ANTIBIOGRAM OF DIARRHOEAGENIC ESCHERICHIA COLI AND NUTRITIONAL STATUS OF DIARRHOEIC PATIENTS UNDER FIVE YEARS ATTENDING SELECTED HOSPITALS IN KADUNA STATE, NIGERIA.

BY

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NOVEMBER, 2015.

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DEPARTMENT OF MICROBIOLOGY,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

NOVEMBER, 2015.

DECLARATION

I hereby declare that the work in this dissertation entitled "Antibiogram of diarrhoeagenic*Escherichia coli* and nutritional status of diarrhoeic patientsunder five years attending selected hospitals in Kaduna State, Nigeria" was carried out by me in the Department of Microbiology, Faculty of Sciences, Ahmadu Bello University, Zaria, Nigeria under the supervision of Prof. C.M.Z. Whong and Prof. S.A. Ado.The information derived from the literature has been duly acknowledged in the text and a list of references provided.

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Chioma Rebecca Date

Ahmadu Bello University, Zaria

CERTIFICATION

This dissertation entitled "Antibiogram of diarrhoeagenic*Escherichia coli* and nutritional status of diarrhoeic patients under five years attending selected hospitals in Kaduna State, Nigeria" by Chioma, OJUNTA (Msc/Sci/5615/2011–2012) meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contributions to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to all children who have died of complications arising from diarrhoealinfections and malnutrition.

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ABSTRACT

Escherichia coli infections and poor nutritional status have implications on the growth and development of children under five years, physically, mentally and healthwise with consequences such as diarrhoea, stunting, wasting, underweight and often times leading to death, depending on their severity. This study evaluated the antibiogram of *Escherichia coli* O157 and Verocytotoxigenic*Escherichia coli*(VTEC)and the nutritional status of diarrhoeic children under five years in Kaduna State, Nigeria, using Conventional isolation methods, latex agglutination tests, VTEC-ELISA tests, Chi-square (SPSS Version 19) and WHO Antro (Version 3.2.2). Purposive sampling was used to select 350 children presenting with diarrhoea in six government hospitals within the three senatorial zones of Kaduna State. The results obtained revealed that 76(21.7%) of the 350 stool samples were positive for *E. coli,* 28(36.8%) of which were positive *for E. coli* O157:H7serotype and 1(1.3%) verocytotoxigenic *E. coli* (VTEC) serotype.High susceptibility to ciprofloxacin, chloramphenicol and high resistance to sulphamethoxazole, cefotaxime, amoxicillin, gentamicin and tetracycline by the isolates were observed. The nutritional status assessment showed 34.3, 24.3 and 13.1% of the children were stunted, wasted and underweight, respectivelyand Kaduna South senatorial zone also recorded the highest prevalence of undernutrition, indicating poor nutrition and poor health accumulated overtime. The study concluded that antibiotics have not been very effective in the treatment of *E. coli*-related diarrhoea, with VTEC now emerging in this part of the world, making it a serious public health issue and its severity enhanced by malnutrition, confirming that the relationship between diarrhoea and malnutrition is bidirectional, resulting in severe limitations in the growth and development of the children, some of

which may be irreversible. The study therefore recommends the implementation of programmes geared towards good hygiene, good nutrition and good health.

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CHAPTER ONE

1.0 INTRODUCTION

Diarrhoea can be defined as the occurrence of three or more loose, liquid or watery stools or at least one bloody loose stool in a 24h period(Gondwe*et al*., 2006).Diarrhoea is also defined as the occurrence of loose or watery stools at least three times per day, or more frequently than normal for an individual (WHO,2013). Diarrhoeal diseases of the bowel make up a veritable augean stable of entities. Microbiological agents cause many diarrhoeal diseases; others arise in the setting of malabsorptic disorder and idiopathic inflammatory. An increase in stool mass, stool frequency or stool fluidity is perceived as diarrhoea by most patients (Crawford, 1999). For many individuals, this consists of daily stool production in excess of 250 gm, containing 70 – 95% water. More than 14 litres of fluid may be lost per day in severe cases of diarrhoea (i.e the equivalent of the circulating blood volumes). Diarrhoea is often accompanied by pain, urgency, perianal discomfort, and incontinence. Low-volume, painful, bloody diarrhoea is known as dysentery (Crawford, 1999).

The World Health Organisation defined diarrhoea as the voiding of more than two unformed watery stools in any 24 h period, or any voiding of watery stools if accompanied by fever, abdominal pain and / or vomiting (Crawford, 1999 and Gondwe *et al*., 2006). Diarrhoea accounts for more deaths in childhood than any other disease in the developing world(Crawford, 1999).It has been pointed out that in developing countries; a child of less than seven years of age still has a 50% chance of dying from diarrhoeal diseases (Crawford, 1999; Guerrant *et al*., 2001; Gondwe *et al*., 2006). Infantile diarrhoea remains

one of the leading causes of childhood morbidity and mortality in developing countries, with children in the developing world having an average of 5-6 episodes a year (Crawford, 1999).There are three major forms of diarrhoea namely: acute watery diarrhoea, acute bloody diarrhoea and persistent diarrhoea (Guerrant*et al*., 2001).Diarrhoeal diseaseforms one of the two major killer diseases in children under five years of age in the developing world. *Escherichia coli* is one of the major bacterial causes of diarrhoeal diseases (Abdullahi *et al*., 2010). The bacterium *E. coli* is one of the best and most thoroughly studied free-living organisms. It is also a remarkably diverse species because some *E. coli* strains live as harmless commensals in animal intestines. External contact and subsequent ingestion of bacteria from faecal contamination can cause detrimental health effects (Money *et al*., 2009).

Acute watery diarrhoea is diarrhoea with a high volume of watery stool occurring over a period of less than 14 days. It usually results in severe dehydration if intervention measures are not sought. Acute bloody diarrhoea is diarrhoea manifested by loose or watery stools with the shedding of red blood cells. Persistent diarrhoea is usually associated with loose or watery stools with or without visible blood occurring over a period of 14 days. Diarrhoeal disease caused by microbial agents is principally a food borne and water borne illness. Foodborne and waterborne illnesses are leading global health problems accounting for more morbidity and mortality than tuberculosis and malaria (Besser *et al*., 2003).According to World health organization (WHO) Report, approximately 11 million children under the age offive, die because of *E. coli*-mediated gastroenteritis (WHO, 2005).

Nutrition is a science that concerns itself with the relationship between food and the functioning of the human body and it encompasses the ingestion of food, digestion,

absorption, metabolism and excretion of waste products. Nutrition plays a key role in physical, mental and emotional development of children and much emphasis has been given to provide goodnutrition to growing populations especially in the formative years of life, which is one thousand (1,000) days. Globally, more thanone- third of child deaths are attributable to under nutrition. Malnutrition refers to a case of faulty nutrition which can spread through a broad spectrum, from under nutrition to over nutrition, causing diseases like Obesity, Marasmus, Kwashiorkor, Protein-Energy Malnutrition (PEM), underweight and other chronic diseases (Alim and Jahan, 2012).Diarrhoeal illness isoften attributed to contaminated water (or food) consumptionalthough the percentage specifically due to waterbornepathogens is still unknown (Hunter *et al*., 2003). Transmission of agents that cause diarrhoea are usually by the faecal oral route, which include the ingestion offaecal contaminated water or food, person to person contact and direct contact with infected faeces. Host factorsthat increase susceptibility to diarrhoea include under nutrition, current or recent measles and immune deficiencyor immunosupression (Andu *et al.*, 2002). Among the bacterial pathogens of diarrhoeal diseases, the most commonly implicated in the endemic form of childhooddiarrhoea on a global scale are strains of *Escherichia coli* (Huilan *et al*., 1991; Iruka *et al*., 2003; WHO, 2005).Diarrhoeal diseases and other related gastrointestinal disorders are one of the most important causes of illness and death all over the world, particularly among infants and young children (Elias *et al*., 1999;Clarke, 2001; Sarantuya *et al*., 2004). The major causes of diarrhoeal illness include, among others, limited access to / or poor quality of water, poor food hygiene, and sanitation. The bacterial pathogens usually responsible for diarrhoeal illness include *Escherichia coli, Shigella, Salmonella, Campylobacter, Yersinia, Aeromonas*(Presterl *et al*., 2003).The relationship

between diarrhoea and malnutrition is bidirectional: diarrhoea leads to malnutrition while malnutrition aggravates the course of diarrhoea. Many factors contribute to the detrimental effect of diarrhoea on nutrition. Reduced intake (due to anorexia, vomiting, andwithholding of food), maldigestion, malabsorption, increased nutrient losses, and the effects of the inflammatory response are some of the factors involved in malnutrition (Nel, 2010).

Diarrhoeal disease may cause, precipitate, or exacerbate protein-energy and micronutrient malnutrition through five possible mechanisms: 1) reduced food intake-reduction of food intake during diarrhoea may be due to the child's anorexia, maternal food-withholding behaviour, or both; 2) decreased absorption of nutrients due to structural damage to the intestine, as well as the physical action of increased intestinal movement and reduced fluid transit time, all interact to produce decreased absorption of nutrients; 3) increased catabolic losses-under the influence of the inflammatory process, diarrhoea of infectious origin induces an average daily negative nitrogen balance of 0.9 g/kg/day, as muscle protein is converted to glucose through gluconeogenesis by the liver; this glucose is used as a fuel by tissues to sustain the hypermetabolism associated with fever; 4) nutrient loss from the intestine-in diarrhoea nutrients are lost directly from the intestinal tract; 5) metabolic inefficiency due to micronutrient deficiency-the increased rate of tissue synthesis displayed by children recovering from protein-energy malnutrition may be hampered by a limited supply of nutrients from the body pool, which in turn may not be replenished fast enough by dietary intake (Martinez and Tomkins, 1993).

1.1 Statementof the research problem

Diarrhoeal diseases ranks second as a major cause of morbidity among the modifiable diseases in Nigeria, where, according to the Federal Statistic Bulletin, 300 children die every day from malnutrition and dehydration caused by diarrhoea (Barua, 1981; FMH, 1987).Severe and prolonged episodes of diarrhoea cause malnutrition in individual patients; while malnourished children are more likely to develop complications with diarrhoea. Many studies, addressing the relationship between diarrhoea and malnutrition, have demonstrated a detrimental effect of diarrhoea on nutritional status(Nel, 2010).

The impact of diarrhoeal illness is most felt in sub-saharan Africa where out of the 25million children born each year, 4.3 million are expected to die by the age of 5 years and about 20% of these deaths will be from diarrhoea (Cunliffe *et al*., 1998). Although no specific organism has consistently been identified as a cause of persistent diarrhoea, in certain geographic areas Entero-aggregative *E. coli, Cryptosporidium*, and *Giardia lamblia* have been identified as important causes of persistent diarrhoea and malnutrition(Nweze, 2010).In Nigeria, the aetiology of diarrhoeagenic bacteria and the virulence of various *Escherichia coli* pathotypes have not been well studied as most currently published data are from the southwestern axis of the country (Nweze, 2010). Although in developed countries and in a few developing countries, the mortality rates have declined considerably in recent times due to improvement in general hygiene and advances in health care. The problem still persists in so many other countries where outbreaks of diarrhoeal diseases continue to affect millions of infants and young children (Hart, 1989; Presterl*et al*., 2003).

Illnesses such as irritable bowel syndrome, Crohn's disease and ulcerative colitis cause the patient to exhibit similar symptoms to individuals who have developed gastroenteritis due to infection with a pathogenic organism capable of causing diarrhoeal disease. After initial

diagnosis of an inflammatory bowel disease, there is typically no further investigation if a patient suffers a relapse due to the underlying cause of the symptoms being known. There is however, the potential that the onset of inflammatory bowel disease is triggered by infection with a pathogenic organism such as *Escherichia coli* which results in gastroenteritis. As such this is the primary cause of the symptoms rather than the result of a weakened immune response due to the inflammatory bowel disease which is often considered to be the case (Burke and Axon, 1988).

Similar effects have been seen after a bout of traveller's diarrhoea with patients still suffering the effects of the infection two weeks after initial contraction. In some individuals this long term effect of the traveller's diarrhoea results in the onset of irritable bowel syndrome, again suggesting that these pathogens are the primary cause of such illnesses (Connor, 2005).The occurrence and distribution of bacterial pathogens causing diarrhoea in humans has beenshown in various studies (Reyes *et al*., 2009). Bando *et al*. (2009) reported that a high incidence of infantile diarrhoea is associated with atypical Enteropathogenic *E. coli* (EPEC)

1.2 Justification

Worldwide, diarrhoeal diseases are estimated to cause over 2 million deaths annually (WHO, 2004), accounting for approximately 4.3% of the total number of deaths and as a result ranking diarrhoeal diseases as the 5th highest cause of death globally (UNICEF/WHO, 2011). Deaths due to diarrhoeal diseases are more common in third world countries with a lower socio-economic standing and which has a significant impact on child health and mortality rates (WHO, 2004).Diarrhoea is one of the leading causes of death

among children under five globally. More than one in ten child deaths – about 800,000 each year – are due to diarrhoea. Today, only 44% of children with diarrhoea in low-income countries receive the recommended treatment, and limited data in literature suggests that there has been little progress since 2000 (UNICEF/WHO, 2013).

Acute diarrhoeal disease is a major public health problem throughout the world, with over two million deaths occurring each year, and affecting mostly children under 5 years of age in developing countries (Kosek *et al*., 2003; Bryce *et al*., 2005). This disease is especially common in developing countries with poor hygiene and sanitation, and with limited access to safe drinking water. Underlying conditions such as malnutrition, which increase the risk of contracting diarrhoea, are also common in these countries. These factors may result in a signficant disease burden with economic effects due to direct medical costs, loss of work, lower quality of life and mortality (Bonkongou *et al*., 2010; Bonkongou*et al*., 2013).Bacteria such as *Escherichia coli, Shigella* and *Vibrio cholerae* are all known to cause diarrhoeal diseases and are identifiable through electron microscopy and culturing of stool samples (Gillespie and Bamford, 2007).In the United States and infact, all over the world, few laboratories culture stool specimen routinely for *E. coli* O157: H7, resulting in the actual incidence of infection with this organism being unknown.

The number of outbreaks of *E. coli* O157:H7 infection reported to the Centre for Disease Control and Prevention (CDC) has increased to 30 in 1994 (Boyce *et al*., 1995) and may be more now. This increase may be attributed in part to increased reporting and screening of *E. coli* infection by laboratories.Malnutrition is the syndrome that resultsfrom the interaction between poor diets anddiseases which leads to most of the anthropometricdeficits observed among children inless developed countries (WHO, 1995).

1.3Aim

This study was aimed at determining the antibiogram of diarrhoeagenic *Escherichia coli*in diarrhoeic patients under-five years and their nutritional status.

1.4 Objectives

The specific objectives of this work were to:

1. isolate and characterize*Escherichia coli* O157, Verocytotoxigenic *E. coli*from diarrhoeal stools of children under 5yrs in Kaduna State.
2. determine the antibiotic susceptibility pattern of the *Escherichia coli* isolates.
3. assess the nutritional status of the diarrhoeic children using Z-scores (internationally accepted reference standards).
4. obtain demographic data and risk factors associated with diarrhoea using pre-adopted questionnaire.

1.5 Research questions

1.Is the target organism present in the faeces of the children presenting with diarrhoea?

2. Is the organism isolated pathogenic?

3. Is the target organism susceptible or resistant to the antibiotics to be used in the study?

1.6 Research hypothesis

H_o:*E. coli* is not the causative agent of diarrhoea in children under five years.

H_0: The organism isolated is not pathogenic in children under five years.

H_0: The target organism is not susceptible or resistant to the antibiotics used.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Escherichia coli*

Escherichia coli (or *E. coli*) is the most prevalent infecting organism in the family of gram-negative bacteria known as enterobacteriaceae (Gerald *et al*., 2000). *E. coli* bacteria were discovered in the human colon in 1885 by German bacteriologist Theodor Escherich (Feng *et al*., 2002).*Escherichia coli (E. coli)* were first isolated by Escherich in 1885 from stools of infants with enteritis and itbelongs to the tribe Escherichiae and family Enterobacteriaceae.It is a predominant normal flora of the intestinal tractsof humans and animals. Most of the *E.coli* strains are harmless commensals with a few being pathogenic.

E. coli is adiverse species both phenotypically and genetically and is identified on the basis of _'O', _'H' and _'K' antigens, whichtogether constitutes the serotype. The serological typing is determined by somatic antigens (O:O1-O173) and flagellaantigen (H:H1-H56).The categorization is based on virulence, pathogenicity and serogroups.Some strains of the bacterium are 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 (James, 2000; Gerald *et al*., 2000). More than 700 serotypes of *E. coli* have been identified (Griffin and Tauxe, 1991; Gerald *et al*., 2000).

The bacterium can be grown easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. The "O" and "H" antigens on the bacteria and their flagella distinguish the different serotypes.

Escherichia coli is a Gram-negative, facultatively anaerobic, rod-shapedbacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms) (Singleton, 1999). Most *E. coli*strains are harmless, but some

serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination (Vogt and Dippold, 2005; CDC, 2012).The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2(Bentley and Meganathan, 1982) and preventing colonization of the intestine with pathogenic bacteria (Hudault *et al*.,2001; Reid *et al*., 2001). 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 dysenterae* type 1 (Griffin and Tauxe, 1991). The best-known and also most notorious *E. coli* bacteria that produce Shiga toxin is *E. coli* O157:H7.

2.2 Classification/Taxonomy

*Escherichia coli*t is a non-spore forming short rod.Eosin methylene blue agar is the selective media for the organism. The bacteria is cultivated with an optimum temperature of 37^{o}C (Buxton and Fraser, 1977), however, other strains of *E. coli* can multiply at a temperature of 49^{o}C. *Escherichia coli*is a facultative gram negative bacilli, which ferments lactose.Since 1977, it has beenrecognised that some diarrhoegenic strains of *E. coli* produce toxins that have an irreversible cytopathic effect on cultured Vero cells (Konowalchuk *et al*., 1977). Such verocytotoxigenic *E. coli* (VTEC) have been shown to belong to over 100 different serotypes (Johnson *et al*., 1996; Strockbine *et al*., 1998). They are also described as Shiga toxin-producing *E. coli* (STEC) due to the similaritydemonstrated between verocytotoxins (VT) and Shiga toxins (Stx) of *Shigella dysenteriae* (O'brien and Laveck, 1983.). In the past twodecades, VTEC O157:H7 has increased in importance world-wide as a public health problem. Each strain has a typical gram-negative cell wall containing lipopolysaccharide.*Escherichia coli*encompasses an enormous population of bacteria that

exhibit a very high degree of both genetic and phenotypic diversity. Genome sequencing of a large number of isolates of *E. coli* and related bacteria shows that a taxonomic reclassification would be desirable.

However, this has not been done, largely due to its medical importance (Krieg and Holt, 1984) and *E. coli* remains one of the most diverse bacterial species: only 20% of the genome is common to all strains (Lukjancenko *et al*.,2010).The genera *Escherichia* and *Salmonella* diverged around 102 million years ago, which coincides with the divergence of their hosts: the former being found in mammals and the latter in birds and reptiles (Battistuzzi *et al*., 2004). This was followed by a split of the escherichian ancestor into five species (*E. albertii*, *E. coli*, *E. fergusonii*, *E. hermannii* and *E. vulneris*). The last *E. coli* ancestor split between 20 and 30 million years ago(Lecointre *et al*., 1998).The genus belongs in a group of bacteria informally known as "coliforms", and is a member of the Enterobacteriaceae family ("the enterics") of the Gammaproteobacteria(Brener *et al*., 2005).

2.3 Habitat

Escherichia coli are normal inhabitants of the human gastrointestinal tract and are among the bacterial species most frequently isolated from stool cultures.*E. coli* and other facultative anaerobes constitute about 0.1% of gut flora (Eckburg *et al*., 2005). Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for faecal contamination (Feng *et al*., 2002; Thompson, 2007).There is, however, a growing body of research that has examined

environmentally persistent *E. coli* which can survive for extended periods outside of the host (Ishii and Sadowsky, 2008).

2.4 Cell Structure and Physiology

Escherichia coli cells are typically rod-shaped, and are about 2.0 micrometers (μm) long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm^3(Kubitschek,1990). Optimal growth of *E. coli* occurs at 37 °C but some laboratory strains can multiply at temperatures of up to 49 °C (Fotadar *et al*., 2005).Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids, and the reduction of substrates such as oxygen, nitrate, fumarate, dimethyl sulfoxide and trimethylamine N-oxide (Ingledew and Poole, 1984).

2.5Pathogenesis of *Escherichia coli*

Over 700 antigenic types (serotypes) of*E. coli* are recognized based on O, H, and K antigens. At one time serotyping was important in distinguishing the small number of strains that actually cause disease. Thus, the serotype O157:H7 (O refers to somatic antigen; H refers to flagellar antigen) is uniquely responsible for causing hemolytic uremic syndrome (HUS). Nowadays, particularly for diarrhoeagenic strains (those that cause diarrhoea) pathogenic *E. coli* are classified based on their unique virulence factors and can only be identified by these traits (Todar, 2012).*Escherichia coli* O157:H7 is the VTEC of greatest public health concern in most countries. Its carriage in the intestinal tract of healthy animals, particularly cattle, represents a source of direct and indirect infection to humans. All pathogenic strains of*E. coli* contain genomic regions (islands) loaded with a

suite of virulence genes that encode key traits for adherence/colonization, invasion, secretion of toxic compounds and transport functions, as well as siderophore production (Touchon *et al.*, 2009).

2.5.1Enterotoxigenic *Escherichia coli*

The enterotoxigenic *E. coli* (ETEC) strain produces one or both of two distinct (the heat stable and heat labile) plasma-mediated enterotoxins responsible for diarrhoea. The heat stable enterotoxin binds to a glycoprotein receptor that is coupled to guanylatecyclase which stimulates the production of cyclic guanosine monophosphate (cGMP). This leads to the release of electrolytes and water into the lumen of the small intestine resulting in watery diarrhoea. This is a distinct characteristic of ETEC (Sears and Kaper, 1996). The heat labile enterotoxin has affinity and binds to specific gangliosides on the epithelial cells and activates membrane-bound adenylase monophosphate by the choleratoxin resulting in hypersecretion of electrolytes and water into the lumen of the intestine.

2.5.2Enterohaemorrhagic *Escherichia coli*

The common type of enterohaemorrhagic *E. coli* (EHEC) is *E. coli* O157:H7. The EHEC was first observed as a cause of illness in 1982, and since then, the infections have been reported with increasing frequency (Fitzpatrick, 1999). Prescott *et al.* (2000) reported the presence of attaching effacing region on this strain which causes haemorrhagic colitis with associated abdominal cramps and pains accompanied by bloody diarrhoea. *Escherichia coli* O157:H7 is reported to have been responsible for several outbreaks of haemorrhagic colitis in developed countries especially the United States of America, with a minimum

occurrence of about 200,000 cases of morbidity and 250 cases of mortality (Prescott *et al.*, 2000). Enterohaemorrhagic *E. coli* is due to a cytotoxin that damages vascular endothelial cells and is associated with serogroup O157:H7. It is seldom called verocytotoxin producing *E. coli* because of its toxicity to vero monkey cells in cultures.

Serotype O157:H7 is the prototype of increasing importance and is associated with hemorrhagic colitis, watery diarrhoea, bloody diarrhoea and a potentially fatal condition called hemolytic uremic syndrome (HUS) characterized by acute renal failure (Wilson *et al.*, 1997). The recognition and perception of *E. coli* O157 associated diarrhoea as declining has changed with re-emerging strains (Griffin and Tauxe, 1991). A variety of *E. coli* serotypes have been linked with human illness, of which *E. coli* O157:H7 is reported to be the most important (Aibinu *et al.*, 2007).

2.5.3Enteropathogenic *Escherichia coli*

The enteropathogenic *E. coli* (EPEC) strains attach to the brush border of intestinal cells. The affinity for this site results in cell damage which is specific and causing effacing lesions or attaching effacing lesions. These lesions results in the destruction of the brush border of microvilli adjacent to attaching bacteria. The destruction of the brush border of the microvilli by this pathogen leads to diarrhoea. Furthermore, it has been reported that this strain of *E. coli* is an important aetiology of the causes of diarrhoea in paediatrics especially in developing nations (Nataro and Kaper, 1998). The terminology attaching effacing lesions is solely associated and used to describe the effect of this strain.

2.5.4Enteroinvasive *Escherichia coli*

The pathogenesis of disease caused by enteroinvasive *E. coli* (EIEC) involves cellular invasion and spread that requires specific chromosomal and plasmid-borne virulence genes. The EIEC have the ability to invade the epithelial cells. This strain is associated with the presence of a large plasmid and has the ability to also produce a cytotoxin and enterotoxin. The EIEC causes a broad spectrum of human diseases. They cause an invasive inflammatory colitis, and may also elicit a watery diarrhoea syndrome distinguishable from that caused by other pathotypes via the penetration and multiplication of the bacterium within the epithelial cells of the intestine (Casalino *et al.*, 2003; Todar, 2007).

2.5.5Enteroaggregative *Escherichia coli*

The enteroaggregrative *E. coli* (EAEC) are a heterogenous group of bacteria that display a wide array of virulence factors. The EAEC strains are pathogens associated with persistent diarrhoea in the developing world. They adhere to the tissue cells often in stacks or aggregates. They have been implicated as causes of both outbreaks and sporadic diarrhoea among AIDS patients in developed countries (Sousa, 2006). The infection with the strain of EAEC depicts varying clinical features which include watery and mucoid diarrhoea, low grade pyrexia with or without vomiting (Bhan *et al.*, 1989).

2.6 Laboratory Diagnosis of *E. coli*

The stool samples are examined for consistency, colour, presence of blood, mucus or pus using direct microscopy technique. A sterile wire loop is used to transfer a portion of the stool sample to inoculate onto MacConkey agar and Eosin Methylene Blue (EMB) agar using streak plate method. The inoculated plates are incubated at 37 and 44.5^{0}C for 18- 24

h.On EMB medium, there are black colonies with greenish-black metallic sheen while on MacConkey agar, pink or deep red colonies are produced to confirm the lactose fermenter organism resulting to drop in pH, leading to darkening of the medium. All the plates are examined for growth and pure isolates are Gram stained and subjected to series ofbiochemical tests such as the Indole test, Methyl-red test, Voges-proskaeur test, Citrate utilization test, (IMViC). Indole (red ring for positive test) and Methyl red (bright red for the positive test), but Voges-Proskaeur negative (no colour changes) and Citrate negative (colour remains greenish). *E. coli*tests negative to Gram's reaction, Methyl-red positive, Voges-proskaeur negative, Indole positive and Citrate negative.

A colony of the pure culture is emulsified in distilled water on a clean grease free slides and spread to make asmear for Gram stain. This is allowed to air dry and heat-fixed by passing it gently over a bunsen flame. The smear is then flooded with crystal violet and allowed to stand for 1 min. This is then rinsed withwater and Lugols iodine is added for 1 minute. The complex formed is washed with water and acetone isapplied and allowed to stay for 5 seconds. The slide is rinsed with water and counter stained with neutral red for 2 minutes. The slide is rinsed with water and allowed to dry and examined using oil immersion objectives(x 100) for retention or non- retention of the stainas described in district laboratory practice in tropical countries (Cheesbrough, 2005).*E. coli* is a gram –ve bacilli and hence, tests negative to Gram's reaction.

ETEC should be considered a possible etiologic agent of watery diarrhoea for which no pathogen has been identified (Dalton *et al.*, 1999). EPEC should beconsidered a possible pathogen in outbreak of severe non-bloody diarrhoea ininfants or young toddlers, particularly in nursery or day care settings. EIEC shouldbe considered a possible etiologic

agent in outbreaks of diarrhoea (bloody or non-bloody),when routine bacterial agents are ruled out (Bopp *et al*., 2003).Diagnostic procedures for VTEC have been developed, primarily for *E. coli*O157:H7, and seek to overcome the problems of isolating low numbers of target organisms from complex matrices such as animal faeces, food and clinical specimens.

For *E. coli* O157:H7 in stool, ELISA test, direct immunoflorescent microscopy of filters and immunocapture techniques using magnetic beads are screening tools that precede rapid testing for the presence of *E. coli* without prior culturing of the stool (De Boer and Heuvelink, 2000).Molecular methods also allow the separation of organisms that otherwise appear to be identical. Nonpathogenic *Escherichia coli* in normal stool cannot be separated from diarrhoea-causing *E. coli* by standard methods; however, identification of virulence genes or factors distinguishes five groups of *E. coli* that cause illnesses ranging from cholera-like watery diarrhoea to neonatal diarrhoea, persistent diarrhoea, and bloody diarrhoea (Nataro and Kaper, 1998).

Non-sorbitol-fermentingcolonies are confirmed biochemically as *E.coli*and by serum or latex agglutination as possessingthe O157 somatic antigen and/or H7 flagellar antigen. Potential virulence for humans is confirmedby the demonstration of verocytotoxin production by Vero cell assay, enzyme-linked immunosorbentassay (ELISA) or agglutination tests or the demonstration of genes encoding verocytotoxin bypolymerase chain reaction.

2.7 *Escherichia coli*Infections in Humans

E. coli is the leading Gram-negative cause of bacteraemia after Staphylococci and streptococci.*E. coli* bacteraemia in children may present without fever, especially in neonates and is often community-acquired,ampicillin-resistant and associated with underlying immune deficiency, urinary infection, or lesions in the gastrointestinal tract; it carries 18% mortality(Bonadio *et al*., 1991; Jacoby, 1997).In addition to its important role in causing watery, inflammatory colitis, and hemorrhagic diarrhoeal illnesses, it may cause neonatal meningitis(Klein *et al*., 1986).

2.8 Antimicrobial Susceptibilities (ETEC, EPEC, EIEC and other diarrhoeagenic *E.coli* strains)

Treatment with appropriate antibiotic can reduce the severity and duration ofsymptoms of ETEC infection (Nataro and Kaper, 1998). Antimicrobial resistance,particularly to tetracycline is common among ETEC strains isolated from outbreaks inthe United State (Dalton *et al*., 1999). Antibiotic treatment may be helpful fordiarrhoea caused by EPEC (Nataro and Kapper, 1998). Most EPEC strains associatedwith outbreaks are resistant to multiple antimicrobial agents. Little informationabout the efficacy of antimicrobial treatment or the prevalence of resistance isavailable for EIEC or other putative diarrhoeagenic *E.coli* strain (e.g EaggEC); butdetermination of antimicrobial susceptibility pattern may be helpful in establishingwhether isolates are associated with an outbreak (Bopp *et al*., 2003).

2.9 Prevention of diarrhoeal infections

2.9.1Promotion of exclusive breast-feeding

Exclusive breast-feeding means no other food or drink, not even water, is permitted, except for supplements of vitamins and minerals or necessary medicines. The optimal duration of

exclusive breastfeeding is six months (WHO, 2001).Exclusive breastfeeding protects very young infants from diarrhoeal disease in two ways: first, breast milk contains both immune (specific) and non-immune (nonspecific) antimicrobial factors; second, exclusive breastfeeding eliminates the intake of potentially contaminated food and water. Breast milk also provides all the nutrients most infants need up to age 6 months. When exclusive breastfeeding is continued during diarrhoea, it also diminishes the adverse impact on nutritional status (Westphal *et al*., 1995)

2.9.2Improved complimentary feeding practices

Ideally, complementary foods should be introduced at age 6 months, and breastfeeding should continue for up to two years or even longer to increase birth intervals (WHO, 2003). There is a strong inverse association between appropriate, safe complementary feeding and mortality in children age 6 to 11 months. Malnutrition is an independent risk predictor for the frequency and severity of diarrhoeal illnesses. There is a vicious cycle in which sequential diarrhoeal disease leads to increasing nutritional deterioration, impaired immune function, and greater susceptibility to infection. The cycle may be broken by interventions to decrease infection incidence to reduce malnutrition (Keusch and Scrimshaw, 1986) or improving nutritional status to reduce the burden of infection (Victora *et al*.,1999).Improved feeding practices to prevent or treat malnutrition could save as many as 800,000 lives per year (Jones *et al*., 2003).

2.9.3 Improved water and sanitary facilities and promotion of personal and domestic hygiene

Human faeces are the primary source of diarrhoeal pathogens (WHO, 1997). Poor sanitation, lack of access to clean water, and inadequate personal hygiene are responsible for an estimated 90 percent of childhood diarrhoea (WHO, 1997). It works best when it is part of a package of behavioral change interventions.

2.10 Control and treatment of infections

There have been major advances in the management of childhood diarrheal dehydration over recent years; foremost among these is the widespread use of oral rehydration therapy (ORT). This therapy is feasible, efficacious, and safe in hospital treatment and at the primary care level in developing countries, in the prevention of metabolic complications of diarrhea and dehydration and in reduced hospitalization(Gracey, 1993). ORT is gradually becoming accepted in the West (Walker-Smith, 1990), although it has been referred to as "the underused simple solution" because of the resistance of pediatricians and other medical practitioners in countries like the United States (Avery and Snyder, 1990; Snyder, 1991), where there may be widely-held perceptions that intravenous fluids are usually needed in children with acute diarrhoeal episodes. Dietary management is a very important but often, poorly-managed aspect of treatment of childhood diarrhoea.Earlier approaches that permitted the bowel to "rest" for some days are outmoded and tended to worsen the potentially-negative nutritional impacts of stool losses, vomiting, infection and widespread inappropriate feeding practices in nutritionally vulnerable infants and young children (Gracey, 1993; Rivin and Sanosham, 1993).There are several important basic principles, these include:

1. Breast-feeding should be continued during diarrhea and additional fluids should be given to replace fluids and electrolytes that are lost with abnormal fecal losses.

2. Most young children with acute diarrhea can be successfully managed with continued feeding of undiluted nonhuman milk. Routine dilution of milk and routine use of lactose-free formula, without a clinical indication, are therefore not needed, especially when ORT and early feeding, in addition to milk, comprise the basic approach to management (Brown *et al*., 1994).
3. In children who are on milk other than breastmilk, this can be reintroduced after rehydration has been achieved. Lactose-free formulas may be needed for patients with severe mucosal damage and secondary lactose intolerance, while a small proportion of children, particularly those who are young and malnourished, may be intolerant to all dietary carbohydrates including monosaccharides (Salazar-Lindo *et al*., 1986; Gracey and Anderson, 1993).
4. Food-based ORT (e.g. based on rice powder or other cereals or local staples) can be used to help reduce fecal losses.
5. In children aged 4-6 months or more, soft or semi-solid foods should be offered as soon as the child is rehydrated - there is no need to withhold or restrict feeding until the diarrhoea has stopped. These foods should be mashed or pureed and offered in small amounts at least six times daily.
6. Extra food should be given for at least two weeks after the diarrhoea has stopped in order for any weight-for-height deficit to be corrected.
7. Attention may be required to correct specific micronutrient deficiencies, e.g.zinc (Sachdev *et al*., 1988) or vitamin A.
8. Drugs have very little role in the management of infants and children with acute diarrhea (Rivin and Sanosham, 1993). Such are often used indiscriminately, particu-

larly antimicrobials and so-called antidiarrhoeal remedies, that may come from pharmaceutical sources but often, from uncontrolled suppliers. There are several reasons why antibiotics should not generally be used in childhood gastroenteritis including the fact that some episodes (e.g. those caused by viruses) would not respond, while many enteric bacteria are already antibiotic-resistant. Indiscriminate usage of antibiotics encourages the development of plasmid-transmitted drug resistance, and antibiotic treatment can prolong carriage for some microorganisms, such as *Salmonella* (Griffiths and Gorbach, 1993). Furthermore, antibiotics are also expensive and have significant side-effects. Antibiotics are appropriate for the treatment of bacterial infections that can produce bacteremia, such as typhoid fever, for which chloramphenicol or ampicillin can be used (Griffiths and Gorbach, 1993). Antibiotics may be indicated for other reasons, such as treatment of a coexistent respiratory infection or other serious infections particularly in young, small and compromised infants and young children (Gracey, 1995).

2.11 Anthropometric / Nutritional status measurements using Z- scores

Malnutrition can be defined as all deviations from adequate nutrition and it still remains a major source of concern in developing countries (de Onis *et al.*, 2012).The consequences of malnutrition can be serious in childhood. Under-nutrition is responsible for 35% of disease burden of under-five year old children. It contributes to about 3.5 million deaths in children within the age group (Black *et al.*, 2008). Present research findings including follow up of intervention trials indicate that chronic malnutrition can have long-term negative consequences on various aspects of child development (Dewey and Begum, 2011).

Nutritional assessment in under-five year old children can be carried out using anthropometric indicators which include stunting, wasting and underweight. In nutritional anthropometry, a child's index is compared with an internationally accepted reference population and children who fall below the recommended cut-offs are classified as malnourished. A malnourished child is one who has failed to attain the expected values for any of the nutritional indicators (e.g length-for-age, weight-for-height or weight-for-age) as compared with a healthy child of the same sex and age in the reference population (WHO Multi-growth Reference Study group, 2006). Depending on the references and descriptors used, malnutrition is defined as an anthropometric index below the -2 standard deviation from the median value of the reference population or below the fifth (5th) percentile for a given anthropometric index.

Low weight-for-height relative to a child of same sex and age in a reference population is referred to as wasting. Wasting is normally used as an indicator of current nutritional status and can be used for screening of children at risk of childhood mortality and for measuring short term changes in nutritional status. It reflects a recent and severe process that has led to substantial weight loss. It may be the consequence of starvation or severe diseases (Diarrhoea in particular) but can also be due to chronic conditions. Weight-for-height reflects proper body proportion or harmony of growth. It is particularly sensitive to acute growth disturbances. Lack of evidence of wasting in a population however does not rule out the absence of current nutritional problem (WHO, 2006).

Weight-for-age reflects body mass relative to age. It is a composite measure of weight-for-age and weight-for-height. It combines the effect of long term and short term health and nutritional problems and represents a convenient measure of both linear growth and body

proportion. Weight-for-age can be used for the diagnosis of underweight in children (WHO, 2006).

Low weight-for-age relative to a child of same sex and age in a reference population is referred to as “lightness” while long term “underweight” is commonly used to refer to severe pathological deficits in weight-for-age. Stunting can be defined as failure to achieve one’s genetic potential for height (Golden, 2009). Many genetic and environmental factors modify stature: Growth hormone deficiency, impaired kidney function, psychological deprivation and under-nutrition. Although stature has a genetic basis, there is increasing evidence of the strength that environmental factors such as poverty, and high frequency of infections have on the determination of final stature. Some workers suggest that environmental conditions are stronger determinants of stature compared to genetic factors in adverse conditions. Children throughout the world can reach their growth potential if they are nurtured in healthy environments under optimal health and nutritional care practices (Golden, 2009).Monitoring childhood stunting in the country based on the more recent nutritional standards is important for performance evaluation of impacts of set intervention goals (Akubugwo*et al*., 2013).

2.12 Antibiotics

This is a term used to describe antibiotic agents (usually antibacterial) that can be used to treat infections.

2.12.1 Historical discovery of antibiotics

Antibiotic discovery started in the 1928 by Alexander Fleming who observed the inhibition of growth on agar plate containing *Staphylococcus* species. The bacterium, *Penicillium neonatum* was found to be responsible for the inhibition of the *Staphylococcus* species. This marked the genesis of the discovery of penicillin and other antibiotic drugs. This discovery has saved millions of humans and animals from infectious disease-causing organisms (Byarugaba, 2010). The discovery of antibiotics in the 19th century reduced the threat of infectious diseases against human race.

2.12.2 Entry of antibiotics into the environment

Antibiotics used in poultry production can enter the environment via a number of routes including the drug manufacturing process, disposal of unused drugs and containers, and through the use and application of waste material containing the drugs (Andremont, 2003; Beausse, 2004; Boxall *et al.,* 2002; 2006). The excretion of waste products by animals, atmospheric dispersal of feed and manure dust containing antibiotics and the incident release of products from spills or discharges are also potential pathways of antibiotic residue entry into the environment (Nielsen *et al.,* 2000).Many antibiotics are not completely absorbed in the gut, resulting in the excretion of the parent compound and its breakdown metabolites (Boxall *et al.,* 2002).Also, Feinman and Matheson (1978) suggested that about 25 % of oral dose of tetracycline is excreted in faeces and another 50-60 % is excreted unchanged or as an active metabolite in urine. Furthermore, oral administration of the macrolide tylosin resulted in a maximum of 67 % of the antibiotics excreted, mainly in faeces (Feinman and Matheson, 1978).

2.12.3Mode of action of antibiotic agents

Most antibiotic agents are classified or categorized according to their principal mode of action (Neu, 1992).

2.12.3.1Interference with cell wall synthesis

Some antibacterial drugs work by inhibiting bacterial cell wall synthesis such as penicillin, cephalosporins, vancomycin. They inhibit synthesis of the bacteria cell wall by interfering with the enzymes required for the synthesis of the peptidoglycan layer of the cell wall while others such as vancomycin interfere with cell wall synthesis by binding to the terminal D-alanine residues of the nascent peptidoglycan chain, thereby preventing the cross-linking steps required for stable cell wall synthesis (McManus, 1997).

2.12.3.2 Inhibition of protein synthesis

Macrolides, aminoglycosides, tetracyclines, chloramphenicol and streptomycin produce antibacterial effects by inhibiting protein synthesis. These drugs bind to the 30s and 50s subunit of the ribosome (Neu, 1992).

2.12.3.3 Interference with nucleic acid

Antibiotic drugs such as fluoroquinolones exert their antibacterial effects by disruption of DNA synthesis causing lethal double stranded DNA breaks during DNA replication (Drlica and Zhao, 1997).

2.12.3.4 Inhibition of metabolic pathway

Sulphonamides and trimethoprim block the pathway for folic acid synthesis which ultimately inhibits DNA synthesis (Yao and Moellering, 2003; Petri, 2006). The common drug combination of trimethoprim and sulphonamide inhibits two steps in the enzymatic pathway for bacterial folate synthesis (Tenover, 2006).

2.12.3.5Disruption of bacterial membrane structure

Polymyxins are postulated to exert their inhibitory effects by increasing bacterial membrane permeability, causing leakage of bacterial contents (Petri, 2006).

2.12.4Antibiotic resistance

Antibiotic drug resistance has been recognized as an emerging global problem in both human and veterinary medicine (Sayah*et al.,* 2005). Antibiotic drugs are considered the most important factor for the emergence, selection, and dissemination of antibiotic agent-resistant bacteria (Witte, 1998).Bacteria in the guts of humans and animals are subjected to different types, concentrations, and frequencies of antibiotic agents which results in the development of resistance. Bacteria gain antibiotic resistance genes through mobile elements, such as plasmids, transposons, and integrons (Prescott *et al.,* 2000).Antibiotic usage is possibly the most important factor that promotes the emergence, selection and dissemination of antibiotic-resistant microorganisms in human and animals. This acquired resistance occurs not only in pathogenic bacteria but also in endogenous flora of exposed individuals (Daniels *et al.,* 2009; Nsofor and Iroegbu 2012).Due to intricate balance of microflora of different habitats within the ecosystem, the transfer of resistance genes

among bacteria occupying different habitats has the potential to occur frequently (Davies *et al.,* 2009).

2.12.5 Onset of antibiotic resistance

After the discovery of penicillin, a number of treatment failures ensued and some bacteria were no longer sensitive to penicillin (Byarugaba, 2010). This was the genesis of resistance to antibiotics.Tetracyclines were discovered in the 1940s and exhibited activity against a wide range of microorganisms including gram-positive and gram-negative bacteria, chlamydiae, mycoplasmas, rickettsiae, and protozoan parasites. They are inexpensive antibiotics, which have been used extensively in the prophlylaxis and therapy of human and animal infections and also at subtherapeutic levels in animal feed as growth promoters. The first tetracycline-resistant bacterium, *Shigella dysenteriae*, was isolated in 1953. Tetracycline resistance now occurs in an increasing number of pathogenic, opportunistic, and commensal bacteria (Chopra and Roberts, 2001).

The presence of tetracycline-resistant pathogens limits the use of these agents in treatment of diseases. Tetracycline resistance is often due to the acquisition of new genes, which code for energy-dependent efflux of tetracyclines or for a protein that protects bacterial ribosomes from the action of tetracyclines.Many of these genes are associated with mobile plasmids or transposons and can be distinguished from each other using molecular methods including DNA-DNA hybridization with oligonucleotide probes and DNA sequencing. A limited number of bacteria acquire resistance by mutations, which alter the permeability of the outer membrane porins and/or lipopolysaccharides in the outer membrane, change the regulation of innate efflux systems, or alter the 16S rRNA(Chopra and Roberts, 2001).

2.12.6 Mechanisms of antibiotic resistance in bacteria

Antibiotics have always been considered one of the wonder discoveries of the 20th century. This is true, but the real wonder is the rise of antibiotic resistance in hospitals, communities, and the environment concomitant with their use. The extraordinary genetic capacities of microbes have benefitted from man's overuse of antibiotics to exploit every source of resistance genes and every means of horizontal gene transmission to develop multiple mechanisms of resistance for each and every antibiotic introduced into practice clinically, agriculturally, or otherwise (Davies and Davies, 2010).Antibiotics have revolutionized medicine in many respects, and countless lives have been saved; their discovery was a turning point in human history. Regrettably, the use of these wonder drugs has been accompanied by the rapid appearance of resistant strains. Resistance processes are widely distributed in the microbial kingdom and have been well described for a variety of commensals and pathogens. Most can be disseminated by one or more distinct gene transfer mechanisms.

Varied mechanisms of antibiotic resistance are readily spread to several bacteria genera. First, the organism may acquire genes encoding enzymes, such as β-lactamases, that destroy the antibacterial agent before it can have an effect (Bester and Essack, 2010). Secondly, bacteria may acquire efflux pumps that extrude the antibacterial agent from the cell before it can reach its target site and exert its effect (Poole and Srikumar, 2001; Schweizer, 2003). Thirdly, bacteria may acquire several genes for a metabolic pathway which ultimately produces altered bacteria cell walls that no longer contain the binding site for the antibiotic agent, or bacteria may acquire mutations that limit access of antibiotic agents to the intarcellular targets site via down-regulation of porin genes (Singh *et al.*,

2005; Maurelli, 2006; Bester and Essack, 2010). This may occur either through one of several genetic mechanisms such as transformation, conjugation or transduction (Bester and Essack, 2010).

2.13 Antibiotic susceptibility patterns of *Escherichia coli* isolates in diarrhoeic children under five years in Nigeria and other countries.

In India, persistent diarrhoea accounted for 5% of episodes but 14% of deaths, and a mortality rate three times higher than briefer episodes (Bhan *et al.*, 1989). In Pakistan, persistent diarrhoea accounted for 8-18% of episodes but 54% of deaths (Khan *et al.*, 1993). In Bangladesh, persistent diarrhoea associated with malnutrition was responsible for nearly half of diarrhoea deaths, and the relative risk for death among infants with persistent diarrhoea and severe malnutrition was 17 times greater than for those with mild malnutrition (Fauveau *et al.*, 1992).

The long-term consequences of diarrhoeal diseases remain poorly studied, and analyses of global trends have not considered them. Niehaus *et al.* (2002) recently evaluated the long-term consequences of acute diarrhoeal disease on psychomotor and cognitive development in young children. Following a cohort of 47 children in a poor urban community in northeastern Brazil, they correlated the number of diarrhoeal episodes in the first two years of life with measures of cognitive function obtained four to seven years later. They found a significant inverse correlation (average decrease of 5.6%) between episodes of early diarrhoeal disease and overall intellectual capacity and concentration, even when controlling for maternal education or helminth infection, which are known to be independent predictors of malnutrition and cognitive defects. Test scores were also 25-65%

lower in children with an earlier history of persistent diarrhoea.Recent evidence suggests that genetic factors may also be involved in the developmental response to repeated diarrhoea (Oria *et al.*, 2005). Better and more sensitive assessment tools are needed to define the relationships between diarrhoeal diseases and developmental disorders and to calculate individual and societal costs and the cost-effectiveness of interventions. In addition, early childhood malnutrition resulting from any cause reduces physical fitness and work productivity in adults (Dobbing 1990).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study area consisted of six (6) hospitals namely General Hospital Makarfi, Gambo SawabaMemorial Hospital Zaria, Yusuf Dantsoho Memorial Hospital Tudun-wada, Gomna Awan General HospitalKakuri, Kwoi General Hospital and Kafanchan General Hospital, selected from the three senatorialzones in Kaduna State, Nigeria.

3.2 Study Population

The study population consisted of children between the ages of 0-5 years, presenting with diarrhoea, whose parents gave consent and the exclusion criteria were children under the ages of 0-5 years, whose parents did not give their consent.

3.3 Sample Size

The sample size was determined using the formula of Sarmukaddam and Gerald (2006) which is as follows:

$N = Z^2Pq/L^2$

Where N is sample size

Z is the standard normal distribution at 95% confidence interval = 1.96

P is the prevalence rate, which is taken as 34.1% (Sule *et al*., 2011)

qis 1 –P

L is the allowable error, which is taken as 5% = 0.05

Therefore $N = \frac{(1.96)^2 \times 0.341 \times (1-0.341)}{(0.05)^2}$

$= \frac{3.8416 \times 0.341 \times 0.65}{0.0025} = 345.3$

The sample size calculated is 345.3 samples.

A total of three hundred and fifty samples were collected from the diarrhoeic children for this study.

3.4 Collection of Samples and Processing

Stool samples were collected from children under 5 years of age visiting hospital due to acute diarrhoea and from healthy controls of similar age. Stool samples were collected

using sterile stool containers and transferred to the microbiology laboratory on ice packs for laboratory analysis.

3.5 Preparation of media

The media used in this study include: Eosin Methylene Blue Agar, Sorbitol MacConkey Agar, Citrate Agar, Methyl Red-Voges Proskaeur medium, Peptone water Agar,Nutrient Agar and Mueller-Hinton Agar. They were all prepared according to manufacturers' instructions and the prepared media were then stored at 4°C for use in stool culture.

3.6 Isolation and Characterization of *Escherichia coli*

Bacterial isolates were identified according to the standard microbiological procedures as described by Gillespie and Hawkey (2006), which includes examination of specimens to detect, isolate, andidentify pathogens or their products using, Microscopy, Culture techniques, Biochemical tests, and Immunological (antigen) tests.

3.6.1 Isolation of Escherichia coli from stool

Approximately 10µl volumes of a micropipette of faecal samples were innoculated directly on toEMB (March and Ratnam, 1986) and CT-SMAC (Zadik *et al*., 1993). The plates wereincubated at 44.5 and 37^0C respectively for 18 - 24 h. Non-sorbitol fermenting (NSF), colonies on CT-SMAC thatappear as colourless colonies on the plates were stored on nutrient agar slants.A growth on EMB agar with greenish-metallic sheen was also transferred to nutrient agar slants forstorage. All typical *Escherichia coli* isolates from

EMB (selective media) and CT-SMAC (differential media) stored on slants were confirmed by Gram- reaction, MRVP test, Citrate utilization, Indole,Motility tests.

3.6.2 Gram Stain Reaction

Smears of all stored isolates were made on slides and heat-fixed, covered with crystal violet for 1 min and rinsed immediately with clean water. The smears were then covered with Lugol's iodine for another minute and rinsed again. Alcohol was used to decolorize it for a few seconds, rinsed and placed in a draining rack to air dry. The slides were examined microscopically with x100 objective lens to view the microscopic appearance of the gram negative rods (Cheesbrough, 2000).

3.6.3 Biochemical characterization of presumptive Escherichia coli isolates

The basic microbiological characteristics of *Escherichia coli* were obtained when cultured on standard lactose-containing EMBagar.*E.coli*lactose-fermenters produce green-black colonies with metallic sheenand dark colonies for other fermenters, while non-lactose fermenters appear colourless(Tarr and Neil,2001).

3.6.3.1Methyl red Voges-Proskaeur (MRVP) test

A colony of the isolate was inoculated into Methyl red Voges-Proskaeur broth (MR-VP) and incubated for 24h at 35-37^{0}C. 0.6ml of α-naphthol solution and 0.2ml of 40% potassium hydroxide was added to 2.5ml of the culture broth and shaken properly. Formation of a pink-red product within 5-15min of dilutionindicates a positive(acid) result which indicates the presence of *Escherichia coli*.

3.6.3.2Citrate utilization test

A light suspension of the organism was made in saline and then stab inoculated onto Simmon's citrate agar using a sterile inoculating needle. Growth indicated by blue colour in Simmon's agar, indicates positive result. This means that citrate has been utilized and hence the absence of *E. coli*, since it is citrate negative.

3.6.3.3Indole test

A colony of the *E.coli* isolate wasinoculated into test tubes containing Sulphur Indole Motility medium using a sterile wire loop and incubated for 48 h at 37^0C.Two drops of Kovac's reagent were added to the medium and shaken. Formation of a pink layerindicates a positive result and hence, the presence of *E. coli.*

3.7 Biochemical characterization of presumptive isolates.

The isolates were further subjected to biochemical tests using *E.coli*(Microgen^{TM} GnA- ID System)Microgen^{TM} Kits.The data obtained by the Microgen GnA-ID microwell strip was designed to generate a 4 digit octal code which was used to interpret the result by the Microgen Identification System Software (Appendix I).

3.7.1Confirmation of *E. coli* using Microgen^{TM} GnA-ID Kit.

A colony from an 18-24 hour culture was emulsified in 3ml sterile 0.85% saline for the GN A microwell test strips. The adhesive tape sealing the microwell test strips were carefully peeled. Three to four drops of the bacterial suspension were added to each well of the microwell test strips. After inoculation, wells 1, 2, 3 and 9 were overlaid with 3-4 drops of mineral oil. The top of the microwell test strips were sealed with adhesive tape and were incubated at 35-37^0C. The microwell test strips were read after 18-24 hours incubation. The adhesive tape was removed and all positive reactions were recorded with the aid of the

colourful chart. Two drops ofKovac's reagent were added to well 8 and read after 60 seconds. One drop each of VP I and VP II reagent were added to well 10 and read after 15-30 minutes. One drop of TDA reagent was added to well 12 and read after 60 seconds. The adhesive tape was removed and all reactions read and recorded according to manufacturer's instructions.

3.7.2Serological identification of *Escherichia coli*isolates

*Escherichia coli*O157 isolates were identified serologically, using specific serological methods that are rapid. The methodused was the Oxoid *E. coli* latex test which demonstrates by slide agglutination *E. coli* strains possessing the O157 sero-group antigen. A bacterial colony of pure growth wasemulsified in physiological saline on a slide and antiserumcontaining specific antibody was added. The antibody binds tothe bacterial antigen, resulting in the agglutination of the bacterialcells (Bettleheim and Beutin, 2003).

3.7.2.1Latex agglutination test

The Oxoid *Escherichia coli* latex agglutination test demonstrates by slide agglutination *Escherichia coli* strains possessing the O157 sero-group antigen.Non-sorbitol fermenting *E.coli* strains were tested with the latex reagents to determine whether they belong to the O157 Sero group.A drop of the test latex was dispensed a circle on the reaction card, close to the edge of the circle. A drop of saline was placed on the other end of the reaction card, so that they do not mix yet. A loop was used to transfer a portion of the colony to be tested and emulsified carefully in the drop of saline until the suspension was smooth, the test latex and the saline were mixed together and spread to cover the reaction area using the loop,

which was then flamed. The card was then rocked and observed for agglutination within one minute.

3.7.2.2Detection of E.coli Verotoxin

The detection of verocytotoxins produced by *E.coli* cultured from faecal samples was done by Enzyme Linked Immunosorbent Assay. The test is a double antibody(sandwich) ELISA using anti-verotoxin antibodies to capture the antigen from the stool supernatant. A second anti-verotoxin monoclonal antibody cocktail is then added, which binds to the complex. This reaction is visualized by the addition of anti-mouse antibodies conjugated to peroxidase. The resulting blue color following the addition of the chromogen indicates the presence of verotoxin being bound by anti-verotoxin antibodies.In this test, microtitre wells are coated with rabbit anti-verotoxin antibodies and the antibody-enzyme conjugated is a monoclonal antibody chemically linked to the enzyme horse radish peroxidase (HRP). The conjugate binds specifically to the solid phase toxin (Bettleheim, 2001).

The wells needed, which includes the number of samples plus two (2) for controls, were broken off and placed in a strip holder. 100ul each of the negative and positive (undiluted) controls were added to wells 1 and 2 respectively and then 100ul of the stool supernatant added to the appropriate test wells and incubated for 30 minutes at room temperature and then washed (this involved using the distilled wash buffer to fill to the top of each well, shaking out the contents and refilling the wells for a total of 3 minutes). Then 2 drops of Reagent 1(blue solution) was added to each well and incubated for 30 minutes and washed, thereafter, 2 drops of Reagent 2 (red solution) was also added to each well and incubated again for 30 minutes before washing and each well was rinsed three times with distilled

water, then 2 drops ofChromogen was added to each well and it was incubated for 10 minutes also at room temperature. 2 drops of Stop solution was added to each well and mixed by tapping the strip holder. Results were read and interpreted using an ELISA reader on a spectrophotometer using bichromatic reading, with the filters set at 450nm-630nm.

3.8 Antibiotic susceptibility testing

The antibiotic susceptibility testing of the isolates were carried out using the disc diffusion techniqueaccording to the methods recommended by the Clinical Laboratory and Standards Institute (2013) to determine the susceptibility or resistance profiles of the isolates. About 3-5 discrete colonies of theisolates from EMB agar were inoculated into 5ml of physiological saline and incubated overnight at 37^{o}C. The overnight culture was then standardized with 0.5 MacFarland standard suspensions. Sterile cotton swab was used in inoculate the bacterial suspension to freshly prepared pre-dried Mueller-Hinton agar plates prepared according to manufacturer's instructions. The antibiotic sensitivity discs were aseptically and spaciously placed on the inoculated Mueller-Hinton agar plates. The antibiotic discs used were Amoxicillin (10µg), Cefotaxime (30µg), Ciprofloxacin (5µg) Chloramphenicol (30µg),Tetracycline (30µg), Trimethoprim (25µg), Gentamicin (10µg) (Oxoid Ltd, UK).The reference standard strains used as control in this study include *Escherichia coli* ATCC 25922. It was obtained from the National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria.

3.8.1 Measurement of diameter of the inhibition zone

After incubation, the test plates were examined for confluent growth and zone of inhibition. The diameter of each zone of inhibition was measured in millimetre (mm) using a ruler on

the underside of the plate. The interpretation of the measurement as sensitive, intermediate and resistant was made according to Clinical Laboratory Standards Institute (CLSI, 2013)manual.

3.8.2 Multiple-antibiotic resistance index

The multiple-antibiotic resistance (MAR) index was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested (Olayinka *et al*., 2004).

$$\text{MAR index} = \frac{\text{Number of antibiotics the isolate is resistant to}}{\text{Total number of antibiotics tested.}}$$

3.9Assessment of Nutritional Status

Anthropometric assessment: Anthropometry refers to comparative measurements of the human body. The four measures used for anthropometric assessment are: Age, sex, length (or height) and weight. Each of these variables provides one piece of information about a person and when they are used together they can provide important information about a person's nutritional status. Collection of anthropometricdata, was done using a standard measuring tape tomeasure height and the children were weighed usinga UNICEF UNISCALE to the nearest 100grams (Alim and Jahan, 2012).Medical history, dietary history, socioeconomic/demographic records were obtained using the proffered answers to the questionnaires.

3.9.1 Reference standards

References were used to standardize a child's measurement by comparing the child's measurement with the average measure for children at the same age and sex. The WHO (2006) International Reference Standardwasused for standardizing the anthropometric data using Z-scores. The Z-score or standard deviation unit (SD) is defined as the difference between the value for an individual and the median value of the reference population for the same age or height, divided by the standard deviation of the reference population. The distribution of Z-scores follows a normal distribution, and the commonly used cut-offs are -3,-2, and -1 Z-scores. The use of a cut-off enables the different individual measurements to be converted into prevalence statistics. The most commonly used cut-off with Z-scores is -2 Standard deviations. Hence, children with a Z-score for underweight, stunting or wasting below -2 SD were considered moderately or severely malnourished.

WHO reference standards that were used for standardizing the anthropometric data obtained includes,weight-for-length/height(45to110cm),weight-for-age(65to120cm), BMI-for-age(22kg/m^2) (WHO, 2006).

3.10Questionnaire Administration

The nutritional status of individuals revolves around general well-being. Questionnaires containing questions on demographic factors, such as socioeconomic backgrounds of the children and their families, dietary history, medical history, risk factorswere administered to the parents and caregivers of the children who consented to this study. The data obtained therefrom, were used to assess the nutritional status of the children and also to show further, the relationship between diarrhoea and nutrition.

3.10.1 Inclusion and exclusion criteria

The inclusion criteria includeddiarrhoeic children (0-5yrs), whose parents gave consent. Childrenbelow or above 5years,whose parents didnot give their consent, were excluded from the study. Diarrhoeic cases in this study was defined as the occurrence of three or more loose or watery stools in a 24h period at least one bloody loose stool (Gondwe *et al*., 2006).

3.11 Statistical analysis

Data collated from the questionnaires and the results obtained from the study, were subjected to the appropriate statistical analysis. Chi-square analysis was used to determine association between variable and infection at 95% CI and at 0.05 significant levels (SPSS, version 19) and nutritional status assessment was done using WHO Anthro statistical software (Version 3.2.2).

CHAPTER FOUR

4.0 RESULTS

4.1.1Prevalence of *Escherichia coli* in diarrhoeic stool samples according to hospital location

Table 4.1 shows the distribution of *E. coli* isolates in the stool samples, with respect to the hospital location from where the samples were obtained. There was statistical difference observed in the prevalences obtained from the various hospitals ($p > 0.05$). General Hospital Kafanchan, had the highest percentage prevalence of 40.74%, while those in Yusuf Danstoho Memorial Hospital had a prevalence of 34.94%. The prevalence of *E. coli* infection on patients in Gambo Sawaba Memorial Hospital was 19.64%, General Hospital Makarfi (16.18%) , General Hospital Kwoi had (15.79%) and Gomma Awan General Hospital had (11.34%).

4.1.2 Microgen GnA identification of *E. coli* isolates obtained from diarrhoeic children under five years

Table 4.2 shows the representation of some of the positive *E.coli* isolates obtained through biochemical characterization using the Microgen GnA-ID system of *E. coli* isolates obtained from the diarrhoeal stools of children under five years of age.

4.1.3Prevalence of *Escherichia coli* O157 and Non-O157 from the total *E. coli* isolated from diarrhoeic stool samples of children (0-5 years) with respect to hospital location.

Fig 4.1 shows the distribution of the prevalence of O157 and non- O157 isolates obtained from the total *E.coli*. The samples obtained were analysed for *Escherichia coli* O157 and non- O157 strains. Of the 76 isolates obtained from 350 diarrhoeic stool samples obtained from under-five children, 28 were O157 positive and 48 non- O157 *E.coli* and generally showing 11 isolates from Gambo Sawaba Memorial Hospital, 11 from General Hospital Makarfi, 29 from Yusuf Dantsoho Memorial Hospital, 11 from Gomna Awan General Hospital, 11 from General Hospital Kafanchan, and 3 from Kwoi General Hospital.

4.3.1 Susceptibility patterns to antimicrobials of *E.coli* isolated fromdiarrhoeic stool samples of children under 5-years.

Table 4.3.1 shows the antibiotic susceptibility pattern of *E. coli* isolated from diarrhoeic stool samples of children under five years in Kaduna state. The antibiotics with the highest antibacterial activity were chloramphenicol and ciprofloxacin with 100% activity against all the isolates. This was followed by moderate resistance (50%) of the isolates to amoxicillin, tetracycline and gentamicin. High resistance was seen in the activity of the isolates to cefotaxime (97.5%) and sulphamethoxazole (88.9%).

4.3.3 Multiple antibiotic resistance indices of *E.coli*isolated from diarrhoeic stool samples of children under 5-years.

Multi-antibiotic resistance (MAR) can be regarded as resistance to two or more antibiotics. The resistance pattern of *E. coli* isolated from faecal samples of diarrhoeic children under

five years is shown in Table 4.3.2. No resistance pattern was obtained for the single antibiotic used in the study rather the isolates were found to be resistant to more than one antibioticcombinations. Two multiple resistance patterns were obtained with varying combinations of 4 and 5 antibiotic combinations.All the *E. coli* isolates from the faecal samples of the diarrhoeic children exhibited MAR.

Table 4.1: Prevalence of *Escherichia coli* isolated from diarrhoeic stool samples according to hospital location

Location	**No of Samples Collected**	**Samples Positive(%)**	X^2	**p-value**
GSMH	56	11(19.64)	22.192	0.000**
GHM	68	11(16.18)		
YDMH	83	29(34.94)		
GAGH	97	11(11.34)		
GHK	27	11(40.74)		
KGH	19	3(15.70)		
Total	**350**	**76(21.7)**		

Key: X^2=chi-square,p-value<0.05, (*)=Statistically significant, (%) =Prevalence,**GSMH**- Gambo Sawaba Memorial Hospital, **GHM-** General Hospital Makarfi, **YDMH**- Yusuf Danstoho Memorial Hospital, **GAGH**- Gomna Awan General Hospital, **GHK**- General Hospital Kafanchan, **KGH**- Kwoi General Hospital

Table4.2 Microgen GnA identification of *E. coli*isolates obtained from diarrhoeic children under five years

CODE	Lys	Orn	H_2S	Glu	Man	Xyl	ONPG	Ind	Ure	VP	Cit	TDA	Octal Code	ID
YD1	+	+	-	+	+	+	+	+	+	-	-	-	6770	*E.coli*
YD4	+	+	-	+	-	+	+	+	-	-	-	+	6561	*E.coli*
YD6	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E.coli*
MK3	+	+	-	+	-	+	+	+	-	-	-	-	6560	*E.coli*
GS27	+	+	-	+	+	-	+	+	-	-	-	-	6660	*E.coli*
GA35	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E.coli*
KAF16	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E.coli*
KG19	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E.coli*

KEY:Lys- Lysine, Orn- Ornithine, H2S- Hydrogen sulphide , Glu-Glucose, Man-Mannitol, Xyl- Xylose, ONPG- Ortho-nitrophenol- galactosidase, Ind - Indole, Ure- Urease, VP- Voges Proskauer, Cit- Citrate, TDA- Tryptophan deaminase acid , ID-Identifications

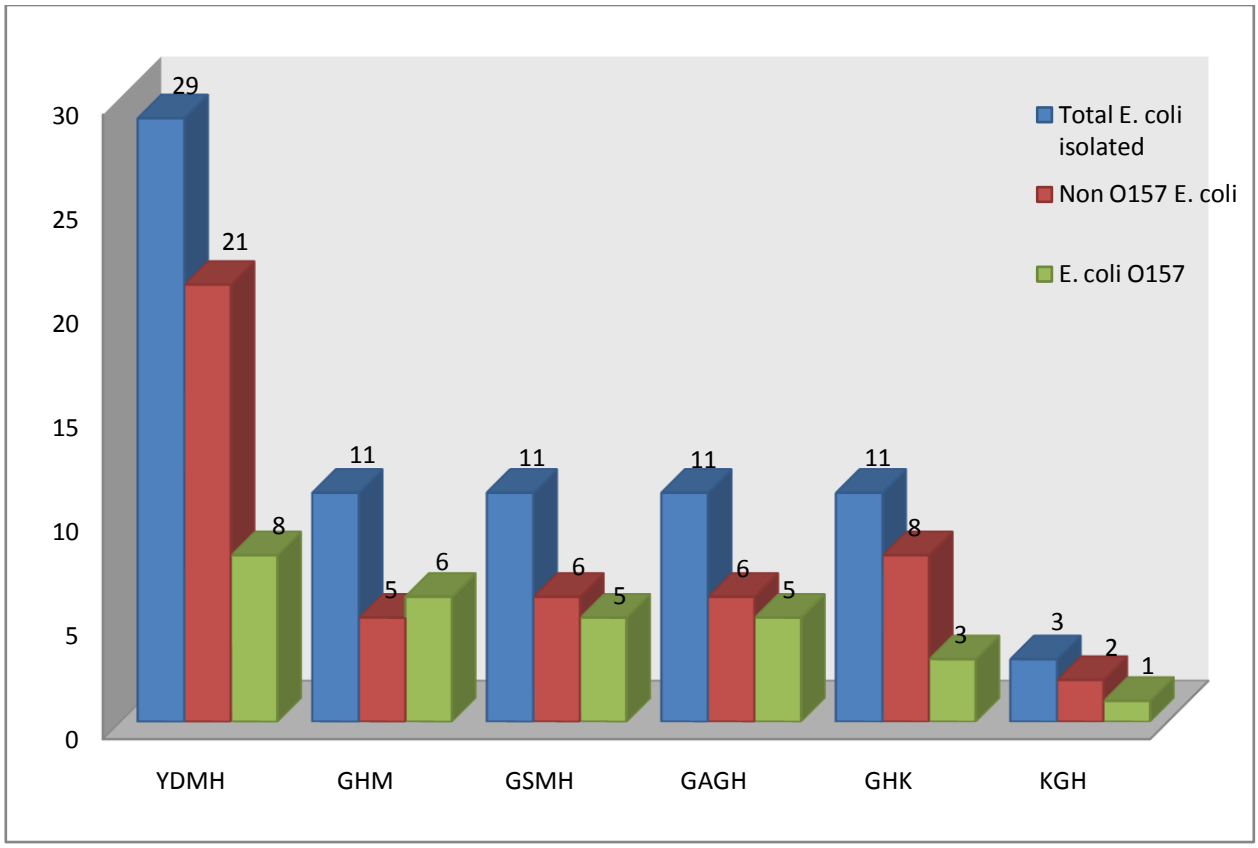

FIGURE 4. 1 Prevalence of *Escherichia coli* O157 and Non-O157 from the total *E. coli* isolated from diarrhoeic stool samples of children (0-5 years) with respect to hospital location.

KEY:GSMH- Gambo Sawaba Memorial Hospital, **GHM**- General Hospital Makarfi, **YDMH**- Yusuf Danstoho Memorial Hospital, **GAGH**- Gomna Awan General Hospital, **GHK**- General Hospital Kafanchan, **KGH**- Kwoi General Hospital.

Table 2.Susceptibility patterns to antimicrobials of *E.coli* isolated from samples of diarrhoeic stool of children under 5-years.

Antibiotic	Disc Potency(µg)	R(%)	I(%)	S(%)
Chloramphenicol	30	0	0	100.0
Gentamicin	30	53.6	5.9	40.5
Tetracycline	30	57.3	3.0	39.7
Cefotaxime	30	97.5	2.5	0
Sulphamethoxazole	25	88.9	7.4	3.7
Ciprofloxacin	5	0	0	100
Amoxicillin	10	67.6	1.5	30.9

Key: R=Resistance, I=Index, S=Susceptible, %= percentage,CEF=Cefotaxime, GN=Gentamicin, AM=Amoxicillin, SXT=Sulphamethoxazole, TET=Tetracycline, CIP=Ciprofloxacin, CH=Chloramphenicol.

Table 4.3.2 Resistance patterns of *E.coli* isolated from diarrhoeic stool samples of children under 5-years (n=76)

Single antibiotic	Resistance Pattern	Multiple Antibiotic Resistance(No. of	No. of isolates (%) with pattern	Resistance pattern

Resistance		antibiotic combinations)		
0(0)	NR	2	NR	NR
		3	NR	NR
		4	VTEC 1(1.3)	GN, TET, CEF, SXT
		5	Non- O157 *E. coli*47(61.8), *E. coli* O157:H7 28(36.84)	GN, TET, CEF, SXT, AM
		6	NR	NR
		7	NR	NR

Key: NR= No resistance, CEF=Cefotaxime, GN=Gentamicin, AM=Amoxicillin, SXT=Sulphamethoxazole, TET=Tetracycline

4.2 Percentage prevalence of underweight, stunting and wasting among diarrhoeic children under five years in Kaduna state

The nutritional status of subjects within the study population can be seen in Fig 4.2. The results obtained, shows the percentage of children underweight as 13.1%, 34.3% of the population were stunted and 24. 3% of the children were wasted.

4.3 The weigh-for-Age Z-score of diarrhoeic children under five years in Kaduna state

Fig 4.3 shows the graph of weight-for-age Z-score of diarrhoeic children under five years. Severe pathological deficits in weight for age are known as long term underweight. Weight-for-age reflects body mass relative to age. It is a composite measure of weight-for-age and weight-for-height. It combines the effect of long term and short term health and nutritional problems and represents a convenient measure of both linear growth and body proportion. Weight-for-age can be used for the diagnosis of underweight in children. The graph shows that the subjects were underweight for their ages in comparism with World Health Organization standard.

4.4 The weight-for-height Z-score of diarrhoeic children under five years in Kaduna state

The graph in Fig 4.4 shows the weight-for-height Z-score of the diarrhoeic children under five years. Low weight-for-height which is also referred to as wasting is normally used as an indicator of current nutritional status and can be used for screening of children at risk of childhood mortality and for measuring short term changes in nutritional status. It reflects a recent and severe process that has led to substantial weight loss. The subjects in the study population did not meet the required World Health Organization standard for weight-for-height as they fell below the -2 standard deviation cut off for weight-for-height.

4.4 Mean anthropometric characteristics of the diarrhoeic male and female children (0-5 Years) in Kaduna State.

Table 4.4 shows the mean ranges of the anthropometric characteristics of the diarrhoeic children under five years of age in the study population. The mean values for weight and height of the 207 male subjects obtained were 11.30±3.50 kg and 84.86±14.55 cm respectively whereas values obtained for the 143 females were 10.63±3.21 kg and

83.87±14.48 cm respectively and the differences in the two mean values were not actually significant. Also reflected in this table are the average Z-scores of weight-for-age, weight-for-height, height-for-age (WAZ, WHZ and HAZ) for both male and female, all of which had negative values or mean Z –scores of less than 0.

4.5 The height-for-age Z-score of diarrhoeic children under five years in Kaduna state

Low height-for-age (Stunting) or linear growth retardation is the best measure of child health inequalities as it is a multi-facet nutritional indicator which captures various dimensions of child health, development and environmental influence. It reflects chronic malnutrition accumulated during pre or post natal periods because of poor nutrition and health (e.g chronic insufficient protein and energy intake). The subjects in study population were highly stunted as they had cut-offs below the -2 standard deviation for height-for-age.

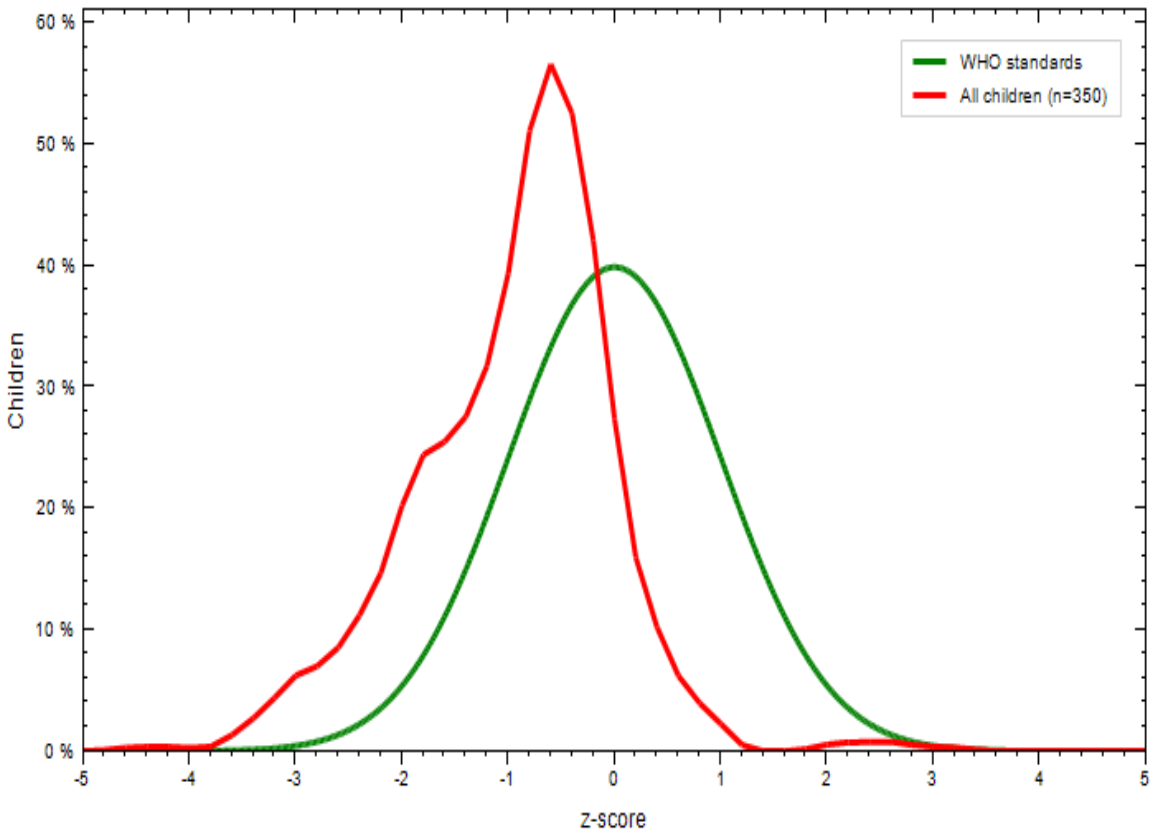

Figure 4.3 Weight for age Z-score of diarrhoeic children (0-5years) in Kaduna

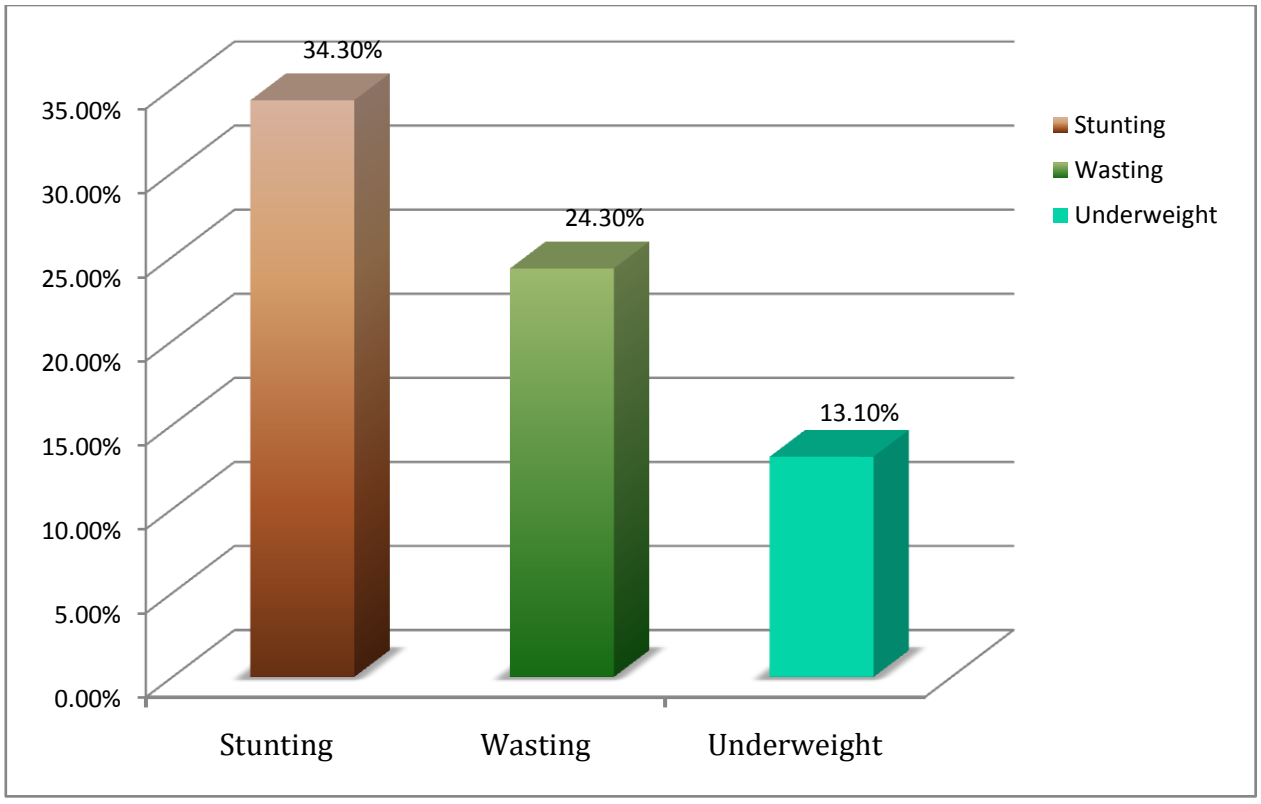

Figure 4.2 Prevalence of underweight, stunting and wasting amongst children (0-5years) in Kaduna State

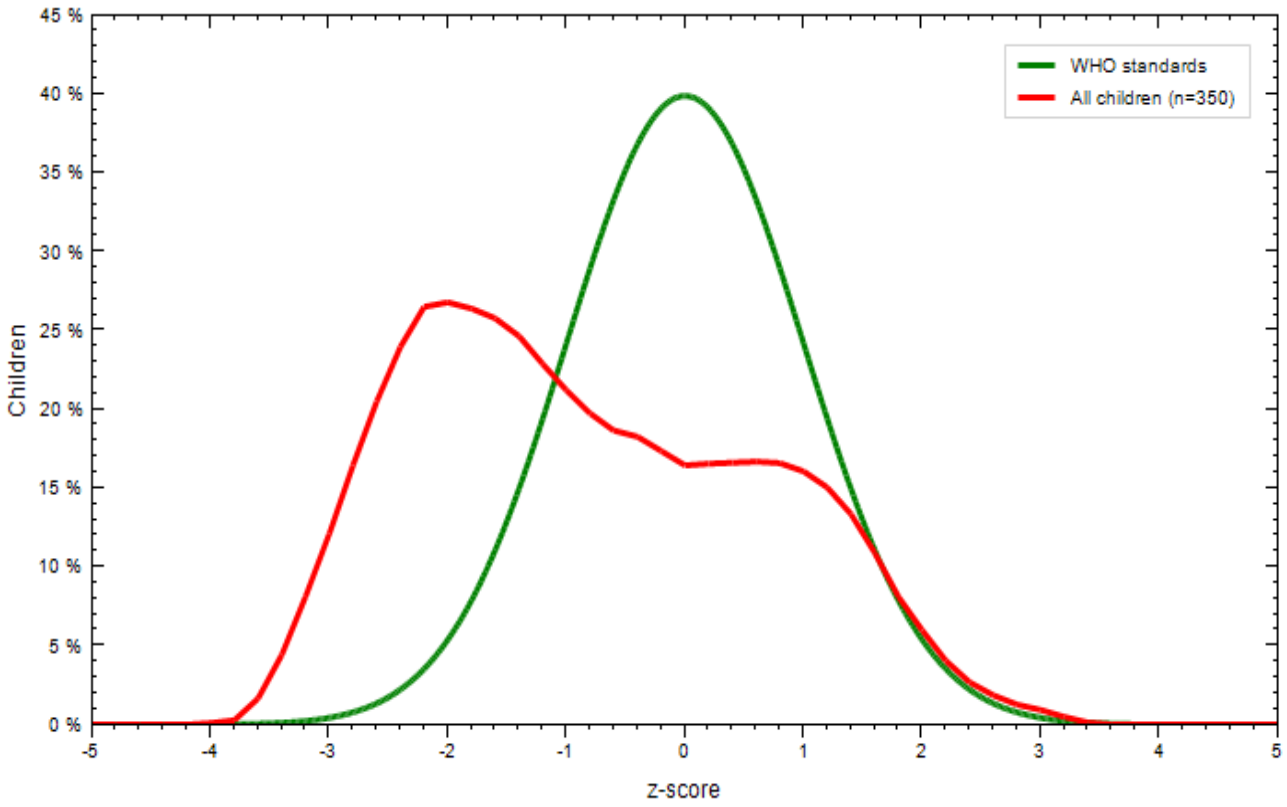

Figure 4.4 Weight-for-height Z-score of diarrhoeic children (0-5years) in Kaduna

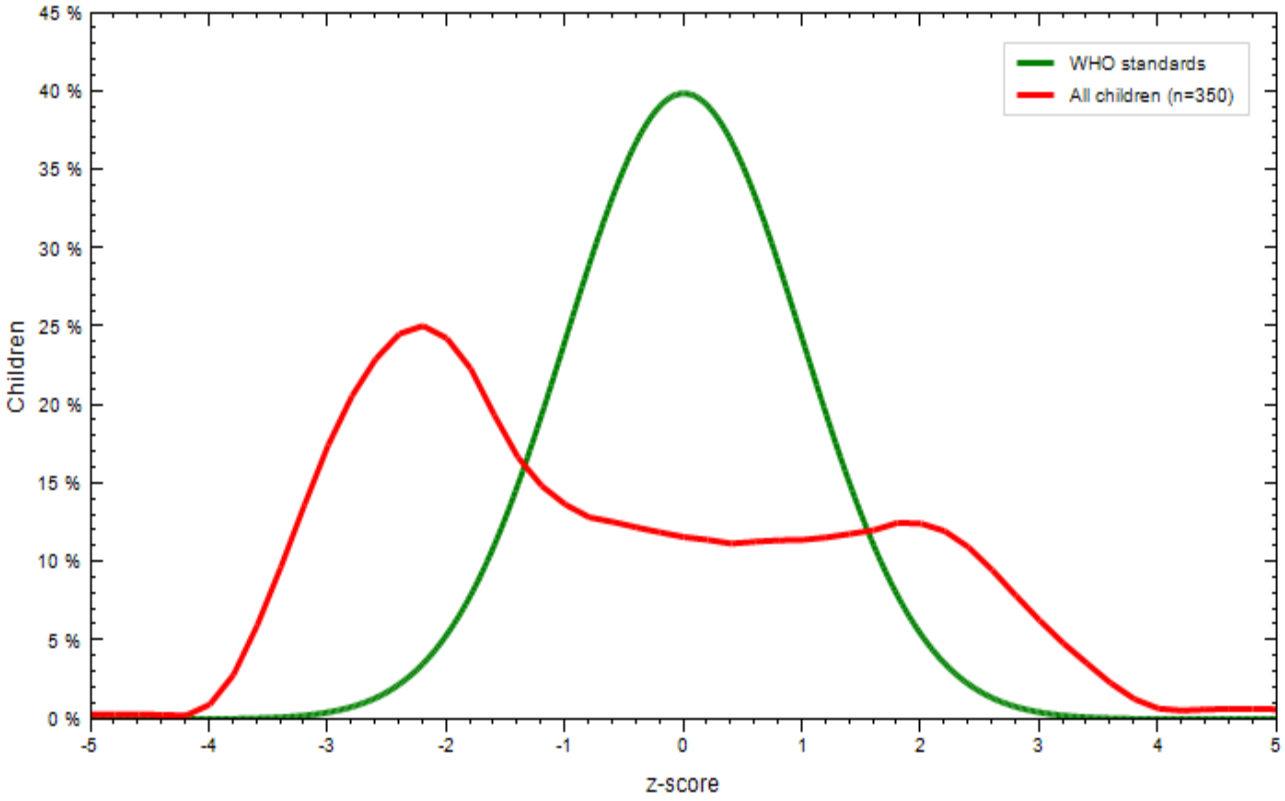

Figure 4.5 Height for Age Z-score of diarrhoeic children (0-5years) in Kaduna

Table 4.4 Mean Anthropometric Characteristics of the Diarrhoeic male and female Children (0-5 Years) in Kaduna State.

Gender	Male(N=207) Mean value ± SD	Female (N=143) Mean value ± SD
Anthropometric indices		
Height (cm)	84.86 ± 14.55	83.87 ± 14.48
Weight (kg)	11.03 ± 3.50	10.63 ± 3.21
Weight for Age (Z-Score)	-1.14 ± 0.96	-0.66 ± 0.76
Weight for Height/length(Z-Score)	-0.90 ±1.41	-0.68 ± 1.44
Length/height for Age	-0.86 ± 1.76	-0.18 ± 2.13

KEY: WAZ= Weight for Age Z-score, HAZ= Height for Age Z-score, WHZ= Weight for Height Z-score,SD= Standard Deviation

4.5 Prevalence of low weight-for-age (underweight) by gender and age group in a sample of 350 diarrhoeic children in Kaduna State.

This table shows the low weight-for-age ranges with respect to age and gender of the diarrhoeic children used in the study population. This information was obtained because it is a very good overall indicator of a population's nutritional health and a composite index

for both weight-for-height and height-for-age. Hence, a child can be underweight for his age if he is either/or both stunted and wasted.It also showed a significant difference in the distribution of the ranges of underweight, (p-value = 0.016). The highest prevalence of underweight in the children was observed amongst those in age group 13-24 months and ≤12 months. The male subjects in the study population had a higher prevalence (16.4%) than the female subjects (8.4%). Underweight recorded the highest rate of severely underweight children in the population.

4.6 Prevalence of low weight-for-length/height (wasting) by gender and age group in a sample of 350 diarrhoeic children in Kaduna State.

In this table, the prevalence of wasting can be obtained and children who are below minus two standard deviations (-2 SD) and minus three standard deviations (-3 SD) from the median of the reference population are regarded as either wasted or severely wasted. Wasting indicates acute malnutrition. The highest prevalence of wasting across the age groups was observed in 25-36 months and the lowest observed amongst ages 37-48 months, with children that were severely wasted falling between age groups 13-24 months. Males also recorded a slightly higher prevalence (26.1%) in this category than females (21.7%).

Table 4.5 Prevalence of low weight-for-age (underweight) by gender and age group in a sample of 350 diarrhoeic children in Kaduna State.

Age range (Months)	Gender	N	wt/age Z-Score Below -3SD n(%)	wt/age Z-Score Below -2SD n(%)	Mean±SD
	Male	60	2(3.3)	13(21.7)	-1.13±1.21
≤12	Female	54	1(1.9)	7(13.0)	-0.67±0.93
	Combined	**114**	**3(2.6)**	**20(17.5)**	-0.91±1.11
	Male	62	4(6.5)	14(22.6)	-1.46±0.94
13-24	Female	38	0(0.0)	4(10.5)	-0.67±0.77

	Combined	**100**	**4(4.0)**	**18(18.0)**	-1.16±0.96
	Male	27	1(3.7)	3(11.1)	-1.12±0.72
25-36	Female	23	0(0.0)	1(4.3)	-0.64±0.58
	Combined	**50**	**1(2.0)**	**4(8.0)**	-0.90±0.69
	Male	26	0(0.0)	4(15.4)	-1.16±0.68
37-48	Female	18	0(0.0)	0(0.0)	-0.68±0.43
	Combined	**44**	**0(0.0)**	**4(9.1)**	-0.96±0.63
	Male	32	0(0.0)	0(0.0)	-0.56±0.42
49-60	Female	10	0(0.0)	0(0.0)	-0.58±0.60
	Combined	**42**	**0(0.0)**	**0(0.0)**	-0.57±0.46
	Male	**207**	**7(3.4)**	**34(16.4)**	-1.14±0.96
Grand Total	**Female**	**143**	**1(0.7)**	**12(8.4)**	-0.66±0.76
	Combined	**350**	**8(2.3)**	**46(13.1)**	-0.95±0.91

Male: X^2=9.777, p-value=0.44,Female: X^2=4.747, P-value=0.314,Total: X^2=12.147, P-value=0.016*

Table 4.6 Prevalence of low weight-for-length/height (wasting) by gender and age group in a sample of 350 diarrhoeic children in Kaduna State.

Age range (Months)	Sex	N	wt/ht Z-Score Below -3SD n(%)	wt/ht Z-Score Below -2SD n(%)	Mean±SD
	Male	60	0(0.0)	8(13.3)	-0.74±1.41
≤12	Female	54	0(0.0)	5(9.3)	-0.33±1.34
	Combined	**114**	**0(0.0)**	**13(11.4)**	-0.54±1.39
	Male	62	1(1.6)	25(40.3)	-1.60±1.09
13-24	Female	38	0(0.0)	15(39.5)	-1.48±1.10
	Combined	**100**	**1(1.0)**	**40(40.0)**	-1.56±1.09
	Male	27	0(0.0)	13(48.1)	-1.56±1.04
25-36	Female	23	0(0.0)	9(39.1)	-1.29±1.43
	Combined	**50**	**0(0.0)**	**22(44.0)**	-1.43±1.22
	Male	26	0(0.0)	3(11.5)	-0.16±1.27
37-48	Female	18	0(0.0)	1(5.6)	-0.23±1.21
	Combined	**44**	**0(0.0)**	**4(9.1)**	-0.03±1.25
	Male	32	0(0.0)	5(15.6)	0.10±1.42
49-60	Female	10	0(0.0)	1(10.0)	0.29±1.56
	Combined	**42**	**0(0.0)**	**6(14.3)**	0.14±1.44
	Male	**207**	**1(0.5)**	**54(26.1)**	-0.90±1.41
Grand Total	**Female**	**143**	**0(0.0)**	**31(21.7)**	-0.68±1.44
	Combined	**350**	**1(0.3)**	**85(24.3)**	-0.81±1.43

X^2 =0.894, P value=0.344

4.7 Prevalence of low height/length-for-age (stunting) by gender and age group in a sample of 350 diarrhoeic children in Kaduna State.

Table 4.7 shows the prevalence of low height-for-age with reference to gender and age of the children. Low height for age,which reflects whether a child is stunted (below -2 SD) or severely stunted (below -3 SD), indicates a condition of cumulative chronic malnutritionin the children. There was statistical difference in the distribution of the prevalence of stunting. Approximately 50% rate of stunting was observed amongst children in age group 37-48 months and the lowest rate of stunted children found in age group 25-36 months. A slightly higher prevalence was also recorded amongst the male subjects (36.7%) than the female subjects (30.8%) in the study population.

4.8 Prevalence of underweight, wasting and stunting amongst diarrhoeic children under five years in Kaduna state.

Table 4.8 shows the distribution of the three malnutrition indices obtained from diarrhoeic children under five years of age in relation to location. It can be seen from the table that Kaduna South senatorial district, under which General Hospital Kafanchan falls, was mostly affected nutritionally, as it recorded the highest prevalences in all three indices.

4.9 Prevalence of *E. coli* isolated from diarrhoeic stool samples with respect to age and gender.

Table 4.9 shows the distribution of *E. coli* isolated according to age and gender. With respect to gender, the result shows that out of the 350 diarrhoeic stool samples obtained, 208 where from male subjects and 142 from female subjects. The females had a higher prevalence of 23.9% as against the males which was 20.2% but the difference in the distribution was not statistically significant (p =0.403, odds ratio = 0.804, CI= 0.481-1.342).With respect to age, the differences in the distribution observed were also not statistically significant within ages.

Table 4.7Prevalence of low height/length-for-age (stunting) by gender and age group in a sample of 350 diarrhoeic children in Kaduna State.

Age range (Months)	Gender	N	ht/age Z-Score Below -3SD n(%)	ht/age Z-Score Below -2SD n(%)	Mean±SD

	Male	60	0(0.0)	26(43.3)	-0.90±1.84
≤12	Female	54	0(0.0)	21(38.9)	-0.41±2.21
	Combined	**114**	**0(0.0)**	**47(41.2)**	-0.67±2.03
	Male	62	2(2.3)	20(32.3)	-0.65±2.05
13-24	Female	38	0(0.0)	9(23.7)	0.67±2.25
	Combined	**100**	**0(0.0)**	**29(29.0)**	-0.15±2.21
	Male	27	0(0.0)	6(22.2)	-0.16±1.66
25-36	Female	23	0(0.0)	3(13.0)	0.42±1.79
	Combined	**50**	**0(0.0)**	**9(18.0)**	0.11±1.73
	Male	26	0(0.0)	14(53.8)	-1.78±1.18
37-48	Female	18	0(0.0)	8(44.4)	-1.43±1.40
	Combined	**44**	**0(0.0)**	**22(50.0)**	-1.63±1.27
	Male	32	0(0.0)	10(31.3)	-1.05±1.34
49-60	Female	10	0(0.0)	3(30.0)	-1.27±1.36
	Combined	**42**	**0(0.0)**	**13(31.0)**	1.10±1.33
	Male	**207**	**2(1.0)**	**76(36.7)**	-0.86±1.79
Grand Total	**Female**	**143**	**0(0.0)**	**44(30.8)**	-0.18±2.13
	Combined	**350**	**2(0.6)**	**120(34.3)**	-0.58±1.96

Male:X^2=7.797, p-value=0.099,Female:X^2=7.542, p-value=0.110,TotalX^2=14.594, p-value=0.006*

Table 4.8 Prevalence of underweight, wasting and stunting amongst the diarrhoeic children with respect to region

Region	**N**	**Wasting(wt/ht)**		**Underweight(wt/age)**		**Stunting(ht/age)**	
		<-3 (%)	**<-2**	**<-3 (%)**	**<-2**	**<-3 (%)**	**<-2**
Kaduna-Central (YDMH and GAGH)	180	0.6	22.8	2.2	14.4	0.0	35.0
Kaduna- North (GSMH and GHM)	124	0.0	20.2	2.4	7.3	0.8	30.6
Kaduna – South (GHK and KGH)	46	0.0	41.3	2.2	23.9	2.2	41.3
Total	350	0.3	24.3	2.3	13.1	0.6	34.3

KEY: GSMH- Gambo Sawaba Memorial Hospital, **GHM**- General Hospital Makarfi, **YDMH**- Yusuf Danstoho Memorial Hospital, **GAGH**- Gomna Awan General Hospital, **GHK**- General Hospital Kafanchan, **KGH**- Kwoi General Hospital.

Table 4.9 Prevalence of *E. coli* isolated from diarrhoeic stool of children under five years with respect to age and gender.

Factor	No Examined	No Positive (%)	X^2	P. value
Gender				
Male	208	42(20.2)		
Female	142	34(23.9)	0.699	0.403
Age (months)				
≤12	114	31(27.2)	3.631	0.458
13 -24	100	21(21.0)		
25 -36	50	10(20.0)		
37 – 48	44	7(15.9)		
49 – 60	42	7(16.7)		
Total	**350**	**76(21.7)**		

Gender Odds Ratio= 0.804, CI= 0.481-1.342,Key: x^2-chi-square, p-value, (%) =Prevalence, No=Number

4.10 Prevalence of diarrhoeagenic *E. coli* with respect to some demographic factors

Table 4.10 shows the demographic factors considered in this study as, parent's level of education, mother's occupation and an estimated level of income. From the results obtained, there was a statistical significance (p-value = 0.026) in the distribution of differences obtained in the parent's level of education, in which children whose parents had Primary or no education at all had higher prevalence of 34.6%, children whose mothers were unemployed had a prevalence of 23.7% and 36.7% prevalence was obtained for those whose parents earned 10,000 and below. A statistically significant difference (p= 0.003) was observed amongst those whose earnings are ₦10,000 and below.

4.11 Prevalence of *E. coli* in relation to some risk factors associated with diarrhoea in children under five years.

Table 4.11 shows the risk factors considered in this study include, source of water, type of water treatment, mode of feeding and type of toilet. In the source of water category, a prevalence of 25% was obtained for those whose source is river/stream, 25.6% obtained for those who did not employ any form of treatment for their water, 28% prevalence obtained for children whose mode of feeding is the feeding bottle and 25% prevalence obtained for those whose toilet is an open field. Although, the above listed risk factors are routes for contamination and infection, the results obtained shows that statistical significance was observed in the treatment of water as a risk factor (p = 0.012), where it can be seen that not treating water before use could expose these children to risk of diarrhoeal infections.

Table 4.10Prevalence of *E. coli*isolated from diarrhoeic stool of under five children in relation to some demographic factors

Demographic Factors	No Examined	No Positive (%)	X^2	p-value
Level of Parent's Education				
None	10	3(30.0)	11.068	0.026*
Primary	52	18(34.6)		
Secondary	170	38(22.1)		
Tertiary	98	12(12.2)		
Adult Literacy	18	5(27.8)		
Mother's Occupation				
Unemployed	139	33(23.7)	1.071	0.899
Civil servant	53	12(22.6)		
Trader	75	16(21.3)		
Farmer	30	5(16.7)		
Others	53	10(18.9)		
Estimated Level of Income (₦)				
<5000	30	9(30.0)	16.068	0.003*
5001-10000	60	22(36.7)		
10001-20000	154	33(21.4)		
20001-40000	53	5(9.4)		
>40000	53	7(13.2)		
Total	**350**	**76(21.7)**		

Key:x^2-chi-square, p-value, (%) =Prevalence, No=Number, (*)=Statisticallysignificant

Table 4.11 Prevalence of *E. coli* in relation to some risk factors associated with diarrhoea in children under five years.

Risk Factors	Total Examined	Positive (%)	X^2	p-value
Sources of water				
Pipe borne	125	28(22.4)	2.347	0.672
Well	142	33(23.2)		
Borehole	51	7(13.7)		
River/stream	4	1(25.0)		
Others	28	7(25.0)		
Treatment of water				
Boiling	37	3(8.1)	10.912	0.012*
None/Raw	258	66(25.6)		
Filtering	39	3(7.7)		
Others	16	4(25.0)		
Mode of feeding				
Personal plate	147	30(20.4)	3.087	0.214
Feeding bottle	93	26(28.0)		
Communal	110	20(18.2)		
Type of toilet				
Pit latrine	203	48(23.6)	1.353	0.717
Open field	12	3(25.0)		
Water closet	112	21(18.8)		
Others	23	4(17.4)		
Total	**350**	**76(21.7)**		

Key: x^2-chi-square, p-value, (%) =Prevalence, No=Number,(*)=Statistically significant

4.12 Relationship between occurrence of diarrhoea and the child's physical examination

Table 4.12 shows the relationship between diarrhoea and the child's physical examination. The results obtained shows the percentage prevalence of severely malnourished children as being higher than the other parameters under general condition, severity in dehydration level and slow recovery in the examination of pinched skin, although the different distributions were not statistically significant. The general condition of the subjects in the study showed 30% prevalence of severely malnourished children, the dehydration level

was severe among the subjects with a prevalence of 32.1 and 33.3% prevalence for the children whose skinwhen pinched returned very slowly. These three conditions are related in that a severely malnourished child would also be severely dehydrated and this dehydration leads to slow skin reponse.

4.13 Prevalence of diarrhoea with respect to clinical symptoms presenting in the diarrhoeic children

Table 4.13 shows the prevalence of diarrhoea with respect to clinical symptoms and symptoms associated with diarrhoea such as, fever, blood in stool, number of episodes and duration in days were all taking into consideration in the course of this study. The subjects in this study who experienced diarrhoeal episodes of more than 7 times had a prevalence of 41.7 and 42.9% for those who had the symptoms for a duration of 4-6 days. The results obtained showed statistical significance in the differences in distribution in terms of number of episodes ($p = 0.009$)and the duration in days ($p = 0.046$).

Table 4.12 Relationship between occurrence of diarrhoea and the child's physical examination

Physical Examination	No Examined	No Positive (%)	X^2	p-value
General condition				
Well/Alert	91	20(22.0)	1.575	0.813
Severely malnourished	30	9(30.0)		
Restless/Irritable	94	20(21.3)		
Normal Eyes	54	10(18.5)		
Sunken Eyes	81	17(21.0)		
Dehydration Level				
None	121	28(23.1)	5.674	0.059
Moderate	173	30(17.3)		
Severe	56	18(32.1)		
Pinched skin				

Quickly	170	39(22.9)	4.615	0.100
Slowly	144	25(17.4)		
Very slowly	36	12(33.3)		
Total	**350**	**76(21.7)**		

Key: X^2-chi-square, p-value, (%) =Prevalence, No= Number.

Table 4.13 Prevalence of diarrhoea with respect to clinical symptoms presenting in the children

Clinical Symptoms	No Examined	No Positive (%)	X^2	P-Value
Fever				
Yes	211	49(23.29)	0.711	0.399
No	139	27(19.4)		
Bloody stool				
Yes	44	12(27.3)	0.915	0.339
No	306	64(20.9)		
No of Episodes				
≤3	141	27(19.1)	9.409	0.009*
4-6	173	34(19.7)		
≥7	36	15(41.7)		
Duration (days)				
≤3	321	66(20.6)	6.173	0.046*
4-6	21	9(42.9)		
≥7	8	1(12.5)		
Total	**350**	**76(21.7)**		

Key: X^2-chi-square, p-value,(*)=Statistically significant (%) =Prevalence,No= Number

CHAPTER FIVE

5.0 DISCUSSION

A total of seventy six (76) isolates identified as*E. coli*were obtained from three hundred and fifty (350) diarrhoeal stool samples of children under five years of age, in Kaduna state,giving a prevalence of 21.7%. Data from other researches showed22.37% prevalence reported in Bosnia and Herzegovina (Dedeic-Ljubovic *et al*., 2009), and the 22.6% reported by Mandomando *et al*(2007) in a study conducted in Mozambique. Higher prevalence of 44.74%was obtained in a study by Nweze (2010), 34.1% by Raji *et al.,* (2008) in Tanzania and 60% reported by Eseigbe *et al.*(2013) in a previous study conducted in Kaduna. Behiry *et al.*(2011) reporteda percentage total of *E. coli* isolates in children under five years as 51.5%.Nweze, (2010), reported the isolation of 119 out of 520 stool samples of children, with 22.88% prevalence in Southeast Nigeria, Akingbade *et al*.(2013) also reported a 51% identification of *E. coli* from children with diarrhoea in Abeokuta. Dhanashree and Shrikar Mallaya, (2008) reported a prevalence of 73.96% *E. coli*in India.

Onanuga *et al*.(2014), reported that 18.4% of diarhoeagenic *E. coli* isolated from children with diarrhoeain Gwagwalada Nigeria and 62.8% prevalence obtained from diarrhoeic children, also in Abuja, Nigeria by Ifeanyi *et al*.(2010). Al-Dawmy and Yousif, (2013) in a study carried out in Baghdad reported a prevalence of 54.7%. Twenty eight 28(36.84%) of the 76 isolates obtained in this study, were found to be non-sorbitol fermenting,and this

prevalence obtained was found to be higher than the 22.4% prevalence reported by Asamole-Osuocha(2006) in Abuja, Nigeria.

The isolates were further confirmed to be *E. coli* O157 by latex agglutinationkit. A total of twenty eight (28) of the 350 stool samples gave positive cultures of *E. coli* O157: H7, and hence a prevalence of 8.0%, this is higher than the prevalence of 5.9% reported from a study conducted in Ondo state Nigeria (Ademokoya *et al.*, 2014) and 4.78% prevalence reported by Al-Dawmy and Yousif, (2013). Studies carried out in Abuja by Asamole-Osuocha in (2006)showed that the faecal samples did not yield *E. coli* O157.

One(1) isolate (1.3%) produced the VTEC toxin also known as STEC, this agrees with the 1.9% recorded in Mozambique by Mandomando *et al*.(2007) but was much lower than the 15.09% prevalence of VTEC reported by Asamole-Osuocha, (2006).

This study is also consistent with previous studies conducted by Villa *et al*.(1999), Cunin *et al*.(1999); Galane and Le Roux(2001) and Presterl *et al*.(2003) in South Africa, Swaziland, sub-Saharan Africa and Tanzania with reports that there was low frequency of verotoxin-producing *E. coli* in the sample population. The 1.3% detection of VTEC in this study is similar to the 1.3% detected and reported by Verma *et al*.(2013) in India, but lower than the 3.6% prevalence reported by Raji *et al*.(2008) and 5.1% incidence rate reported by Ogunsanya *et al*.(1994) in studies carried out in Lagos, Nigeria. Khan *et al*.(2002)also from India reported a low incidence of 2% prevalence of STEC in humans, Dhanashree and Shrikar Mallaya(2008) also reported a low incidence of1.4% prevalence of STEC from diarrhoeal stools.In this study, the prevalence of *Escherichia coli* aetiology of diarrhoea is 21.71% which is lower than the research conducted Lagos, Nigeria which was reported to be 39.5% (Ogunsanya *et al*., 1994) and also in Kaduna State which was found to be 34.1%

by Sule *et al.*(2011). A study conducted in Dhaka, Bangladesh reported that among 200 stool samples collected from patients aged, 0 to 60 months, *E. coli*was isolated in 135 (67.50%), as reported by Roy *et al*.(2013).

Escherichia coli was the most encountered of the organisms isolated in the study of aetiologic agents of diarrhoea, with a prevalence of 77.8% (Olowe *et al.,* 2003). Mengistie *et al*.(2013) reported a prevalence of diarrhoea aetiology in under five years old children in Ethiopia, to be 22.5%. Seventy eight percent (78%) of the three hundred and fifty diarrhoeal cases investigated had no *Escherichia coli* isolated from them, which suggests the possible presence of other bacterial pathogens, viral pathogens or non pathogenic factors, and this also shows relation to the work done by Sule *et al*.(2011).It was observed in this study that Kafanchan General Hospital had the highest prevalence (40.74%) of *E. coli* isolated from the samples obtained therefrom and this could be as a result of the insurgencies and unrest ongoing at the time of sampling which could have led to displacement of the people, rendering them homeless and having to rely on refugee camps which may not be very hygienic, as they are too crowded and do not possess the necessary social amenities and facilities to satisfy the need of the people housed therein.A prevalence by location (in this case, hospitals) showed Gambo Sawaba Memorial Hospital, Zaria having 19.64% as percentage of occurrence of *E. coli* in stools as against 5.4% reported by Vincent *et al*. (2010) showing an increase in the rate of infection in the city, which could be tied to negligence on the part of the parents and care-givers as regards both personal and environmental hygiene.

The antibiotic susceptibility patterns of the isolates as shown on table 4.3.1 and 4.3.2 indicates resistance of the isolates to cefotaxime, tetracycline, gentamicin,

sulphamethoxazole and amoxicillin, and multiple antibiotic resistance in the isolates. Verma *et al.* (2013) in India, reported resistance to antibiotics such as tetracycline, ampicillin, streptomycin, nalidixic acid, neomycin, cefalothin and cotrimoxazole, and also showed multi-drug resistance in 14 strains. Asamole-Osuocha (2006) reported a 100% resistance of isolates to all the six antimicrobials such as ciprofloxacin, sulphamethoxazole, tetracycline, amoxicillin, chloramphenicol and streptomycin used in the study. The level of resistance observed in this study for trimethoprim-sulfamethoxazole reflects the results from several studies by other authors who demonstrated high rates of resistance towards enteric *E. coli* against this drug. One explanation for this could be its widespread use in the treatment of diseases associated with Gram-negative bacteria, especially in children under two years of age with acute infectious diarrhoea(Aranda *et al*., 2004). Eseigbe *et al.*(2013), in a study in Kaduna, reported that the *E. coli* isolates were more sensitive to cefuroxime, ceftriaxone and streptomycin than ciprofloxacin, and this was in contrast to the more commonly used drugs, in the treatment of diarrhoea and this disagrees with the level of sensitivity of ciprofloxacin, observed in this study. However, there has been limited use of the drug, except in very rare cases due to its reported effect on growth (Oshikoya, 2006).

This study also however, agrees with that of Eseigbe *et al.*(2013), with respect to the limited sensitivity of the isolates to most of the commonly prescribed antibiotics. Akingbade *et al*.(2013) also reported that most of the *E. coli* isolates were resistant to cotrimoxazole, erythromycin, ampicillin and tetracycline, due to widespread and indiscriminate use of these antimicrobials.No resistance pattern was obtained with the single antibiotic used in this study, rather they were found to be resistant to more than one antibiotic. This data is in accordance with earlier reports by Dhanashree and Shrikar

Mallaya, (2008), were approximately more than half of the *E. coli* isolates tested displayed resistance to one or more antimicrobials. Akingbade *et al*.(2013) also reported antimicrobial resistance of the isolates to more than half of the antibiotics used. Schroeder *et al*.(2002) and Bettleheim *et al*.(2003) also had similar data reports on the susceptibility pattern of *E. coli* isolates.Regarding the gentamicin and tetracycline, low levels of intermediate resistance were found, corroborating data in the literature which suggest a good activity of these antimicrobials against enteric Gram-negative bacilli. Moreover, such drugs are considered as antimicrobials used in hospitals, and resistant bacteria originating from the community are not expectedto thrive (Usein *et al*., 2009).

Multiple resistance patterns were obtained in this study, with varying combinations of 4 and 5 antibiotic combinations. Vincent *et al.*(2010) reported multidrug resistance to 8 and 9 antibiotic combinations in a study carried out in Zaria, Kaduna state.The VTEC isolate in this studywas resistant to four (4) of the seven (7) antibiotics which includes tetracycline, gentamicin, cefotaxime and sulphamethoxazole. The*E. coli* O157 and non-O157 isolates were resistant to cefotaxime, tetracycline, sulphamethoxazole, gentamicin and amoxicillin indicating that chloramphenicol and ciprofloxacin were the most active antibiotics used.The high prevalence of multiple antibiotic resistance obtained in this study may be because *E. coli*acts as a reservoir for resistance available to enteric pathogens (Akingbade *et al*., 2013) or may be due to the fact that antimicrobial resistance in *E. coli* has increased worldwide and its susceptibility patterns show substantial geographic differences and variations (von Baum and Reinhard, 2000).Kibret and Abera (2011) in a study carried out in Ethiopia, reported high resistance rates of *E. coli* to amoxicillin and tetracycline and also

high rates of multiple antibiotic resistance, and this agrees with the findings obtained in this study.

The overall resistance to antimicrobials was found to be high in this study, just as was observed in previous studies carried out in Trinidad (Orett and Shurl, 2001).The *Escherichia coli* isolates obtained from this study showed resistance to more than half of the antimicrobials used in this study and this is in agreement with previous studies, this could either be due to the fact that antimicrobials are unnecessary in the treatment of majority of infantile diarrhoea cases but are often misused in this condition, non-adherence to duration of antibiotics prescribed or that the use of these drugs has been a key factor in the emergence of antimicrobial-resistant *E. coli* (Raji *et al.,* 2008; Schroeder *et al.,* 2002; Ifeanyi *et al.,* 2010). It could also be because the misuse of antimicrobials generally impacts resistance in enteric organisms heavily and directly because they readily acquire resistance genes from the faecal flora.

Prevalence of the *E. coli* isolates in relation to risk factors as seen in table 4.11 indicates thattwenty five percent (25%) was obtained for children whose source of drinking water is from rivers and streams, showing high contamination of these untreated waters by *E. coli*. This is in agreement with work done by Vincent *et al*.(2010) in Kaduna. It can also be tied to the high percentage of *E. coli* in the stools of the children who are given water from wells, since these raw waters are the surface waters treated for drinking, irrigation, recreation. Poor sewage disposal with the rains washing these surface waters into rivers and streams.

The 8% prevalence of *E. coli* O157 isolated from the stools of diarrhoeic children used in the study, though higher than the 5.4% prevalence obtained by Vincent *et al.*(2010) further suggests that the epidemiological situation in Nigeria still needs to be looked into properly. Omoikhodon *et al.*(1998) in Ibadan, reported that 33% of children of market women had diarrhoea and this was low in comparison with other occupations. This however, shows a similarity with the low rate of the bacteria recorded in this study with regards to trading as an occupation, and this could be attributed to the fact that most markets these days have received government attention and unions are established to ensure cleanliness and orderliness.

In this study, 7.4% of the children presenting with diarrhoea showed no signs of dehydration, and this was second to the 9.2% obtained in moderately dehydrated cases which follows the trend of reports made, in which majority of the children did not show any signs of dehydration. The severity of dehydration obtained, which was 5.4%, tallies with the 5.2% obtained by Ahmed *et al.* (2009). The higher rate of *E. coli* being isolated from females (23.9%) more than males (20.2%) correlates the result obtained by Sule *et al.*(2011) though disagreeing with the reports of Abdullahi *et al.*(2010) and Nweze (2010) in relation to gender. However there is an agreement on the necessity for prolonged breastfeeding of children until about age one to reduce their risk of infection, though this can be only achieved if the mother takes strictly to personal hygiene.

The odds of having diarrhoea was not significantly associated with sex (females, 23.9% and males, 20.2%). This agrees with a study done in teaching hospital in Nigeria where males affected were 54% and female 40%, with the odds of having diarrhoea not significantly related to sex (Donald *et al.,* 1993), but disagrees with previous study carried

out in Kaduna, in which 156 males were affected, as against the 114 females affected (Eseigbe *et al.,* 2013). A study done on the children living in urban slums in Salvador, Brazil on incidence of diarrhoea revealed that male children were associated more with episodes of diarrhoea as compared to their female counterparts (Yilgwan and Okolo, 2012), a similarity was also reported in a study conducted by Ogunsanya *et al*.(1994) in Lagos, Nigeria.

A similar study done in Denmark found that being a male, one stands a chance of getting diarrhoea (Maria Clotildes *et al.,* 2008), and this contradicts our finding in this study. Children in the age group less or equal to 1year had the highest prevalence of diarrhoea followed by those in age group 1 to 2years and this agrees with the reports of Mengistie *et al*.(2013)Ifeanyi *et al*.(2010)Desalegn *et al.*(2011) and Boadi and Kuitunen (2005) on the prevalence of diarrhoea with respect to age, attributing this high prevalence to changes like the introduction of contaminated weaning foods and risk of ingesting contaminated materials while crawling (Dewey and Adu-Afarwuah, 2008; Motarjemi *et al*., 1993). Ogunsanya *et al*.(1994) had a contrary result from this study, as regards diarrhoea and its relationship with age group. Asamole-Osuocha, 2006 reported ages 0-5years as the most afflicted with diarrhoeal diseases in Abuja. According to a study done in Saudi Arabia by Mazrous and others, incidence of diarrhoea was significantly associated with fathers' occupation ($p = 0.0011$) but no significant association was found between the incidence of diarrhoea and parents'education (Ifeanyi *et al.,* 2010).

In this study we obtained an association between the parent's level of education, mother's occupation, estimated level of income and diarrhoea, as seen in table 4.10. Mengistie *et al*.(2013) also reported an association between the parent's level of education and

diarrhoea, and this disagrees with our findings. Bui *et al.* (2006) reported that the risk of diarrhoea in children whose mothers had only primary education is 2.2 times higher than those whose mothers had higher level of education. Comparing with primary education, higher levels of education of mothers, such as secondary and tertiary, are associated with decreased risk of diarrhoea. This is contrary to our findings in which children whose mothers have only secondary or quranic education were more at risk of the disease. They also reported low income in particular and poor economic status in general as associated with increased diarrhoea, this also agrees with our findings.

The nutritional status of the subjects used in this study as shown in fig 4.2 showed underweight percentage as 13.1%, higher in prevalence and disagreeing with work done inKaduna (11%), Niger (7%) and Anambra (0.8%) states which reported lower rates of underweight in the children, suggesting that the State, thus far has not done enough in the area of eradication of childhood malnutrition.It has long been recognized that there is an intimate relationshipbetween diarrhoeal disease and undernutrition in pediatricpopulations in developing countries(Anigo*et al*., 2009; Akubugwo *et al*., 2014).The results obtained in this study on the prevalences of underweight, stunting and wasting with respect to location (Kaduna-central, Kaduna-north and Kaduna-south senatorial districts) with Kaduna-south senatorial district having the highest prevalences in all three indices, agrees with reports obtained from previous studies conducted in Anambra (Akubugwo *et al*., 2013).

The rate of stunting amongst the children was also on the high side with 34.3% and this was higher than the 1.1% reported by Akubugwo *et al*., 2014, showing that the population is retarded in linear growth, which reflects chronic malnutrition accumulated during pre or post natal periods due to poor nutrition and poor health (Cogil, 2003; Dewey and Begum,

2011; de Onis *et al*., 2012) and this high rate of stunting only goes to show that the children are more at risk of having low mental development, reduced work capacity by the time they attain adulthood and increased obstetric risks (Hautvast *et al*., 1999). Also the rate of stunting (34.3%) reported in this study, indicates a rise towards the 42% stunting rate of National average rate of stunting recorded by the Nigerian Food Consumption and Nutrition Survey (NFCNS, 2004).

This study presents a higher prevalence of 34.3%, as seen on fig 4.2 for stunting amongst the test subjects, as against the reported expected global prevalence of stunting which was supposed to reduce from26.7% to 21.8% by the year 2010, (de Onis *et al*., 2012). Available data from the United Nations Children's Fund (UNICEF) estimates that the prevalence of childhood stunting in Nigeria at about 41% making it a major public health problem in the country, and needing quick attention (UNICEF, 2009). Adekanmbi *et al*.(2013) reported that48.7% of under five children in Kaduna State are stunted, this is more than the 34.3% obtained in this study and although showing a lot of decrease, it is also still on the high side and there is a need for more action to be taken to fully combat this menace, so as to achieve the standard nutritional status possible for these children.

The prevalence of 34.3% obtained in this study for stunting agrees with the report of Adekanmbi *et al*.(2013) about childhood stunting in Nigeria being higher in the Northern States, further buttressing the point that these variations could be attributed to "special causes", noting the fact that Northern States have higher poverty rates and health deprivation index than other states.

The result obtained in this study as seen in fig 4.2reflects that 24.3% of the population sampled were wasted and the prevalence of wasting decreased as the age ranges increased,

this is in consonance with the 24.9% prevalence reported by Akubugwo *et al*.(2014) and this could be due to poor access or scarcity to staple foods due to increased poverty levelsand the period of sampling, which was during the farming season, in the north.This study records a much higher rate of wasting (24.3%), an indication of chronic malnutrition than that reported in 2006 by the Nigerian Demographic and Health Survey (DHS) which indicated that 14% of Nigerian children aged 0-59months were wasted (NDHS, 2006) and studies conducted by Anigo *et al*.(2009)also showed Kaduna State as having a much lower rate of wasting (10.38%) in children of the same age (0-5 years).Sufiyan *et al*., 2012, also reported a 7% rate of wasting in children in Zaria city, which is lower than the 24.3% prevalence of wasting obtained in this study.

Another prevalence of 3.7%, also lower than our own findings was reported by Aliyu *et al*.(2012) as the prevalence obtained in a study conducted in Northern Nigeria.From this study, we see that the most prevalent form of malnutrition observed in the State was stunting (34.3%) .

The data obtained on tables 4.5, 4.6 and 4.7shows the percentage prevalence of children classified as malnourished, according to height-for-age, weight-for-height and weight-for-age indices by age and gender. Generally, males in this study had slightly higher mean weight and height than the females in this study. This is contrary to reports on mean weight and height of males and females in another study on Northern Nigeria by Aliyu *et al*.(2012). The results obtained in this study shows that,24.3%of the children under five years of age are considered to be too thin for their age or wasted and 0.3% are severely wasted, it also shows wasting hitting a very high peak between age 2-3years with a percentage prevalence of 44%, this is higher than the 18% and 9% respectively reported for

wasted and severely wasted, and a peak at age 9-11months (27) reported in the 2013 NDHS survey for children in the same age range as well as the 2.4% prevalence of wasting reported by Akor *et al.*(2010).The results obtained in this study for wasting indicates that 24.3% fell below the -2 and -3 standard deviation cut-offs for World Health Organization reference standard, making them severely wasted for their ages.

The results obtained was also higher than the 3.7% prevalence for wasting obtained in a previous study conducted in Kaduna by Aliyu *et al.*(2012). With respect to the percentage prevalence of weight-for-age (underweight) of the said children, a total of 13.1% considered as underweight and 2.3% as severely underweight, with underweight peaking at age 1-2years with a prevalence of 18% and this was less than the 28% underweight and 12% severely underweight, with the proportion of children underweight increasing sharply to 29% at age 6-8months (NDHS, 2013) and the 15.6% prevalence reported by Aliyu *et al.*(2012) but higher than the 10.3% prevalence reported in a study conducted in Jos-Plateau (Akor *et al.*, 2010). Approximately 34.3% of the children were classified as being too short for their age or stunted, and 0.6% were severely stunted and peaks at age 3-4years with a prevalence of 50%, corresponding with the NDHS, 2013, survey, with data showing 37 percent prevalence reported of children under age 5 considered to be too short for their age or stunted and 21% as severely stunted and peaking in 16% of children under age 6 months to 46%of children 24-35 months.Aliyu *et al.*(2012) reported a 44.9% prevalence of stunting in children under five years in Kaduna and reports from a study in Jos-Plateau showed an 11.1% prevalence of stunting in pre-school aged children (Akor *et al.*, 2010).

The anthropometric data for wasting, stunting and underweight in children under five years of age, obtained from this study as seen on table 4.4 shows some consistency with previous

reports on the trends in nutritional status of children under five years of age from 2003-2013 (NDHS, 2013). The mean Z-scores for stunting (-2.4), wasting (-1.4), and underweight (-2.3), obtained in this study were all negative values and less than zero, suggesting that the distribution of an index has shifted downward, showing that most or all of the children suffer from undernutrition relative to the reference population and this could be due to poverty and poor health in the state.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The dangers of diarrhoeal diseases and its effect on the nutritional status of children under five years of age have long been established, to this end, the following conclusion have been drawn from this study.

1.*Escherichia coli* 0157 (a re-emerging pathogen causing haemolytic-uremic syndrome) and Verocytotoxigenic *Escherichia coli* were isolated from the study population further confirming *E. coli* as an active causative agent of diarrhoeal diseases in Kaduna state, Nigeria

2. The presence of antibiotic resistant strains of the *E. coli* isolates in the study population indicates the often unnecessary and uninformed use of these drugs in the treatment of most infantile diarrhoea cases.

3. The presence of the three indices of malnutrition (stunting, wasting and underweight) reflects a high level of undernutition and a case of current nutritional problems in the study population, posing serious public health issues.

4. The significant results recorded in the study population for demographic factors(level of parent's education, mothers occupation and estimated level of income) and risk factors (source of water, treatment of water, mode of feeding and type of toilet) indicates serious issues of poor hygiene and poor health.

6.2 Recommendations

The following recommendations are made from the findings obtained from this study

I. Breastfeeding of children, which is known to help a lot in improving child's immunity, should be encouraged for longer periods (1000 days) than the usual

6months as this would help ensure that parents, especially the mothers, keep a watchful eye on the kind of things the child ingests.

II. Children within ages 0-3yrs should be monitored more closely by their parents and care-givers, as negligence puts them more at risk of infection.

III. Personal and environmental hygiene strategies should be embarked upon and maintained by parents and society in general.

IV. The Government should improve on existing infrastructural facilities and make provision for new ones, especially in rural areas.

V. The Government should establish programs that are centered around the fortification and supplementation of foods and making them readily available especially in rural areas, and if possible free of charge for the low income earners who reside more in these rural areas. Thiswould help improve the nutritional status of children, thereby reducing the incidences of diarrhoeal diseases which have so far been established to be bidirectional.

VI. Awareness creation and perception on the dangers of diarrhoeal diseases should be intensified as it is one thing to be aware and another to believe that it actually does exist and is a killer.

VII. Handwashing practices should be inculcated and encouraged amongst pre-school aged children as they are the most vulnerable group in the society.

VIII. Care-givers in schools such as daycare centres and nursery schools were we have these children enrolled should be educated on proper hygienic practices as these children cannot handle such things on their own.

IX. Antimicrobials should be conserved for use only when safe and necessary.

X. Strategies for successful empirical treatment can be employed with the doctors requesting for culture and sensitivity tests before prescribing antibiotics.

XI. Agent–specific risk factors identified as existent can now be monitored and eradicated or avoided.

XII. The need for the introduction of feeding practices in schools for pre-school aged children, as is obtainable in more developed societies should not be ignored, as this would afford the government an avenue to help have these children benefit directly from programmes aimed at improving poor diets and in the process reduce the risks of malnutrition and diarrhoea.

XIII. The government should also organize 'intervention programmes' that is, programmes in which staple foods can be stored or preserved overtime and distributed to the people for use during the farming season when most households would have run out of these foods.

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APPENDICES

APPENDIX I: Microgen GN A substrate reference table

Well	Reaction	Description	Positive	Negative
1.	Lysine	Lysine decarboxylase-Bromothymol blue changes to green/blue indicating the production of the amine cadaverine.	Green/Blue	Yellow
2.	Ornithine	Ornithine decarboxylase- Bromothymol blue changes to blue indicating the production of the amine putrescin.	Blue	Yellow/Green
3.	H_2S	H_2S production-Thiosulphate is reduced to H_2S that reacts with ferric salts producing a black precipitate.	Brown/black	Straw
4. 5. 6.	Glucose Mannitol Xylose	Fermentation- Bromothymol blue changes from blue to yellow as a result of acid produced from the carbohydrate fermentation.	Yellow	Blue/green
7.	ONPG	Hydrolysis-ONPGHydrolysis by B-galactosidase results in the production of yellow ortho-nitrophenol	Yellow	Colourless
8.	Indole	Indole is produced from tryptophan and gives a pink/red complex when Kovac's reagent is added.	Pink/red	Colourless
9.	Urease	Hydrolysis of urea results in the formation of ammonia leading to an increase in pH which turns phenol red from yellow to pink/red.	V.Deep Pink	Straw to pale salmon pink colour
10.	VP	Acetoin production from glucose is detected by the formation of a pink /red complex after the addition of alpha naphthol and creatine in the presence of KOH.	Deep Pink/Red	Colourless to Pale Pink
11.	Citrate utilization	Utilization of citrate (only carbon source) leading to a colour change in bromothymol blue from green to blue.	Blue	Yellow/Pale Green

12.	TDA	Indolepyruvic acid is produced from tryptophan by tryptophan deaminase giving a cherry red colour when ferric ions are added. Indole positive isolates may give a brown colour- this is a negative result.	Cherry red	Straw colour

Key: H_2S-Hydrogen sulphide; ONPG-Ortho-nitrophenol- galactosidase; TDA-Tryptophan deaminase acid

APPENDIX II: Microgen GnA Identification result (*E. coli*)

CODE	Lys	Orn	H_2S	Glu	Man	Xyl	ONPG	Ind	Ure	VP	Cit	TDA	Octal code	ID
YD10	+	+	-	+	-	+	+	+	+	-	-	-	6570	*E. coli*
YD20	+	+	+	+	-	+	+	+	-	-	-	-	7560	*E .coli*
GS44	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E. coli*
GS60	+	+	-	+	-	-	+	+	-	-	-	-	6460	*E. coli*
GA3	+	+	+	+	+	-	+	+	-	-	-	-	7660	*E. coli*
KG5	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E. coli*
MK63	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E. coli*
KAF3	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E. coli*

KEY:Lys- Lysine, Orn- Ornithine, H2S- Hydrogen sulphide , Glu-Glucose, Man-Mannitol, Xyl- Xylose, ONPG- Ortho-nitrophenol- galactosidase, Ind - Indole, Ure- Urease, VP- Voges Proskauer, Cit- Citrate, TDA- Tryptophan deaminase acid , ID-Identifications

APPENDIX III: ZoneDiameter Interpretative Standard Breakpoints (for*Enterobacteriaceae*) of antibiotics used in this study.

Antimicrobial Agents	Disk potency (µg)	Zone diameter (mm)			
		R≤	I	S ≥	**Testing conditions**
Amoxycillin	10	13	14-17	18	**Medium**
Cefotaxime	30	22	23-25	26	Mueller-Hinton agar
Chloramphenicol	30	12	13-17	18	
Ciprofloxacin	5	15	16-20	21	**Inoculum**
Sulphamethoxazole	25	10	11-15	16	Growth method or direct colony suspension equivalent to a 0.5 McFarland Standard
Gentamicin	30	12	13-14	15	
Tetracycline	30	11	12-14	15	**Incubation**
					35 ± 2^0C,
					ambient air;
					16-18hours

Key: R= Resistant, I=Intermediate, S=Sensitive
Source: CLSI, 2013

APPENDIX IV:Media Formulation

1. Eosin methylene blue (EMB) agar (Oxoid, England)

g/L	
Peptone	10.0
Lactose	5.0
Dipotassium hydrogen phosphate	2.0
Eosin-Y	0.4
Metyhlene blue	0.065
Agar	15.0

pH 6.8 ±0.2 @25^{o}C

2. Methyl Red Voges Proskauer Broth (Scharlau, Spain)

g/L	
Peptone	7.0
Dextrose	5.0
Potassium phosphate	5.0

pH 7.0 ± 0.2 @25^{o}C

3. Simmon's citrate agar (Biomark, India)

g/L	
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar	15.0

pH 6.8 ± 0.2 @25^{o}C

4. Buffered Peptone Water (Oxoid, England)

g/L	
Peptone	10.0
Sodium chloride	5.0
Disodium phosphate	3.5
Potassium dihydrogen phosphate	1.5

pH7.2 ±0.2 @ 25^{o}C

5. Mueller-Hinton-Agar (Merck, Germany)

g/L	
Infusion from meat	2.0
Casein hydrolysate	17.5
Starch	1.5
Agar-Agar	13.0

pH 7.4 ± 0.2 @25^{o}C

6. Cefixime – Tellurite Sorbitol MacConkey Agar (Oxoid, England)

Sorbitol MacConkey Agar (Oxoid, CM 813)	500ml
Potassium Tellurite	1.25mg
Cefixime	0.025mg

7. Nutrient broth (agar) (Oxoid, England)

g/L	
Lab- Lemco powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
(Bacteriological agar)	15.0

pH 7.4 ± 0.2 @25^{o}C

APPENDIX V: Composition of reagents

1. Kovàc's Indole Reagent

Para-dimethylaminobenzaldehyde	2g
Isoamyl alcohol (3-methyl-1-butanol)	30ml
Hydrochloric acid, concentrated	10ml

2. Methyl red solution

Methyl red (pH indicator)	0.05g
Absolute ethanol	28ml
Distilled water	22ml

3. 5%-alpha-napthol

Alpha-napthol	5g
Absolute ethanol	100ml

4. 40% Potassium hydroxide

Potassium hydroxide	40g
Distilled water	100ml

APPENDIX VI: Questionnaire

Department of Microbiology,

Faculty of Science, Ahmadu Bello University,

Zaria, Kaduna State.

July 2013.

Research Questionnaire

ANTIBIOGRAM OF ESCHERICHIA COLI AND NUTRITIONAL STATUS OF DIARRHOEIC CHILDREN UNDER FIVE YEARS ATTENDING SELECTED HOSPITALS IN KADUNA STATE, NIGERIA.

DATA COLLECTION QUESTIONNAIRE Questionnaire No.

ELIGIBILITY:Children of 0-5 years who have passed more than three stools in the past 24 hrs

A) Background information

1. Name of child ....., Sex: M/F Age.....
2. Name of caregiver....., Sex: M/F

B) Socio-Economic Variables

1. Occupation of father (a) Civil Servant (b) Trader (c) Farmer (d) Unemployed (e) Others

2. Occupation of Mother (a) Housewife (b) Civil Servant (c) Trader (d) Farmers (e) Others
3. Estimated income of parents (a) < #5,000 (b) #5,001 - #10,000 (c) #10,001 - #20,000 (d) #20, 001 - #40,000 (e) > #40,000
4. Level of parents/guardian's formal education (a) None (b) Primary (c) Secondary (d) Tertiary (e) Quranic/ Adult literacy
5. Source of drinking water (a) Pipe-borne water (b) Well (c) Pond (d) Borehole (e) River/Stream (f) Others
6. Treatment of drinking water (a) Boiling (b) None/Raw (c) Filtering (d) Others (specify)
7. Type of toilet (a) pit Latrine (b) Open-field (c) Water closet (d) Stream (e) Others

C) Traditional Management of Diarrhoea

1. How do you know your child has diarrhoea? .....
2. How do you treat diarrhoea? .....
3. Have you used ORS/SSS? Yes ( ) No ( )
4. Describe how to prepare ORS/SSS

Recipe for SSS; 2 level teaspoon of sugar and one level teaspoon spoon of salt dissolve in two 35cl bottles of coca cola full of clean, boiled and cooled water (about 70cl SSS)

D) Feeding Practice:

1. When your child has diarrhoea, how does it affect his/her appetite? Increase ( ) No effect ( ) Decrease ( )
2. Are you still breast-feeding the child? Yes ( ) No ( )
3. How do you normally feed the child? Personal plate ( ) feeding bottle ( ) Group plate ( )

4. List what you have given your child to eat in the last 24hrs .....

E) Assessment of Child for Diarrhoea

Medical History

1. How many times has your child passed loose stools in the last 24hrs?.....
2. For how long has the child passed loose stool? .....days
3. Has there been any blood in the stool? Yes ( ) No ( )
4. How many times has the child had diarrhoea in the last 3 months? ..... times
5. For how long does the diarrhoea usually last? ..... days
6. Is the child able to drink water? Yes ( ) No ( )
7. If YES, does he/she drink eagerly? Yes ( ) No ( )

F) Physical Examination

1. What is the child's general condition? Well and Alert ( ) Severely malnourished ( ) Restless and Irritable ( ) Normal eyes ( ) Sunken eyes ( )
2. When the skin is pinched, how does it go back? Quickly ( ) Slowly ( ) Very slowly ( )
3. Does the child have fever? Yes ( ) No ( )
4. Degree of dehydration? None ( ) Moderate ( ) Severe ( )
5. Other diseases/ ailment (specify) .....
6. Child's weight (kg) .....
7. Child's height (cm) .....

APPENDIX VII:Ethical approval

MINISTRY OF HEALTH, KADUNA STATE

All Communications to be Addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Tel: (062) 248084
(062) 248252

Independence Way,
P.M.B. 2014,
Kaduna.
Kaduna State, Nigeria

MOH/ADM/744/VOL.I/

30th May, 2013

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PERMISSION TO CONDUCT RESEARCH

I have been directed to convey the Ministry's approval to STEP-B PROJECT TEAM who are students from Department of Microbiology, Faculty of Science Ahmadu Bello University, Zaria Kaduna State Nigeria. To conduct a research on the Topic: **"DIARRHOEAL AGENTS AND THE NUTRITIONAL STATUS OF CHILDREN IN NORTH-WESTERN NIGERIA"** for the award of, Ph.D /M.Sc in the Department of Microbiology Faculty of Science Ahmadu Bello University, Zaria.

It is expected that necessary assistance be accorded to any of the step-B Project team members in the process of the research, please.

However, it is mandatory for the researcher to submit a copy of his/her research finding(s) to the office of the Honourable Commissioner, Ministry of Health, as soon as it is concluded.

Accept the assurances of the Honourable Commissioner's highest regards.

F. A. KURAH (MRS)
SECRETARY, ETHICAL COMMITTEE

APENDIX VIII: Informed consent form

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

INFORMED CONSENT FORM (ICF)

Serial No.: ..... *Hospital No.:* ..... *Age:* ..... *Phone No.:* .....

This Informed Consent Form is basically for parents or caregivers of children aged 0-5 years, presenting with diarrhoea in some hospitals in Kaduna State. We are inviting you to participate in this research work titled "Antibiogram of *Escherichia coli* and Nutritional status of Diarrhoeic children under – five years attending some selected hospitals in Kaduna State, Nigeria". *Escherichia coli* have been implicated as causative agents of diarrhoeal diseases in children and also have been found to have a negative effect on the nutritional status of the said children. The research will involve the collection of stool samples of Children who meet the study inclusion criteria. The results obtained therefrom will be used to improve the understanding and management of the disease in our community. Individual data will be treated with strict confidentiality. Participation is strictly voluntary and refusal to participate in the research study will not in any way affect your right and benefit in this hospital/health centre.

CERTIFICATE OF CONSENT

I ……of …… hereby consent to participate in this study. The full procedures of the test/study have been explained to me by the investigator. I understand that stool will be taken for the research. I therefore give this consent voluntarily without being subjected to any pressure.

Name of Participant ……

Signature/right thumb print of participant …… Date ……

Statement by Witness:

I have witnessed the accurate reading of the consent form and detailed explanation of the study to the potential participant, and the participant has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Name of witness……

Signature of witness…… Date ……

Statement by the Researcher/Person Taking Consent

I confirm that sufficient information, including risks and benefits, to make an informed decision has been provided to the participant. The participant was given an opportunity to ask questions about the research, and all the questions asked by participant have been answered correctly. I confirm that the participant has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher ……

Signature …… Date. ……

APENDIX IX: Eligibility form

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

INFORMED CONSENT FORM (ICF)

This Informed Consent Form is for Diarrhoiec children (0-5yrs) attending clinics in some hospitals in Kaduna State. We are inviting you to participate in this research work titled "Antibiogram of *Escherichia coli* and Nutritional status of Diarrhoeic children under – five years attending some selected hospitals in Kaduna State, Nigeria".

Principal Investigator:	Ojuota, Chioma Rebecca
Collaborating Investigators:	Prof. C.M.Z. Whong Prof. S.A. Ado
Name of Organization:	Department of Microbiology Ahmadu Bello University, Zaria - Nigeria
Name of Sponsor:	Self
Name of Proposal:	Postgraduate Research Proposal

This Informed Consent Form has two parts:

- Information Sheet (to share information about the research with you)
- Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form

PART I: INFORMATION SHEET

Introduction

I am Ojuota, Chioma Rebecca, a postgraduate student of the Department of Microbiology, Ahmadu Bello University, Zaria, carrying out a research work under the supervision of Prof. C.M.Z Whong and Prof. S.A. Ado. We are carrying out a research work on "Antibiogram of *Escherichia coli* and Nutritional status of Diarrhoeic children under – five years attending some selected hospitals in Kaduna State, Nigeria". I am going to give you information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me, the study doctor or the staff.

Purpose of the research

The purpose of this research is to Study the Antibiogram of *Escherichia coli* and Nutritional status of Diarrhoeic children under – five years attending some selected hospitals in Kaduna State, Nigeria.

Participant selection

We are inviting all children with diarrhoea who are attending hospitals in some parts of Kaduna State to participate in the research work on the Study of the nutritional status of diarrhoiec children in Kaduna State.

- *Do you know why we are asking you to take part in this study? YES.....NO.....*
- *Do you know what the study is about? YES..... NO. .....*

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered in this clinic/hospital for diarrhoea patients and we will tell you more about it later. You may change your mind later and stop participating even if you agreed earlier.

- *If you decide not to take part in this research study, do you know what your options are? YES..... NO.....*
- *Do you know that you do not have to take part in this research study, if you do not wish to? YES..... NO .....*
- *Do you have any questions? YES..... NO. .....*

Procedures and Protocol

When you have agreed to participate in this research, we will collect stool samples in wide-mouth containers, and then taken to the laboratory for further analysis.

Description of the Process

During the research you will make just one visit to the clinic.

In the visit, stool samples will be collected into sterile bottles in order to carry out faecal examinations to ascertain the presence or absence of the bacterial organisms, also a questionnaire form will also be filled and used to ascertain the nutritional status. We will also ask a few questions about the general health of the children who meet the study criteria. General health and other personal information will be required too.

Duration

The research will take place over two months. During which time, it will be necessary for you to come to the clinic just once. After which, the research will be concluded.

- *How many times are we asking you to come to the hospital to participate in the research?*.....
- *Can you tell me the number of time we will take your blood sample?*.....
- *Do you have any questions?*.....
- *Do you want me to go through the procedures again?*.....

Risks

There is a risk that you may share some personal or confidential information about your child, or that you may feel uncomfortable talking about some of the topics. However, we do not wish for this to happen. You do not have to answer any question or take part in the research if you feel the question(s) are too personal or if talking about them makes you uncomfortable.

Benefits

If you participate in this research, you will have the following benefits:

- ✓ Your child will have the opportunity of being tested for diarrhoeal infections
- ✓ The nutritional status of the child will be determined.
- ✓ There may not be any benefit to the society at this stage of the research, but future generations are likely to benefit.
- ✓ Apart from these few benefits listed above, your participation is likely to help us find answers to the research questions.

Reimbursements

You will not be provided with any special incentive or travel allowance for you to take part in the research. But we advise that your visit to the clinic will have to be on your normal scheduled clinic days.

Can you tell me if you have understood correctly the benefits that you will have if you take part in the study?.....

- *Do you know that the study will not pay for your travel costs?*.....
- *Do you know the kind of benefit you can get by participating in this research?*.....
- *Do you have any other questions?*.....

Confidentiality

With this research, it is possible that if others in the community are aware that you are participating, they may ask you questions. We will not be sharing the identity of those participating in the research.

The information that we collect from this research will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be allowed access to it. Any information about your