03** (Table 4.2).

4.8.4 Association between Low Dose Prescription Due to Drugs Price and Antibiotic Resistance

The percentage of respondents who used low dose due to drug price was 35.4% and 64.6% of respondents said they did not reduce dose due to drugs price. The association is statistically significant (X^2 = 7.260 p<0.05) p=0.027 (Table 4.2).

Table 4.2: Risk Factors Associated with Antibiotic Resistance

Risk Factor	% Yes	% No	P value
Antibiotics use before hospitalization	39.5	60.5	X^2 8.36 and p>0.05(p=0.01)
Parents Awareness about antibiotic resistance	41.6	58.4	X^2 7.71 and p<0.05(p=0.034)
Low dosage due to high price of Antibiotic	64.6	35.4	X^2 = 7.260 and p<0,05(p=0.027)

Source: Field Data (2014)

CHAPTER FIVE

DISCUSSION

5.1 Introduction

The objective of this study was to investigate the prevalence of enteric bacteria associated with diarrhoea under five years old and their sensitivity to antibiotic in Zanzibar.

5.2 The common Pathogenic Enteric Bacteria in Zanzibar

In the present study four types of enteric bacteria were isolated namely, Diarrheic *Escherichia coli*, *Salmonella*, *Shigella* and *Vibrio parahaemolyticus*. The finding showed that the Shigella bacteria were the predominant pathogenic enteric bacteria isolated in Zanzibar.

High prevalence of Shigella in this study is similar to another study carried out at Ifakara, Tanzania by Goscon *et al.* (2000). The results of the present study are, also supported by Njuguna *at al*. (2012) who found high prevalence *Shigella* in Kenya. Moreover, several studies carried out in developing countries have reported high proportion of *Shigella* in under five children (Jack *et al*., 2010). This may be due to the fact that asymptomatic infection is always common and may have arisen due to the tolerance inducing immune mechanism (*Gascon et al.,* 2000). High prevalence of *Shigella* causes shigellosis in developing countries *suggested* of being third most common pathogen transmitted through food The (CDC, 2010). Association of *Shigella* with contamination of street food has been well documented and several

outbreak of disease include shigellosis which has been traced to consumption of contaminated street food (Abdullsalam *et al*., 1993).

High prevalence of *Shigella* bacteria in our study confirmed highest incidence of shigellosis to less than five years children in study area. The occurrence of *Shigella* in common street vended food include potato salad, vegetables and junky food documented in study area (Hammd *et al*., 2014) reported high prevalence of *Shigella* among foodborne pathogens..The association between prevalence of *Shigella* and food contamination reported in study by (Haley *et al*., 2010), reported that the *Shigella* was the cause of food borne diseases known as Shigellosis and commonly contaminants a potato salad and brought about a big diarrhoea outbreak. This context justifies the relation between high prevalence of *Shigella* from stool of children of under five years to food contamination in study area which contributed by poor food safety practiced may be discussed under the following aspects.

In food safety point of view, a food microbial contamination risk is a public health challenge in the study area. Food vending practices contribute highly to transmission of enteric pathogens, as many food vendors have no or little knowledge regarding to food hygiene and sanitation. This is because there are paucity of knowledge on food hygiene as well as the ways of transmission of the food borne pathogens among consumers and food vendors'. This is supported by study (Curtis *et al.,* 2010) indicated that there is an association between food vending practices and transmission of enteric bacterial pathogens.

In the study area, food hygiene is still public health problem, since food vending is carried out in unofficial areas with poor hygiene including outside schools, markets

and bus stations, more often the food is left open without being covered in which becomes easily reached by flies which play role as mechanical vectors for pathogens transmission.

It should be born in mind that the consumers of those street vended food come from all levels of society including children. Self determination on selection of what is right is low in most children, therefore they have to buy and consume food, whatever they see is right to them regardless that the food complies with proper food hygienic principles or not, including good food handling and hand washing which are among the risk factors for transmission of pathogens and increase the chance for children to be infected with enteric pathogenic bacteria. The study in Indonesia (Rina *et al*., 2013), Dhaka, Manna *et al*., 2011), (WHO 2007) illustrated the association between poor hygienic practice especial food handling behavior, feeding practices in increasing risk of having diarrhoea, up to 70% of diarrhoea episode are actually caused by water and food contamination with pathogens (Motarjemi *et al*., 1993).

At the household level, many parents on the other hand, have poor knowledge on hygiene and sanitation. The finding of this study illustrated that, hand washing practices by mother, after defecation and before preparation of food is frequently neglected or ignored as well as monitoring of correct hand washing behavior yet is a challenge to many people. Probably dirty hands of mother contaminated by feaces during preparation of food may transmit pathogens to the children food during preparation which leads to the infection associated with diarrhoea. Our finding supported by other studies reported the importance of hand washing by mother in

reducing the occurrence of childhood diarrhoea (Mirza *et al*., 1997, Harder *et al*., 2010). Study in Ethiopia (Bezatu *et al*., 2013) showed significant positive association between the availability of hand washing facility with reduction of childhood diarrhoea.

In addition, ignorance, local taboos and cultural practices among people may hamper the transmission of pathogens associated with diarrhoea in the study area. In Zanzibar society, the relationship between diarrhoea and food contamination is not understood by many people. Some have negative attitude and misconception to diseases such as diarrhoea. For example, some parents believe that the feaces of children are no dirty; some believe that diarrhoea is a sign of early teeth development, while some believe that every diarrhoea is the result of unclean milk and blame their own milk. In general, the study indicates that the hygienic condition in the study area was not good. This might be the cause of the observed high prevalence of *Shigella* bacteria and shigellosis incidence in study area. This notifies the need for public health concerned authorities in the study area to improve efforts on their strategies of presentation and control of infectious diseases through hygienic education so as to create awareness about the ways of pathogens transmission.

5.3 Spatial Prevalence of Pathogenic Enteric Bacteria Isolated In Zanzibar

In this study, Urban, North and South districts were involved. The spatial prevalence of the enteric bacteria between the three districts were investigated and found to differ from each other. At least one isolated pathogenic enteric bacteria were found to be isolated in each district. The South and North districts are almost rural areas.

Therefore, the prevalence of enteric bacteria in rural areas and urban area was compared. High prevalence of enteric bacteria was observed in urban area compared to rural areas. The results of the present study are similar to a study by Obi *et al.* (1997) in Nigeria who reported high prevalence of enteric bacteria in urban areas. However, the results concur with the study conducted in Bangladesh by Sumon *et al.* (2013) which reported high prevalence of pathogenic enteric bacteria in rural area.

The study is also consistent with a similar study made in Kenya (Henry *et al*., 2011) and (Brooks *et al*., 2013) who reported highest prevalence of *Shigella* in urban areas. Many urban areas have undergone rapid urbanization, leading to high population density coupled with inadequate sanitary facilities, and unregulated water connections. These may have contributed to the high incidence of Shigellosis in urban areas (Grump *et al*., 2013).

Nonetheless, the high prevalence of pathogenic enteric bacteria in urban area might be attributed to poor infrastructure and distinct geographical variation with diverse socio - economic and cultural factors such as general sanitation practices (Sumon *et al*., 2013). In addition poor urban setting and sanitation, , improper ways of disposal of child faeces and domestic waste, may result to increased flies population which are the mechanical vectors for the pathogens (Bhatacharya *et al*., 2003). In general, a study conducted by Warrouw *et al*., (2002) in Indonesia confirmed that there is relationship between poor urban setting and increased risk of diarrhoea to children. In addition Oseil *et al*., (2008) reported that high prevalence of pathogenic enteric bacteria in urban area is partly due to the uncontrolled urbanization and overcrowding.

In Zanzibar as in other developing countries, rapid urbanization and high rapid population explosion in urban areas; as well as poor urban setting is a problem. The presence of poor housing settlements leads to inadequate spaces for disposal of child wastes. People have to dispose child wastes in open drainage spaces and channels, which all terminate into water sources, include sea and rivers. This leads to contamination of water sources and increases the chance for transmission of pathogenic enteric bacteria to children when coming into contact with such water. Moreover, the outdated waste water sewage systems and frequent blockage of pumps, exarbates the problem. Definitely present study could report that, poor environmental hygiene in urban area is a problem compared to rural area and this is the possible reason to explain the high prevalence of enteric pathogenic bacteria to children less than five years living in urban areas compared to children living in rural areas.

The finding of this study revealed high prevalence of pathogenic enteric bacteria in Southern district compared to Northern district. The explanation to this is probably due to geographical variation factor where the Southern district said to be the driest area in Zanzibar and experiences frequent drought period throughout a year. This affects accessibility of clean drinking water. The people forced to use alternative sources of water, which is not safe and there increasing the possibility of infection of enteric pathogenic bacteria to children less than five years. On the other hand, in the Southern district, many people are engaged in farming practices including animals keeping; people use domestic animals wastes as manures for agricultural activities. Probably such frequent contact with animal manure facilitates pathogens

contamination to parents which may be transmitted to their children. The spatial difference in prevalence of diarrhoea explained in difference studies in developing countries examples in Congo (Mock N.B *et al*., 1993) in Kenya (UN-Habitat, 2005) found that urban residence showed highly association with diarrhoea occurrence in urban area than rural area.

5.4 Prevalence by Season of Pathogenic Enteric Bacteria Associated with Children Diarrhoea with Less than Five Years in Zanzibar

Seasonal cycles of infectious diseases have been variously attributed to changes in atmospheric conditions, the prevalence or virulence of the pathogen, or the behavior of the host organisms. An understanding of the seasonal variation of enteric pathogens would contribute greatly in focusing in healthcare initiatives in a climate of limited resources to a cost- reduction in disease morbidity and mortality which is why it has attracted considerable attention to healthcare researchers around the world with several studies having been conducted in both the developing and the developed countries (Alam *et al*., 2003).

The present study in Zanzibar revealed highest prevalence of enteric pathogenic bacteria observed in rainy season than dry season. These results were similar to the other studies in developing countries, in Mozambique Inacio *et al*. (2007) reported high frequency of isolation of enteric bacteria rainy season. Also a study in Ifakara by Gascon *et al*. (2000) reported high prevalence of enteric bacteria in rainy season In the present study the variation of prevalence of four pathogenic bacteria between rainy and dry season was statistically insignificant ($p > 0.05$). During rainfall, humidity and moderate temperature provide a good condition for growth of bacteria

and that is easy to contaminate food (Adkin *et al.*, 1987) and (Jensen *et al*., 2004). Recent studies have effectively demonstrated that variables such as rainfall and temperature have influence on the prevalence of cholera and other diarrhoea diseases (Koelle *et al*., 2005).

The climate has a greater impact on environmental conditions. Zanzibar experiences heavy rainfall with floods usually between September to December. Poor infrastructure and poor house setting in urban area result into blockage of sewage systems; this leads the rain water to spread to residential areas. Poor house setting associated with poor latrine system during flooding become fully covered with water this increases the possibility of water borne diarrheoa.

Proximity of residential buildings to coastal water has demonstrated to influence cholera and other diarrhoea diseases during rainfall (Borroto *et al*, 2000). In Zanzibar rain fall pulse can flush feacal materials from terrestrial into water ways t such as sewages systems and pumps. The contaminated water with feacal materials is discarded into sea water and estuaries where most of the sewage terminates. This coastal water is Children likes to swim and fish in the waters thus becoming at risk for transmission of enteric pathogens such as *Vibrio parahaemolyticus*.

Sea foods including sea shells and prawns might be contaminated thus increasing the possibility of infection with enteric bacteria.

5.5 Prevalence by Age of Enteric Pathogenic Bacteria Isolated According to Age of Children Isolated in Zanzibar

(This study) reports high prevalence of pathogenic enteric bacteria in children under age from 0-60 months. The prevalence by age of pathogenic enteric bacteria were

distributed as 0-6(4.3%), 7-12 (22.3%), 13-24 (36.44%, 25-36 (19%), 37-48 (12.8) and 49-60(4.5%) respectively.

At least one bacteria was isolated in each group except at the age group 0-6 months where *Salmonella* and *Vibrio parahaemolyticus* were not isolated. The failure of isolation is probably due to its source where salmonella and *Vibrio parahaemolyticus* were found, *Salmonella* is among the major food borne pathogens and transmitted to human through consumption of contaminated water or food.

Vibrio parahaemolyticus is commonly found in sea water and sea products such as fish and sea shell are the main host. Definitely the age of 0-6 months the children have not yet started eating food and drinks since their major diet is breast feeding, therefore the possibility of being infected with bacteria through food consumption is low.

The prevalence by age of pathogenic enteric bacteria that cause diarrhoea varies with age and the highest prevalence was observed in age group 13-24 months at 36.4% and lowest prevalence was observed in 49-60 age groups at 4.5%. The finding of this study does not concur with the report of Mateel *et al*. (2011) who reported the overall prevalence of enteric pathogenic bacteria in age group 7-12 months at 40%, 13-24 months at 16.7% in Dar es Salaam and stated that among the 0-5 years old children the prevalence diarrhoea due to enteric bacteria was at 4%, and that 0-6 years area higher risk of having diarrhoea.

This finding is in agreement with the report from Saudi Arabia which reported high prevalence of enteric bacteria associated with diarrhoea at 1-2 years. Also it is in

agreement with the results of (CSB, 2003) that reported the highest prevalence at ages of 12-23 months at about 14.8% and the lowest prevalence at 6-11 months. The present study also concurs with studies in study in Bangladesh (Piechulek *et al*., 2003), Vietnam (Takanesh *et al*., 2009). Also a study from Kenya by Onyango *et al*., (2010) reported prevalence of 16.7% at the ages of 13-24 months. The WHO (2011) reports that Salmonella is the predominant pathogenic enteric bacteria isolated in 13-24 months age. This observation from WHO is in agreement with the results of the present study.

Protection against diarrhoea at the youngest age group may be conferred by several mechanisms such as maternal antibodies against enteric pathogens and current breastfeeding. The lower prevalence of diarrhoea in the oldest age group may be due to acquired natural immunity. The lower age the study observed low prevalence of enteric bacteria; this could be due to maternal antibodies in which an infant receives against enteric bacteria through breast feeding. In our study it was observed that there is significant association between poor immunity of children and diarrhoea, and many respondents reported lack of vaccination to their children. High reported prevalence of enteric bacteria in children between the ages 13-24 in the present study is partly due to the fact that that many people lack awareness of the importance of vaccines provision to their children and also some have negative attitude against providing vaccines. From this reason they don't engage their children in any vaccination services. Definitely, lack of vaccination to young children results to immune deficiency. However, there are deliberate efforts taken by the Ministry of Health through its Unity of Vaccination through mass. Contaminated weaning food

has been suggested as a major contributor to diarrhoea in low-income settings as up to 70% of diarrhoea episodes are actually caused by water and food contaminated with pathogens (Motarjeni et al., 19930, although observational studies gave inconclusive results (Lanata *et al*., 2003). A study in Gambia failed to document an association between water or weaning food contamination and higher rates of diarrhoeal morbidity (Hamer *et al*., 2001). Two recent studies found an increased risk of diarrhoea associated with the consumption of maize-based weaning foods (Mock et al 1993). However, in one of these studies, this association was only significant in children living in rural communities (Mock *et al*., 1993) Therefore, the association between contaminated weaning foods and diarrhoeal diseases in young children living in urban settings of developing countries remains lacking (Hamer *et al*., 2001).

Transmission of Enterotoxigenic *Escherichia coli* (ETEC) is known to be specifically associated with contaminated weaning foods among younger children in developing countries (Rao *et al*., 2003, Blak et al 1982), and was considered to be responsible for the diarrhoea-induced weight faltering (Motarjeni et al., 1993). This finding may be explained by the fact that weaning foods for young children prepared under in hygienic conditions are frequently contaminated with pathogens and are an important risk factor of diarrhoea transmission (Motarjeni *et al*., 1993). The increased risk of having diarrhoea in children with age less than five years whose mothers had poor food hygiene practices in our study was observed. Direct association between food hygiene practices and diarrhoea in children have been suggested in several epidemiological studies in developing countries, such as Vietnam (Takanashi *et al*., 2009).

In the present study the hygienic practices were evaluated in terms of hand wash practice, food preparation and washing hand after defecation of a child. Several food hygiene practices were assessed in our study such as mother and child's hand washing practice, hand wash during food preparation and after helping children defecation and feeding habits. The present study was able to demonstrate an association between the contamination of weaning foods and diarrhoea, however many respondents reported exclusive breastfeeding of their children.

In Zanzibar exclusive breastfeeding is always very frequently practiced. However, water and other foods are often introduced to young infants at a very early age. Consequently giving infant contaminated food may increase the risk of diarrhoea diseases even during the very early month of life. During weaning period, mothers take direct responsibility for feeding their infants and are given prepared food. Many women prepare large amount of infants' food to save fuels which is cooked at high temperature so as to save time. After food has been prepared, it is stored and cooled for later use. There is a tendency for most mothers to add cold water or cold milk in the process that increases the risk for pathogens transmission. The poor food handling after it has been prepared may contaminate the food and hence increasing the risk of infection.

Moreover, observed increase of the prevalence of enteric diarrhoea at the age between 13-24 on the other hand, is due to the fact that, children at the age between 12-24 months start to eat themselves various kinds of food items without hygienic precaution of washing hands and having habits of washing fresh foods including fruits and vegetables.

5.6 Antibiotic Sensitivity Test of Isolated Pathogenic Bacteria

Multidrug resistance in bacteria pathogens is now a common phenomenon in developing countries. This circumstance is most likely related to the frequent use of over counter drugs without proper medical supervision (Periska *et al.,* 2003). Because of limited budget we just used seven kinds of antibiotics to test the resistance pattern of enteric pathogenic bacteria strains isolated from diarrhoeal children in our study area.

The present study found that all four isolates showed some extent of resistance to all drugs tested. All isolates showed high level of resistance to Ampicilline, Erythromycin, Tetracycline and trimethoprim/ sulfamethoxazole respectively (Figure 4.6(e). This finding is in agreement with the report of other studies in Tanzania. Moyo *et al*., (2011), reported high level of resistance of bacteria to Erythromycin (62.4%), Ampicillin 54.2%) Tetracycline (78%). The finding is also consistent with report of study in Kenya by Willie *et al*., (2012) reported high level of resistance to Ampicillin, Sulfamethoxazole/Trimethoprin, Tetracycline and Erythromycin. A Study in Tehran by Fereshteh *et al*. (2008) reported high level of resistance to Ampicillin (95%) and Tetracycline (91.7%). Kumarel *et al*., (2014) reported high level of resistance to Ampicillin at 92.8%, Trimethoprim/ Sulfamethoxazole at 76.4%. Mshana *et al*., (2013) reported high resistance to Ampicillin at 86%, Tetracycline at 74% and Erythromycin at 83% respectively. Most of the bacteria showed high susceptibility to Gentamycin, Ciproflaxin, and Chloromphenicol.

The finding of this study showed that *Shigella* was more resistant to Tetracycline in at 74% Erythromycin at 60%, Ampicilline at 67% and Trimethoprim/

Sulfamethoxazole at 71%, and low resistant to Gentamtamycine at 12%, Ciproflaxine at 38% and Chloromphenicol at 56% (Figure 4.2(b). This finding is in agreement with study in Vietnam (Nguyen *et al*., 2005) who reported high level of resistance of *Shigella* to Ampicillin at 69.16%, Tetracycline at 64.49%, and Trimethoprim/ Sulfamethoxazole at 67% and highly susceptibility level to Ciproflaxin at 95.5% and Chloromphenicol at 67.41%. Also this is in agreement with (Inacio *et al.*, 2009) who reported higher resistance to Trimethoprim/ Sulfamethoxazole at 84%, tetracycline at 66%. Beletschacheo, 2010 reported resistance rate to Ampicillin at 74%, Trimethoprim/ Sulfamethoxazole at 56% and high level of susceptility to Gentamycin at 15%.

Diarrheic *E.coli* showed high resistance level to Ampicillin (50%), Erythromycin (54.5%), Tetracycline (77.3%) and Trimethoprim/ Sulfamethoxazole (68%) (Figure 4.6(c). This is in agreement with a study by Moyo *et al*., (2011) in Dar es Salaam that reported high level of resistance to Erythromycin at 90.6%, tetracycline (71.4%), Ampicillin (96.9%) and Sulfamethoxazole trimethoprime at 90.6%.

Vibrio parahaemolyticus were more resistant to Ampicillin (68%), Erythromycin 88%, Tetracycline (52%) and Trimethoprim/ Sulfamethoxazole (53%). Similar results have been reported by (Lim *et al*., 2013; Periska *et al*., 2003). All isolated bacteria showed high level of resistance to more than two antibiotics. Such multi drugs resistance level observed in our finding to isolated bacteria associated with children diarrhoea in Zanzibar shows the trend of antibiotic resistance as reported in other developing countries. High level of resistance to Ampicillin, Erythromycin,

Trimethoprim/ Sulfamethoxazole and Tetracycline respectively, is due to its overuse and misuse because they are ready available at the counter (WHO, 2011). In addition, high level of resistance of bacteria to Antimicrobials is due to the gene sharing between enteric bacteria for antimicrobial resistance and continuous selective pressure applied by the over the counter availability of the agents (Pass *et al*., 2004).

In Zanzibar such high observed antibiotic resistance of pathogenic enteric bacteria associated with diarrhoea under five years age, is associated with increasing use of antibiotics by a wide range of health care providers and increasing resistance, occurring by indiscriminate use of antibiotics in human and animals. Antibiotics such as sulfamethoxazole/Trimethoprim and tetracycline have been commonly used to treat other kinds of infections in human. Example sulfamethoxazole is used for the treatment of cold, flue and fever. On the other hand, increasing misuse of antibiotics in poultry sectors by unskilled persons, results in accumulation of burden of antibiotics residues meat, milk and their products in which over and prolonged consumption of those products by human, may bring the selective pressure to microorganism and cause them to develop defense mechanism against effect of drugs hence becoming resistant strains of bacteria.

According to the Ministry of Health in Zanzibar the standard treatment guide line instructs the Trimethoprim/ Sulfamethoxazole, Erythromycin as the first - line drugs recommended antibiotics to treat diarrhoea to under five years old children. These drugs which are currently used to treat diarrhoea are commonly available to every drug dealer store and local pharmacies and are cheaply sold.

This finding illustrates the need for the Sulfamethoxazole/Trimethoprim and Erythromycin to be removed in the treatment of diarrhoea to children under five years old and be replaced by other antibiotics including Gentamycin, Chloromphenicol, and Ciproflaxin which showed high level of susceptibility to isolated bacteria. A call to the Ministry of Heath in Zanzibar to regulate the use of antimicrobial may be necessary and also the Government should encourage the development of new vaccines to help reduce the incidence of diarrhoea disease to under five years old children in Zanzibar. Moreover, this finding confirms the need for the Ministry of Health of Zanzibar to have a long term surveillance program which is essential in identifying the changes in the spectrum of Antimicrobial pattern of bacteria. This will provide appropriate control measures for Antimicrobial resistance pathogens in Zanzibar.

5.7 Risk Factors Associated with Diarrhoea in Children Less than Five Years Age in Zanzibar

The risk factors for diarrhoea vary from one country to another. The study identified some risk factors associated with diarrhoea among children less than five years old in Zanzibar and the possible risk factors investigated were as follows:

5.7.1 Association between Mother Education and Diarrhoea

Mother with education showed high percentage of their children becoming diarrheic than those who had no education. The association is not significant at ($p>0.05$) $p=0.1$. Mother education is not considered as among the factors associated with diarrhoea in less than five years old children. In this study mother education has no significant effect in prevalence of bacterial diarrhoea to under five years since mother with

education from primary, secondary and tertiary their children were in more risk suffer with diarrhoea compare to those who had no education. However study in Indonesia (Nida R. 2007), In Kenya (Onyango *et al*., 2010), In Eritrea (Woldemicael G.2011) showed positive association between mother education and prevalence of bacterial diarrhoea in children where mother had high education level found low prevalence of bacterial diarrhoea to their children than those with no education. Protective effect of mother education on infant diarrhoea varies according to social social-economic where mother live (Molina D.1994). This explained that in study area risk having bacterial is common to both illiterate and literate persons and probable illustrate lack of awareness to knowledge regard to diarrhoea among Zanzibaris. Calling to Ministry of Health and other health stakeholders to keep deliberate effort in public health education provision to public.

5.7.2 Association between Water Access and Diarrhoea

According to WHO (2000), worldwide around 1.1 billion people lack access to pure and safe drinking water and 2.4 billion have no basic sanitation. In Zanzibar however, there is access to tap water in many areas of the Municipality yet the problem of urbanization is currently a challenge to the nation. This leads most of the people to build their residential buildings near the water sources which may result in contamination of the water sources and lead to infections. This study revealed that there is strong association with diarrhoea occurrence among children and source of drinking water at ($p=0.05$). In addition many people have wrong perception about prevention of diarrhoea and have no tendency of boiling drinking water. A household, which boils drinking water and have their own source of drinking water,

had fewer children with diarrhoea than a household with sharing or use public facility drinking water source. Our finding is consistent with study in Burundi (Cairncross *et al*., 2010), that boiling water before consumption was able to reduce diarrhoea prevalence.

5.7.3 Association between Disposal of Child Waste and Diarrhoea

Household disposes the child waste away in open space had children with high incidence of diarrhoea than a mother who buries the waste or put them in the latrine. The disposal of child waste in open surroundings has no association with diarrhoea ($p>0.05$). The incidence of diarrhoea always increases at the household which uses open liquid waste draining or no drainage. This means that the chain of diarrhoea transmission is water borne and flies as another factor has little effect.This finding is consistent with study in Ethiopia (Bezatu *et al*., 2013) and in Indonesia (Rina *et al*., 2013), reported direct association between open damping of child waste facilitate the transmission of bacterial diarrhoea to under five years.

5.7.4 Association between Washing Handing and Diarrhoea

This finding showed high association between promoting hand washing behavior and reduction of diarrhoea incidence. There is a noticeable decrease in the prevalence of diarrhoea if mother practiced regularly hand washing action. Washing hands after helping children regularly ($p=0.02$) is significantly associated with diarrhoea prevalence.

Mother washing hands before preparing their food ($p=0.035$) and mother washing hand after going to the toilet ($p=0.044$) are significantly associated with diarrhoea.

This result illustrates that most of the mothers recruited in this study do not realize that they have no awareness to personal hygiene and sanitation. The finding also shows that among the children whose mothers had little or no washing related practice are in high risk for children to have diarrhoea than those whose mothers paid attention to washing their hands after going to the toilet, after helping child defecation, and before preparing food. Direct positive association between food hygienic practices by mothers and prevalence of bacterial diarrhoea in children has been reported several studies in developing countries e.g. Guinea Bissau (Molbak 2000), India (Karim *et al*., 2001),Vietnum(Takanashi *et al*.,2009) and Senegal(Mannan *et al*., 2011) association between was handing practices and diarrhoea reported in various studies(Molbak 2000,Karim *et al*.,2001) Washing hands is important barrier to transmission and has been cited as being one of the most cost- effective public health intervention (VanDerslice *et al*., 1994).

5.8 Factors Associated with Antibiotics Resistance to Pathogenic Enteric Bacteria

In Zanzibar, there is an increasing use of antibiotics by a wide range of health care providers and increasing resistance, occurring by indiscriminate use of antibiotics in human and animals. There are many factors associated with antibiotic resistance among these are lack of awareness and knowledge to antibiotic, misuse of antibiotics by the public, antibiotic, prescription of antibiotics by physicians, poor quality of antibiotics and economic factors (Murray *et al*., 1999). The study investigated some of those factors by interviewing the mothers whose children attended clinic to seek medication for diarrhoea at Mnazi Mmoja Hospital which is discussed below.

5.8.1 Association between Using Drugs before Hospitalization and Antibiotics Resistance

Self treatment and over use of antibiotics in community caused by the people buying antibiotics after self diagnosis or diagnosed by poorly trained or untrained health care providers are common and uncontrolled behavior among Zanzibaris. People usually use antibiotics without a doctor's prescription once they feel symptoms of sickness. Sometimes they are offered poorly stored left antibiotics to treat in diarrhoeal infection, from their neighbors. Prescription of right antibiotics relies on knowledge of symptoms of infection; since most of the people lack such knowledge and therefore self medication to community leads to incorrect use of antibiotics which increase selection pressure to bacterial strains hence encourage resistance to antimicrobial agents. This finding is similar to other study in developing countries reported that antibiotics are readily available on demand from hospital, pharmacies, patent medicine stalls and hawkers (Goel *et al*., 1998) In rural Bangladesh, for example, 92% of drugs consumed for 1 month by more than 2,000 study participants came from local pharmacies; but only 8% of them had been prescribed by physicians (Hossain *et al*., 1984).

5.8.2 Association between Mother Awareness and Antibiotic Resistance

In developing countries, antibiotics can be bought without prescription, even when the practice is not legal (Bojalil *et al.,* 1994). In Zanzibar, community - based antibiotics resistance surveillance data and antibiotics resistance trend among pathogenic bacteria are absent. As well, there is no national surveillance program for antibiotic resistance done by concerned authority. Antibiotics circulation and

supplying, controlling, among unauthorized drugs dealers yet is a challenge in Zanzibar. The drugs, including antibiotics, are sold by vendors, patent medicine drugstore and local pharmacies. Many people purchase those drugs in the sense that they are cheap and safe. This happens due to the fact that, the antibiotic resistance has not yet been given proper attention by the ministry itself, physicians and health workers. Definitely these results into lack of knowledge among local people hence lack of awareness of antibiotics resistance. Worthily there are no strategies and measures taken by the concerned authorities to address the situation by provision of education to the community, and therefore there are huge limitations of information among Zanzibaris. In that case the situation promotes the behavior of misuse of antibiotics by the community which facilitates increasing of antibiotics resistance to bacteria in the study area.

5.8.3 Association Between Low Dose Prescription Due to Drugs Price and Antibiotics Resistance

Lack of resources hampers implementation of most strategies against antibiotic resistance. Statistics from the World Bank show that developing countries spent 41 USA dollars per person on health in 1990 compared with 1500 dollars per person spent by the industrialized countries (http://www.worldbank.gov/data/).

Since when the government privatized the drugs sector, it opened the door for drugs sellers to import the drugs and now most important drugs are not available in public hospitals. The people have to purchase prescribed drugs from private pharmacies which are expensive to some extent.

In Zanzibar antibiotics are readily available on demand in local pharmacies and hospitals. Most of the quality essential drugs and antibiotics are expensive in such a way that many low income population cannot afford to purchase them and instead they end up by purchasing low quality and counterfeit antibiotics with low active ingredients for treatment of infection. This poor quality and counterfeit antibiotics contain low inhibitory concentration in vivo to inhibit growth of bacteria, which probably increases the selection pressure for bacterial strains and become resistant.

Moreover, to cut costs for purchasing antibiotics drugs, many people purchased incomplete antibiotics dose because they did not have enough money and instead just purchased part of the dose to reduce or treat suspected bacterial infection. This scenario hampered the resistance of strains of bacteria to antibiotic in the study area ($p<0.05$).

5.9 Conclusion

From the present study it can be concluded that the *Shigella*, *salmonella*, Diarrheic *E.coli* and *Vibrio parahaemolyticus* respectively were common types of pathogenic enteric bacteria. *Shigella* was the most prevalent enteric bacterial type of bacteria associated with diarrhoea in the less than five years old children in Zanzibar. Spatial prevalence of pathogenic enteric bacteria was higher in urban district compare to northern and southern districts. Seasonal prevalence of pathogenic enteric bacteria was higher in rainy season compare to dry season in Zanzibar. Moreover, the prevalence by age group was higher to the children in age group 13-24 months among children less than five years in Zanzibar.

Antibiotic resistance has become a public problem in Zanzibar. There is high rate of resistance of enteric bacteria pathogen isolated from diarrhoea patients that provide resistance to antibiotics and mono - resistance to antibiotic is a rare issue in Zanzibar. Isolated enteric bacteria were more resistance to Sulfamethoxazole/ Trimethoprim, Erythromycin, tetracycline and Ampicillin respectively and therefore should not be used as they seemed not to be effective drugs for the treatment of diarrhoea to under five years children and from such case they should not be used any more for the treatment of diarrhoea. All pathogenic enteric bacteria showed multi-resistance to drugs used in treatment of diarrhoea. There were low rate to resistance to Ciproflaxin, chloramphenicol, gentamycin.

Poor environmental and personal hygienic practices as well as poor immunity to children were important factors associated with diarrhoea in less than five years children in Zanzibar. Lack of awareness to antibiotic resistance, self-prescription of antibiotics before hospitalization and low dose utilization due to high price of drugs were associated factors for antibiotic resistance in Zanzibar. The results from this study could have implication to enhancement of measures and strategies in controlling diarrhoea and provide alertness to public in antibiotic resistance to bacteria.

5.10 Recommendations

The hygienic condition and sanitation in the community should be improved particularly in areas of poor urban settings and in the rainy season. Safe disposal should be enhanced by encouraging the population on burying the child waste. The

Ministry of health through its Unity of health education in association with other stakeholders should promote health education campaigns to raise awareness about food hygiene and risk factors for diarrhoea. Encourage wash handing practices with soap before feeding children or after going to the toilet. Good urban planning is necessary by taking into account the proper sewage disposal. Municipality and rural authorities both must have specific intervention both in rural and urban areas to control diarrhoea as well as street food vending in open spaces and in school compounds should be controlled and monitored. Strong effort should be taken to reduce diarrhoea in children under five years old as it is still needed in Zanzibar at policy and program levels, because diarrhoea is among the leading causes of morbidity and mortality in children. The community leadership (sheha) should encourage accessibility of lavatory facilities and promote its use to community.

The Zanzibar Food and Drugs Board and Medical Store Department should provide awareness to antibiotic resistance through mass media, including local radio, local newspapers, and TV programs. Antibiotic resistance surveillance through isolation and identification pathogens by laboratory practioners and physicians should emphasize diagnosis of enteric bacteria through stool culturing and antibiotics test before drugs prescription to patients. Health management team who is responsible to manage health of particular district in Zanzibar, should emphasis antibiotics resistance tests by build capacity of laboratories of district hospitals and as well as to encourage and ensure the proper and rational use of antibiotics to community and health care providers. The antibiotics for treatment of diarrhoea should be given free of charge to children less than five years of age and over counter sales of antibiotics

should be stopped or controlled. Moreover, further study should be conducted in investigation of prevalence of other enteric pathogens associated with diarrhoea including virus, protozoa and worms to children less than five years of age Zanzibar. Ministry of Health in Zanzibar should review its Standard Treatment Guideline, Erythromycine, and Sulfamethoxazole/Trimethoprime should be stopped to treat diarrhoea in Zanzibar.

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APPENDICES

Appendix I: Consensus Form

Title: Determination of the prevalence of enteric bacteria associated with diarrhoea in children less than five years of age; and their resistance to antibiotics in Unguja Island-Zanzibar.

ID-NO

Consent to participate in this study

Greeting! My name is Muhiddin H. Omar I am postgraduate student in Open University of Tanzania conducting study with the objective of determination of the prevalence of enteric bacteria associated with diarrhoea in children less than five years of age; and their resistance to antibiotics in Unguja Island-Zanzibar. We plan to talk with 319 parents or guardians whose children suffering with diarrhoea. We are asking you to take part in this study because your children suffering with diarrhoea now and you have been selected by chance.

We want you to understand the purpose of this study and your role so you may decide if you want to join. If you join, we will ask you to sign this paper (or if you cannot read/ write, make your mark in front of a witness). Please ask us to explain any words or information that you may not understand.

Information about the research

If you participate we will interview you. We will ask you about your personnel hygienic practices include hands washing also children immunity and drugs use. The interview will last about 20 min. After the interview, we will collect stool sample of

your children. Our interview will be private and we will do our best to protect your privacy and study records. However, it is possible that others will learn that you joined the research.

The interview questions may cause you to feel some anxiety. You may refuse to answer any question. You may end the interview at any time. Possible benefits this study has no direct benefits but the results of this study more useful to ministry of health to help them in their effort of controlling diarrhoea to under five years children as well to shape the proper treatment plan on diarrhoea. If you decide not to be in the research. You are free to decide if you want to take part in this research or not.

Confidentiality

We will do our best to protect information about you and your part in this research. We will interview you in a private place. We will not write your name on the interview form. We will use your form number to link your interview answers to our results of laboratory analysis. You will not be named in any reports. Only the study staff and investigators will know your answers to the questions.

Compensation

You will not receive any money by joining this study.

Leaving the research study

You may leave the research at any time. If you leave, it will not change the health care you receive here. If you choose to take part, you can change your mind at any time and withdraw. If so, please tell the research interviewer why you wish to leave.

Your rights as a participant: This research has been reviewed and approved by the Open University of Tanzania, postgraduate and research committee. Also has been approved by Zanzibar Medical Research Committee.

Whom to contact

If you ever have questions about this study, you should contact the study Principal Investigator Muhiddin H. Omar Open University of Tanzania (OUT) Faculty of Natural science, Dar es Salaam Mobile no. 0777491223.

About your rights as a participant, you may contact/call Professor S.P. Kigadye who is supervisors of this study (Mobile: 0754373756).

Signature:

Do you agree?

Participant Agrees

Participants disagree

I ---------- have read/understood the contents in this form. I agree to participate in this study.

Signature of participant ----------

Signature of witness (if participant cannot read) ----------

Signature of research assistant ----------Date of signed consent ---------- 55

Appendix II: Fomu ya Ridhaa

FOMU YA RIDHAA TOLEO LA KISWAHILI

CHUO KIKUU HURIA CHA TANZANIA, KURUGENZI YA SAYANSI YA MAZINGIRA NA SAYANSI YA MAUMBILE

UTAFITI KUHUSU KIWANGO CHA VIMELEA WANAOISHI KWENYE TUMBO NA WANAOSABABISHA KUHARISHA KWA WATOTO CHINI YA MIAKA MITANO NA UFANISI WA DAWA ZINAZOUA VIMELEA KATIKA KISIWA CHA UNGUJA.

Ridhaa ya kushiriki katika utafiti huu

Salamu! Mimi naitwa Muhiddin H. Omar, mimi ni mwanafunzi wa ngazi ya shahada ya pili katika chuo kikuu huria Tanzania. Ninifanya utafiti kuhusu kiwango cha vimelea wanaoishi kwenye tumbo na wanaosababisha kuharisha kwa watoto chini ya miaka mitano na ufanisi wa dawa zinazoua vimelea katika kisiwa cha unguja-Zanzibar. Nimepanga kufanya mahojiano na wazazi au walezi ambao watoto wao wanasumbuliwa maradhi ya kuharisha kwa sasa na ambao tutawapata kwa njia isiyo ya upendeleo yaani bahati nasibu.

Kwa njia hiyo ya bahati na sibu umechaguliwa kuwa miongoni mwa washiriki wa utafiti hou. Tunakuomba ridhaa yako ya ushiriki katika mahojiano haya. Tungependa uelewe malengo ya utafiti huu na umuhimu wa kushiriki utafiti huu ili uweze kuamua ama kukubali kushiriki au kukataa.Tutakuomba kutia sahihi kwenye fomu hii endapo utakubali kushiriki katika utafiti huu au kama hujui kuandika utaweka alama ya dole gumba mbele ya shahidi.

Maelezo kuhusu utafiti huu

Endapo utakubali kushiriki tutakuuliza maswali yaliyopo katika dodoso hili.Tutakuuliza kuhusu usafi wako na mtoto wako pia, taarifa za chanjo na vile vile matumizi ya dawa kwa ujumla..Baada ya mahojiano tutapenda kuchukua sampli kinyesi cha mtoto wako kwa ajili ya kufanya vipimo vya kimaabara juu kuwepo au kutokuwepo vimelea husika.

USIRI:

Nakuhakikishia kuwa taarifa zote tutakazo chukua hazitawekwa bayana kwa mtu yoyote isipokuwa anaefanya au wanofanya kwenye utafiti. Taarifa yetu itatumia majibu ya vipimo vyetu tu na sivinginevyo.

FAIDA YA UTAFITI HUU:

Hakuna faida ya moja kwa moja utakayoipata kutokana na wewe kushiriki katika utafiti huu, isipokuwa majibu yako ya vipimo vya maabara unaweza kupewa ili kujua kimelea kinachosababisha kuharisha kwa mtoto wako. Pia vilele matokeo ya utafiti huu yataisaidia Wizara ya Afya katika jitihada zake za kudhibiti ugojwa wa kuharisha kwa watoto chini ya miaka mitano na kupanga mikakati ya tiba sanifu ya kuharisha.

MADHARA:

Hakuna wasiwasi wa madhara, yoyote yatokanayo na utafiti wetu. Una uhuru pia wa kukataa baadhi ya maswali pia kukataa kushiriki katika utafiti huu wakati wowote. Ni hiari yako kushiriki katika utafiti huu. Uamuzi wako wa kutokushiriki hautakuwa

na madhara yoyote kwako ya kupata huduma zako unazostahiri. Wakati wowote unaweza kujitoa katika utafiti huu, hata baada ya kutoa ridhaa yako hapo awali. Kwa kujitoa kwako hakuna adhabu yoyote wala hutanyimwa haki yoyote unayostairi kupata katika jamii.

MAWASILIANO

Kama una swali lolote unaweza kuwasiliana na mimi Ndugu, Muhiddin H Omar kwa kutumia anuani ya Chuo Kikuu Huria Cha Tanzania, Dar es Salaam, Namba yangu ya simu ya kiganjani ni 0777491223. Ukiwa na swali lolote kuhusu haki yako ya kushiriki utafiti huu unawezo kumpigia Professor. Gideon S.P. Kigadye kwa simu namba 0754373756 ambaye ni msimamizi wa utafiti huu watakupa msaada.

SAHIHI:

Je utakubali?

Mhusika amekubali Mhusika amekataa

Mimi …….. nimesoma na kuelewa kilichoko katika fomu hii. Nakubali kushiriki katika utafiti huu. Sahihi ya mhusika ……...……

Sahihi ya shahidi (endapo mhusika hajui kusoma) ……

Sahihi ya mtafiti mwandamizi ……...……

Tarehe ya kusainiwa ridhaa …….....

PART A: Health facility information

	Name	**District**	**Date**
CLINIC			

PART B: **Patient's information**

File number			
Address			
Telephone No.			
	QUESTIONS	**CATEGORIES**	**CODING**
1	Age of child	1-6 7-12 13-24 25-36 37-48 49-60	**1** **2** **3** **4** **5** **6**
2	Level of mother education	Primary education Secondary education Tertiary education None	**1** **2** **3** **4**
3	Mother occupation	Farmer/Animals keeper	**1**

		Public employ Private employ	**2** **3**
4	`House hold	Having latrine in the house Having no latrine in the house	**1** **2**
5	Current water source access	Public water access Private water access	**1** **2**
6	Drinking water source	Tape water Boiled water well water	**1** **2**
8	Disposal of child waste	Through away in open space Buried Pour away in open space	**1** **2** **3**
9	Feeding behavior	Children buy food from street vendors Not buy food from street vendors	**1** **2**
10	Child feeding status	Bottle feeding Exclusive breast feeding of children in the first six months	**1** **2**
11	Washing hands by mother after helping children defecation	Wash hands Not wash hands	**1** **2**
12	Washing hands by mother before prepare food	Always Not wash hands	**1** **2**
13	Washing hands by mother after going toilet	Wash hands Not wash hands	**1** **2**

PART C: DIAGNOSTIC RESULTS

POSITIVE RESULT OF BACTERIA	
NEGATIVE RESULT OF BACTERIA	

PART D: ANTIBIOTICS RESISTANCE RISK FACTORS INFORMATION

1	Antibiotic utilization	Take antibiotic before hospitalization Don't	1 2
2	Awareness to antibiotics use	Self treatments when children get sick Go to Hospital when get sick Go direct to pharmacy	1 2 3
3	Price of antibiotics	Price of antibiotics acceptable Price not acceptable	1 2