ENTERIC PATHOGENIC BACTERIA AND ASSOCIATED RISK FACTORS IN DIARRHOEIC AND DYSENTERIC CHILDREN IN SOKOTO STATE, NIGERIA

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY OF SCIENCE IN MICROBIOLOGY

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Abstract

Enteric bacterial diarrhoeal and dysenteric illnesses exact a heavy toll on human populations, particularly children. Diarrhoea due to bacterial infections is an important cause of morbility and mortality in infants and young children in most developing countries. Diarrhoea a situation when a person passes more stool than is normal, passing three or more loose or liquid stools per day is caused by a variety of pathogens including bacteria, viruses, and parasites. Among the bacteria, *Campylobacter jejuni, Salmonella, Shigella* and *Yersinia* species are known to be the most common and economically important agents but other bacteria, including diarrhoeagenic *Escherichia coli* (DEC) and *Campylobacter coli* have also been identified as causes of enteric diarrhoeal disease in children. The aim of this study is to detect enteric bacterial pathogens and associated risk factors in diarrhoeic children in Sokoto state, Nigeria. A total of four hundred and twenty diarrhoeic children five years and less from the three senatorial zones of Sokoto State, Nigeria were screened for the presence of enteropathogenic bacteria namely *Escherichia coli, Campylobacter, Salmonella* and *Shigella* species. Stool samples were analyzed by both the conventional culture methods and the standard PCR techniques for the detection of the target enteropathogenic bacterial virulence genes. The antibiogram of the isolates were determined by disk diffusion method. To determine some risk factors associated with incidence of bacterial diarrhoea in children structured questionnaire designed to obtain basic data was used while various water sample from the study area were examine for possible faecal contamination. Of the diarrhoeaic stool specimens analyzed by the culture techniques, *Campylobacter* species, *Escherichia coli, Salmonella enterica* and *Shigella* species were detected in 14 (3.3%), 79 (18.8%), 30 (7%) and 17 (4%) of cases respectively. On the other hand, results of PCR of the stool specimens revealed that, *Campylobacter* spp., *E. coli, Salmonella enterica* and *Shigella*

spp. accounted for 34 (8.1%), 87 (20.7%), 35 (8.3%) and 22 (4.8%) of all cases of childhood diarrhoea respectively. *E. coli* remains the major cause of infantile diarrhoea, followed by *Salmonella enterica* irrespective of the method of stool analysis. It was observed that, the incidences of diarrhoea among children of 0 to 5 years across the study area is higher (19 - 21 %) among children within the 0 to 3 years age bracket compared with the 13 – 15% recorded for children of 4 - 5 years age bracket. Also, a consistent trend of decreasing incidence with increasing age of the children was noted across the study area. Children of both sexes appear to be equally affected by the disease (51.4% males, 48.6% females). The antibiogram studies on the isolates revealed that most were resistant to Amoxycillin-clavulanic acid, Cotrimaxazole and Erythromycin. However the isolates were found highly susceptible to Ofloxacin, Ciprofloxacin, Ceftriaxone and Cefuraxime. The multiple antibiotics index analysis revealed that all the four isolates had a very high index value (≥0.2%). The percentage frequency of multiple antibiotics resistance (MAR) index value greater than 0.2%, were recorded as 8(57.1%), 44(55.7%), 19(63.3%) and 10(58.8%) isolates for *Campylobacter* species, *Escherichia coli*, *Salmonella enterica* and *Shigella* species respectively. The risk factors so far identified are lack of portable safe water, improper sewage disposal system and poor hygienic practices. Assessement of various potential risk factors, the incidence of diarrhoea and infection rate indicated that strong association exist and the difference observed was considered statistically significant ($P<0.05$).

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Diarrhoea is termed as a situation when a person passes more stool than is normal, passing three or more loose or liquid stools per day. Diarrhoea in children can be caused by a variety of pathogens including bacteria, viruses, and parasites. Among the bacteria, *Campylobacter jejuni, Salmonella, Shigella* and *Yersinia* Species are known to be the most common and economically important agents (**Britton and Veraslovic, 2008)** but other bacteria, e.g. enterotoxigenic *Escherichia coli* (ETEC) and *Campylobacter coli* have also been identified as causes of enteric disease and diarrhoea in children (**Britton and Veraslovic, 2008).**

Every year, diarrhoeal diseases contribute to 1.7 million deaths worldwide, mainly in children under the age of 5 years particularly in developing countries (WHO, 2009). Even in industrialized countries, the burden of diarrhoeal diseases is substantial, but in most cases, the etiology is unknown (Richard *et al.,* 2006). Infectious diarrhoea still represents a common public health issue. More than three-quarters of the world population is affected and 1–2 illnesses per person are recorded annually. However, in about 50% of cases, the causative agent remains unknown.

At present, the widespread occurrence of infectious diarrhoea has become one of the major public health problems worldwide. Therefore, a rapid response, which includes identification of the pathogens and prevention of the spread of these pathogens in the community, is crucial for the control of disease outbreak and case investigations (Yuanhai *et al.,* 2008). Transmission of enteric pathogens often occurs through the consumption of contaminated food and water. Although a detailed diagnosis and treatment is usually not required, in outbreak situations, especially in community that share facilities or camps, the causative organism must be identified quickly to prevent further spread. Furthermore, clinicians need an early diagnosis for the effective treatment of the severely ill patients (Guerrant *et al.,* 2001).

Bacterial culture is still the 'gold standard' for the identification of enteropathogenic bacteria, but the procedures are time consuming and require 3–5 days (Pawlowski *et al.,* 2009). Most medical microbiology laboratories use conventional diagnostic procedures, such as culture and microscopy, for routine detection of enteric pathogens. These culture procedures include enrichment steps, use of selective culture media, biochemical identification, serotyping, and resistance profiling (Pawlowski *et al.,* 2009). Final results are obtained after 3 to 5 days, making these procedures laborious and time-consuming. Furthermore, the detection of pathogens in stool specimens by culture is complicated. For instance, bacteria belonging to the normal gastrointestinal flora can present with the same colony morphology as enteric pathogens (**Cunningham *et al.,* 2010**). The resultant misidentification increases hands-on time and delay in reporting of a definite negative result. Other problems are the viable but non-culturable state of some organisms such as *Campylobacter jejuni* (LaGier *et al.,* 2004) and the

limited viability of shigellae outside the human body. These also compromise the sensitivity of culture methods (**Cunningham *et al.,* 2010**).

More rapid diagnostic tools are therefore required to circumvent these problems (Weimer *et al.,* 2011). The invention of molecular diagnostics, in particular Polymerase chain reaction (PCR), has allowed rapid identification of microorganisms (Espy *et al.*, 2006). However, in the past, even the PCR methods were laborious and prone to error. But the introduction of hydrolysis and hybridization probes have substantially improved the specificity of the test, and reduced the problem of cross-contamination considerably. Several PCR assays use either gel or hydrolysis probes combining enrichment, DNA extraction, PCR amplification, and detection by hybridization and colour development. The most recent PCR thermo-cyclers allow the simultaneous amplification and detection of several targets (maximum 5) in multiplex PCR (Wilhelm and Pingoud, 2003). So far, only a few attempts have been made to use multiplex PCR for the detection of enteropathogenic bacteria in stool specimens (Linton *et al*., 1997, Douglas *et al.,* 2003 and Weimer *et al.,* 2011).

1.2 Statement of research problems:

Infectious diarrhoeal disease in children is a leading cause of morbidity and mortality worldwide particularly in developing countries. There are also significant economic and social costs associated in addition to increased morbidity and mortality in Nigeria (Aminu *et al.,* 2007). The prevalence of diarrhoea in Nigeria is 18.8% and is one of the worst in sub-Sahara Africa and above the average of 16% (WHO, 2010). It accounts for over 16% of child deaths in Nigeria. An estimated 150,000 deaths mainly amongst children under five occur annually due to this disease mainly caused by bacterial organisms arising from poor sanitation and hygiene practices (WHO, 2009).

Large proportion of diarrhoeal illnesses in children in developing countries is ascribed to an unknown aetiology because the commonly available methods of diagnosis, such as microscopy and culture, have been associated with low sensitivity (Ajjampur *et al.,* 2007). Conventional culture methods remain the norm for the isolation of bacterial enteric pathogens in clinical laboratories. Diarrhoeal diseases due to known bacterial pathogens require rapid diagnosis of the causative pathogenic bacteria which included *Escherichia coli, Vibrio cholerae, Vibrio parahaemolyticus, Salmonella enterica, Campylobacter jejuni, Shigellae, Yersinia enterocolitica,* and *Listeria monocytogenes (*Yuanhai *et al.,* 2008). All these aetiologic agents are often unidentified by traditional methods as both the clinical and analytical sensitivity are low, and require a longer time, as such the diagnostic outputs to allow for earlier treatment, epidemiological investigations and infection control interventions are hindered (Cunningham *et al.,* 2010).

1.3 Justification of the study

Conventional methods of isolation of *bacterial* strains from faecal samples take 3–5 days to complete and are therefore laborious and require substantial manpower. Besides, very small numbers of viable organisms present in the faeces may fail to grow in artificial laboratory media (Pawlowski *et al.,*

2009). Traditional methods for detection of the aetiological agents of diarrhoea have ranged from microscopy and bacterial culture to immunoassays, and vary for each enteric pathogen.

This has resulted in a large proportion of samples being ascribed to an unknown aetiology. This study is aimed at decreasing the diagnostic gap in diarrhoeal disease detection by the application of PCR techniques. Identifying the enteric pathogens that contribute significantly to the causes of diarrhoea in children will help to provide an accurate and region-specific estimate of disease burden. Also, the PCR-based methods provide an easy to perform, rapid and uniform method of testing for a wide range of pathogens. This study, therefore, is designed towards assessing the sensitivity of the PCR assays for detecting common enteric pathogens with the view to enhance the identification of aetiological agents of diarrhoea in children. Enteric bacteria such as *Campylobacter jejuni, Salmonella, Shigella* and *Yersinia species* are major cause of bacterial gastroenteritis worldwide (**Britton and Veraslovic, 2008)**. A rapid method for their detection in clinical specimens is therefore needed. Multiplex-PCR can obtain results within a shorter time with only a few processing steps, which minimizes processing errors. The cost of PCR is comparatively low and, above all, no infectious waste is produced, and results can be obtained even from inactivated material. The high sensitivity and speed of the test allows clinicians to start early antibiotic treatment in critically ill patients. In the management of outbreak scenarios, the source of infection can be pinned down more quickly, and early action to control the spread can be initiated (Weimer *et al.,* 2011).

1.4 Aim

To detect enteric pathogenic bacteria and determine associated risk factors in diarrhoeic and dysenteric children in Sokoto state, Nigeria.

1.5 Objectives

1. To determine the prevalence of bacteria in children with diarrhoea using conventional culture method.

2. To determine the antibacterial susceptibility pattern of the enteropathogenic bacteria detected.

3. To determine some risk factors associated with incidence of bacterial diarrhoea in children.

4. To screened for total and faecal coliforms in various sources of water from the study areas.

5. To detect some enteropathogenic bacteria directly from the stool sample by PCR technique.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diarrhoeal disease

Diarrhoea is both a symptom and a sign (Binder, 2006). As a sign, diarrhoea is defined, quantitatively, as an increase in stool weight or volume of more than 200 gms in 24 hours for a person on a good diet (Binder, 2006). As a symptom, diarrhoea is defined by each patient differently: a decrease in consistency, increase in the amount of stool per bowel movement, and/or increase in frequency of bowel movements. Diarrhoea can last several days, and can leave the body without the fluid and electolyte that are necessary for health. Most people who die from diarrhoea actually die from severe dehydration and fluid loss (Binder, 2004). Children who are malnourished or have impaired immunity as well as people living with HIV are most at risk of life-threatening diarrhoea (Parashar *et al.,* 2003).

According to the World Health Organization (2009) and the United Nations Children's Fund (2009) about two billion cases of diarrhoeal disease occur worldwide every year, and 1.9 million children younger than 5 years of age perish from diarrhoea each year, mostly in developing countries. This amounts to 18% of all the deaths of children under the age of five and means that more than 5000 children are dying every day as a result of diarrhoeal diseases. Diarrhoea poses a very serious problem in developing countries where it is the leading cause of morbidity and mortality and ranks second as a major cause of morbidity among the notifiable diseases in Nigeria where, according to the Federal Statistic Bulletin 300 children die every day from dehydration and malnutrition caused by diarrhoea. This could be an underestimate, as very few patients have access to the very limited number of hospitals and health center that are available (Ogunsanya *et al.,* 1994).

Diarrhoea in most developing countries still remains one of the principal causes of morbidity in children with each child reported to experience a median of between two and three episodes of diarrhoea every year. Where episodes are frequent, young children may spend more than 15% of their days with diarrhoea. About 80% of deaths due to diarrhoea occur in the first two years of life (Kosek *et al.,* 2003). Diarrhoea is also reported to be one of the six conditions identified in the fourth Millennium Development Goal (MDG): reduce child mortality that leads to the majority of child deaths. In 2010 alone, diarrhoeal diseases caused 1.1 million deaths and 66.5 million years of productive life lost in developing countries (WHO, 2010).

Diarrhoea is a leading cause of death during complex emergencies and natural disasters. The infection can be spread through food or water contamination, or from person to person as a result of poor hygiene (Kosek *et al.,* 2003). Displacement of populations into temporary, overcrowded shelters is often associated with polluted water sources, inadequate sanitation, poor hygiene practices, contaminated food and malnutrition – all of which affect the spread and severity of diarrhoea. At the same time, the lack of adequate health services and transport reduces the likelihood of prompt and appropriate treatment of diarrhoea cases (Parashar *et al.,* 2003). Children with poor nutritional status and overall health, as well as those exposed to poor environmental conditions, are more susceptible to severe diarrhoea and dehydration than healthy children (Black *et al.,* 2003). Children are also at greater risk than adults of life-threatening dehydration since water constitutes a greater proportion of children's bodyweight. Young children use more water over the course of a day given their higher metabolic rates, and their kidneys are less able to conserve water compared to older children and adults (Black *et al.,* 2003). The Millennium Development Goals call for a reduction of child mortality by two thirds between 1990 and 2015. As the deadline approaches, the reality is that although progress is being made, much more remains to be done. Nearly nine million children under five years of age die each year. Diarrhoea is more prevalent in the developing world due, in large part, to the lack of safe drinking water, sanitation and hygiene, as well as poorer overall health and nutritional status. According to the latest available figures, an estimated 2.5 billion people lack improved sanitation facilities, and nearly one billion people do not have access to safe drinking water. These unsanitary environments allow diarrhoea-causing pathogens to spread more easily. Diarrhoea's status as the second leading killer of children under five is an alarming reminder of the exceptional vulnerability of children in developing countries. Saving the lives of millions of children at risk of death from diarrhoea is possible with a comprehensive strategy

that ensures all children in need receive critical prevention and treatment measures (UNICEF and WHO, 2009).

2.2 Causes of diarrhoeal disease

It must be clearly emphasized that each human being has his or her own bowel habit that is normal to him or her: some individuals empty their bowels or pass faeces or stools once daily; some others once in two days; some others twice in a week, whilst some others do so twice in a day. When a person who normally empties his bowels once in a day starts to do so three times or more a day, or instead of passing formed stools , passes loose or frankly watery stools like the so called rice-water stool that typifies the diarrhoea caused by *Vibrio cholerae*, the individual is said to have developed diarrhoea. The term diarrhoea must be differentiated from dysentery which connotes the passage of blood and mucus in the stool (Kosek *et al.,* 2003).

To put this whole area of diarrhoea in proper perspective, it is important to provide the fundamental basis upon which the development of diarrhoea rests, irrespective of the cause of diarrhoea. Seven to nine litres of fluid pass through the gastrointestinal tract (Fig. 2.1), which may be likened to a tunnel or pipe with two open ends, one of the two ends being the mouth, the other being the anus, every 24 hours or every day. The 7 to 9 litres of the fluid come from a number of sources as follows: 2 litres come from fluids (water) taken in the diet every day; saliva contributes a litre, gastric juice produced in the stomach 2 litres; bile 1 litre, juice from the pancreas 2 litres and the small intestine itself accounts for another 1 litre. Most of the fluid is reabsorbed from the lumen into the wall and ultimately into the blood in the small intestine (small bowel) such that only 1 to 2 litres are passed unto the large bowel, the colon, which absorbs nearly 90 percent of the 1 to 2 litres presented to it, thus allowing only 100 to 200mls of fluid to come out in the faeces or stool. This process whereby the colon reabsorbs 90 per cent of the fluid reaching it is the last defense against allowing diarrhoea to ensue and is referred to as colonic salvage mechanism (Stefano and Haleh, 2010). Diarrhoea occurs when this colonic salvage mechanism fails as the last critical step. For diarrhoea to take place, irrespective of the underlying cause, the colonic salvage mechanism has to be overwhelmed (Binder, 2006).

The inner surface of the gastro-intestinal tract (Fig. 2.2) or tunnel/pipe is characterized by the present of finger-like folds called villi whose aggregate surface area is as large as a lawn tennis court to provide an abundant absorptive surface to achieve great efficiency in absorption of digested food products, water, salt and other materials. This normal gut physiology relies on a functioning enteric nervous system that coordinates the gut ion transport and motor activity (Binder, 2006).

The Specific causes which increase the likelihood of diarrhoea include:congenital or inherited causes (intolerance to gliadin protein contained in wheat, inability to absorb chloride ions, post surgery/post radiotherapy, neoplastic (colo-rectal cancer, medullary carcinoma of the thyroid, villous papilloma of the rectum, oat-cell cancer of the lungs), reactions to allergens (milk, pepper, beer, flour) and drugs (magnesium salts, lactulose, antibiotics,colchicine, neomycin, etc), endocrine (diabetes mellitus, thyrotoxicosis, carcinoid syndrome, zollinger-ellison syndrome, pancreatic pseudo-cholera syndrome), functional and infectious causes (causes due to germs) (Steffeno, 2004).

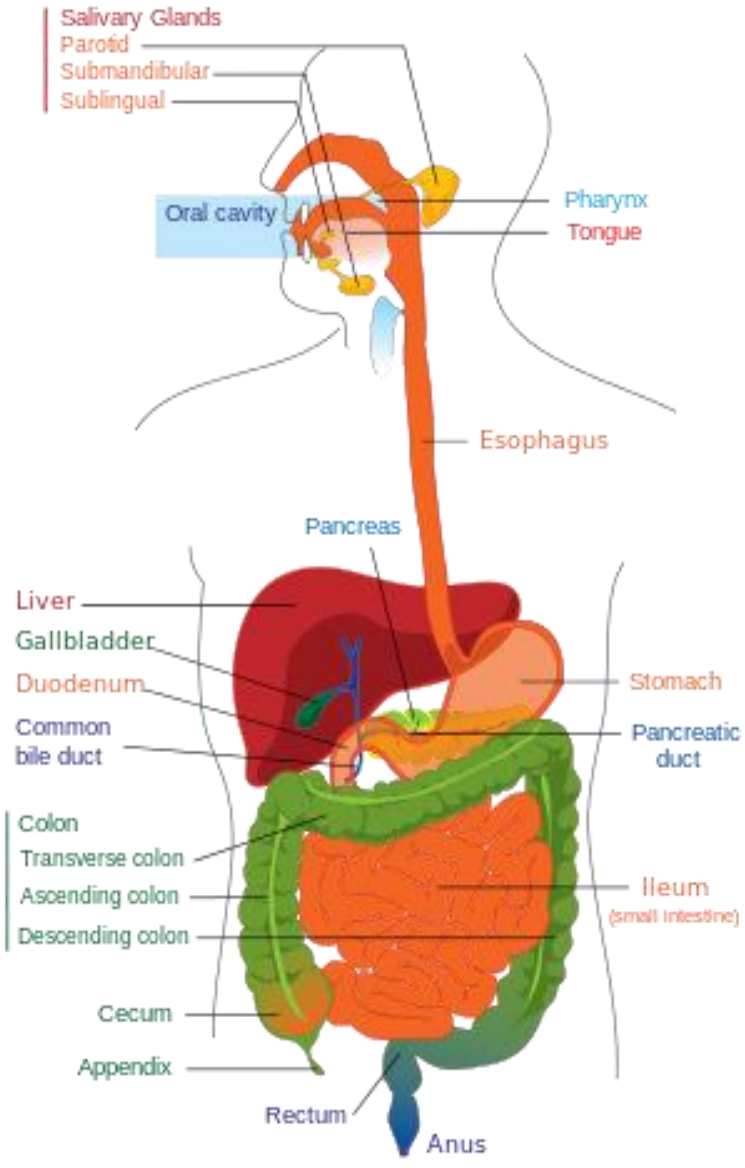


Figure 2.1: Diagram of human Upper and Lower human gastrointestinal tract

(Vander *et al*., 2001)

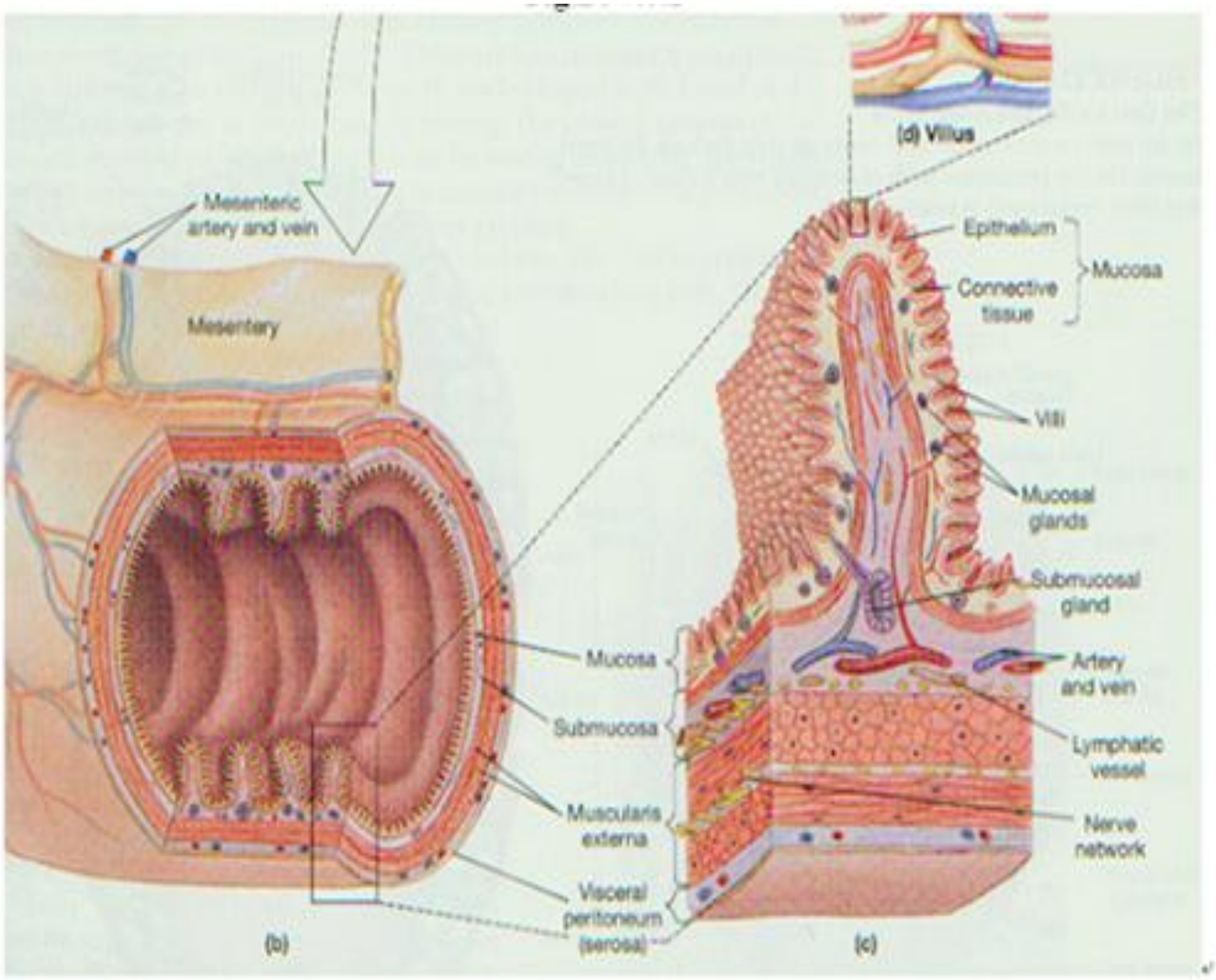


Figure 2.2: Diagram of the inner surface of the gastro-intestinal tract

(Vander *et al*., 2001).

2.2.1 Congenital or Inherited Causes

Diarrhoea with an onset in the early days of life is rare and generally caused by congenital malabsorptive disorders (Guarino and De Marco, 2004). Congenital glucose-galactose malabsorption (CGGM) is a rare autosomal recessive disorder, which presents as a chronic refractory diarrhoea in early neonatal life. It is due to a defect in sodium-coupled transport of glucose and galactose in the enterocyte (Abad-Sinden *et al.,* 1997). In the Arab countries where consanguineous marriage is quite common, CGGM appears to be a common problem as compared to the western populations. There is no data about the incidence or the prevalence of this problem in the Arab world. Lebenthal *et al.* (1971) have reported an Iraqi adult with CGGM and subsequently Abdullah *et al.* (1996) reported 8 Arab children with a similar problem in Saudi Arabia. Assiri *et al.* (2013) published five Arab children with CGGM and one of them developed gangrene of both legs as a complication of hypernatremia and dehydration, necessitating bilateral amputation. Intolerance to gliadin protein contained in wheat was reported as another cause of congenital diarrhoea known as celiac disease (Rewers, 2005). Upon exposure to gliadin, and specifically to three peptides found in prolamins, the enzyme tissue transglutaminase modifies the protein, and the immune system cross-reacts with the small-bowel tissue, causing an inflammatory reaction that leads to a truncating of the villi lining the small intestine (called villous atrophy). This interferes with the absorption of nutrients because the intestinal villi are responsible for absorption. The only known effective treatment is a lifelong gluten-free diet while the disease is caused by a reaction to wheat proteins; it is not the same as wheat allergy (Rewers, 2005).

Congenital chloride diarrhoea (CCD) is caused by a variety of mutations in the SLC26A3 (solute linked carrier family 26 member A3) gene, which encodes for an epithelial anion exchanger. Most reported cases are in Finland, Poland, or Arab populations (Guarino and De Marco, 2004). Because of excessive fecal losses of fluid and electrolytes, affected individuals present in the neonatal period with hyponatremia, hypochloremia, and metabolic alkalosis; there may be a history of polyhydramnios (Guarino and De Marco, 2004). The diagnosis of CCD is based on the finding of excessive fecal secretion of chloride, in which the chloride concentration exceeds the concentration of cations. However, this finding is only reliable when the patient is in fluid and electrolyte balance. In the untreated patient, the volume and chloride content of the diarrhoea may be artificially reduced.

Congenital sodium diarrhoea (CSD) has a syndromic form which includes choanal or anal atresia and is associated with mutations in the SPINT2 gene, which are not seen in patients with isolated CSD. The stool is alkaline and fecal sodium concentrations are high; metabolic acidosis and hyponatremia are typically present; there may be a history of polyhydramnios (Guarino and De Marco, 2004). Microvillus inclusion disease (MID) also called microvillus atrophy was associated with mutations in type V myosin motor protein (MYO5B) in several kindred. The disorder typically presents with intractable secretory diarrhoea shortly after birth, rapidly progressing to hypotonic dehydration and metabolic acidosis. A milder form of MID presents a few months later.

Tufting enteropathy (also known as intestinal epithelial cell dysplasia) appears to be caused by mutations in the EpCAM (epithelial cell adhesion molecule) gene. The disorder presents with secretory diarrhoea shortly after birth; the diarrhoea volume is often less than in MID, and may partially respond to fasting. Epithelial tufts also may be observed in congenital diarrhoea associated with other gene defects, such as in the case of SPINT2-related disease. In such cases, genetic testing can be particularly helpful to define the diagnosis, prognosis, and potential therapy. Enteric anendocrinosis (also known as congenital malabsorptive diarrhoea 4) is caused by mutations in neurogenin-3 (NEUROG3) and is associated with a paucity of enteroendocrine cells in the pancreas and intestine. It is characterized by an osmotic diarrhoea and later development of insulin-deficient diabetes, without anti-islet cell antibodies. Enteroendocrine and/or enterochromaffin cell paucity can be caused by a variety of other mechanisms (Guarino and De Marco, 2004).

2.2.2 Post Surgery/Post Radiotherapy

Extensive activities in the ileum and right colon lead to diarrhoea due to lack of absorptive surface and hence fat and carbohydrate malabsorption, decreased transit time, or malabsorption of bile acids and smaller bile acid pool (Thomas *et al.,* 2003). Loss of the small intestinal length by 50% is usually well tolerated; in 50-75% loss, a special diet is needed; if more than 75% of the small intestine is lost, long term total parenteral nutrition (TPN, given intravenously) is usually required. Loss of the jejunum is usually tolerated well, since the remaining ileum may adapt (absorptive villi increase in size and amount); the adapting process may take up to 2 years. Loss of the ileum (the main site of bile acids and vitamin B12 absorption) cannot be compensated, though. If bile acids cannot be absorbed, fats and fat soluble vitamins (A, D, E, and K) would not be absorbed and would be lost with the stool. If the valve

between the small and large intestine (ileocecal valve) is removed, colonic bacteria might invade the small intestine and cause diarrhoea (Suhr *et al.,* 1988).

Almost all patients undergoing radiation to the abdomen, pelvis, or rectum will show signs of acute enteritis resulting to diarrhoea. Injuries clinically evident during the first course of radiation and up to 8 weeks later are considered acute. Chronic radiation enteritis may present months to years after the completion of therapy, or it may begin as acute enteritis and persist after the cessation of treatment (O'Brien *et al.,* 1987)

2.2.3. Endocrine disorders

Diarrhoea in diabetic patients has often been ascribed to abnormalities of small bowel motility due to autonomic neuropathy, although other factors such as steatorrhoea and bile acid malabsorption may be involved. Its prevalence is estimated at 2–10%, predominantly occurring in type 1 diabetic with other manifestations of autonomic neuropathy (Saslow and Camilleri, 1995). Diarrhoea may be continuous or intermittent, the latter often being difficult to distinguish from "functional" symptoms; diagnosis is difficult, other than by implication when autonomic neuropathy has been demonstrated (Valdovinos *et al.,* 1993). Diarrhoea is also reported as one of the most common symptoms of diabetic enteropathy. The diarrhoea is typically painless, may be associated with fecal incontinence and occurs during the day but more often at night. Characteristically, it is seen in patients with poorly controlled diabetes who have peripheral and autonomic neuropathy (Lysy *et al.,* 1999). Other causes of diarrhoea in diabetics include pancreatic insufficiency, bile salt malabsorption, steatorrhea and drugs (Metformin). These should be excluded by appropriate investigations before making a diagnosis of diabetic enteropathy (Lysy *et al.,* 1999). Diabetic diarrhoea manifests as profuse, watery, typically nocturnal diarrhoea, which can last for hours or days and frequently alternates with constipation and abdominal discomfort is commonly associated. The pathogenesis of diabetic diarrhoea includes reduced gastrointestinal motility, abnormalities in gut transit time reduced alpha 2 adrenergic receptor mediated fluid absorption, bacterial overgrowth, pancreatic insufficiency, co-existent celiac disease and abnormalities in bile salt metabolism. Fecal incontinence, due to anal sphincter incompetence or reduced rectal sensation is another manifestation of diabetic intestinal neuropathy (Valdovinos *et al.,* 1993 and Lysy *et al.,* 1999).

Both diarrhoea and constipation have been reported in patients with thyrotoxicosis. Although theories support disturbances in gastrointestinal motility as the basis for these symptoms, the responsible mechanisms have yet to be defined (Thomas *et al.,* 2003). Steatorrhea has been reported in hyperthyroidism, and both hyperphagia and hypermotility are believed to play important roles in this form of steatorrhea (Saslow and Camilleri, 1995).

2.2.4. Neoplastic diarrhoea (caused by cancer)

Neuroendocrine tumors affecting the gastrointestinal tract are rare in children; they tend to cause secretory diarrhoea, gastrinoma syndrome, also known as Zollinger-Ellison syndrome (unregulated secretion of gastrin causes hypersecretion of gastric acid, with consequent peptic ulcer disease) and

chronic diarrhoea. Fewer than 5% of patients present this during adolescence. The disorder may be suspected in a patient presenting with unexplained peptic ulcer. Diarrhoea may be caused by colorectal neoplasia (Thomas *et al.,* 2003). Studies of screening colonoscopy in asymptomatic individuals have shown a prevalence of colonic adenomas of between 14.4% and 37.5% (7.9% with adenomas >10 mm) (Thomas *et al.,* 2003). This prevalence is strongly influenced by age, male sex, and a history of a first degree relative with colorectal cancer. Few studies have addressed the frequency of neoplasia in symptomatic patients, and none has specifically addressed the prevalence of adenomas in patients undergoing colonoscopy for diarrhoea (Guillem *et al.,* 1992 and Lieberman *et al.,* 2000). However, Neugut *et al*. (1993) showed a prevalence of colonic neoplasms of 27% in those patients undergoing colonoscopy for a change in bowel habit, a value which approached the yield of 33.6% in patients with a history of rectal bleeding. A large proportion (approximately50%) had neoplasia proximal to the splenic flexure, indicating the need for full colonoscopy rather than flexible sigmoidoscopy in these patients. Lymphocytic and collagenous colitis (collectively called microscopic colitis) are conditions with a similar natural history and often (in 25–30%) overlapping features. These conditions have increasingly been identified as a cause of diarrhoea in patients with macroscopically normal mucosa (Neugut *et al*., 1993). Although the diagnosis has often relied on biopsies obtained at flexible sigmoidoscopy, recent studies have pointed to the high false negative yield from rectosigmoid histology (34–43%) (Lieberman *et al.,* 2000).

2.2.5. Diarrhoea due to allergic reactions

A food allergy occurs when the immune system responds to a harmless food as if it were a threat. The first time a person with food allergy is exposed to the food, no symptoms occur; but the body sensitized (primed) to respond the next time. When the person eats the food again, an allergic response can occur and adverse reactions to ingested foods are extremely common, especially in children (Stefano and Haleh, 2010). Different pathogenic mechanisms underlie them, only those mediated by immune processes being defined as food allergies. Allergic reactions to food are due to several distinct immune reactions and can lead to a number of signs and symptoms including diarrhoea. It should be noticed that not all adverse effects of food are due to allergic responses. In fact, adverse reactions to ingested foods can be classified as either non-immune mediated (due to a variety of other conditions such as disorders of intestinal digestion, absorption or pharmacological reactions to chemicals in foods) or immune mediated. Of the latter, only a few represent a true hypersensitivity reaction (immunoglobulinE-mediated pathogenenesis). Nevertheless, the term ‘food allergy’ is used to encompass all specific reactions to offending food proteins that have an immunological basis, whether IgE or non-IgE mediated (Stefano and Haleh, 2010). Several food proteins, both from fluid and solid foods, can act as allergens in humans and cause an immune reaction. Cow’s milk proteins are most frequently implicated as cause of food intolerance during infancy. Soybean proteins rank second as antigens in the first months of life, particularly in infants with primary cow’s milk intolerance who are switched to a soy formular (Sichener, 2003). From school age on, egg protein intolerance becomes more prevalent in childhood; there is also evidence of growing prevalence of allergy to peanuts. Clinical reactions to all these proteins were reported to be very heterogenous in children were gastrointestinal symptoms typically diarrhoea predominate (Stefano and Haleh, 2010).

2.2.6. Drug-related diarrhoea

Up to 4% of cases of diarrhoea may be due to medications (particularly magnesium containing products, antihypertensive and non-steroidal anti-inflammatory drugs, theophyllines, antibiotics, antiarrhythmics, and antineoplastic agents) and food additives such as sorbitol and fructose, and these should be carefully sought (Thomas *et al.,*2003). Drug-related diarrhoea is a relatively frequent adverse event, accounting for about 7% of all drug adverse effects (Chassany *et al.,* 2000). More than 700 drugs have been implicated in causing diarrhoea. And several mechanisms have been reported to be involved in inducing drug related diarrhoea, such as altered gastrointestinal defences, mucosal damage of the small and large intestine and/or disruption of normal pathophysiological processes of fluid and electrolyte absorption and secretion; sometimes more than one mechanism may be involved (Chassany *et al.,* 2000). Epidemiological survey carried out in Italy demonstrated that children with diarrhoea were taking a significantly higher number of drugs than patients without diarrhoea. Moreover, a significant increase in the prevalence of diarrhoea occurred in patients who were treated with a progressively higher number of drugs, reaching a prevalence of 11.0% in patients who were taking 3-5 drugs and a prevalence of 11.7% in patients who were taking 6 drugs or more. In this population of children, the drugs significantly associated with the presence of diarrhoea were antibiotics, proton pump inhibitors, allopurinol, psycholeptics, selective serotonin reuptake inhibitors and the antihypertensive angiotensin II receptor blockers (Fabio *et al.,* 2009).

2.2.7 Purgative abuse

Example of this are “maganin basir”, “agbo jedijedi” etc. that may cause profuse diarrhoea and dehydration leading to death in severe cases (Akande *et al.,* 2012). Purgation was adopted worldwide as a therapy for diseases. The idea behind this broad application was that something in the body, the entity or toxin that caused the disease, needed to be flushed out. The elimination of this pathological influence was thought to be accomplished by sweating it out through the pores, vomiting it out through the mouth, or disposing of it through the intestines or urinary tract (Akande *et al.,* 2012). In the ancient Chinese system of therapy, prevalent during the Han Dynasty, the three methods of diaphoresis, emesis, and purgation were widely used for this purpose. Although the reliance on such approaches to treating diseases faded at times (some medical authorities considered the methods too drastic, debilitating the patient), they were later revived. For example, these methods were again promoted during the Jin-Yuan medical reform (12th-13th centuries) by proponents of the Purgation school (or attacking school) (Xing and Soffer, 2001). In America, treatment of numerous diseases by elimination techniques, especially purgation, has became a hallmark of the natural healing profession since the 19th century and is still deemed an important component by its practitioners, including use of such questionable therapies as colonics (intestinal cleansing with water) (Xing and Soffer, 2001). The proliferation of herbal concoction has been of great concern to stake holders in the health sector. Some Nigerians delve into the act of herbal medicine without any form of education or training. Most of them also do it without considering the dangers it posses to its consumers. Medical experts have cautioned that some purgative preparations are dangerous to the health since they don’t undergo any form of laboratory test and are not prepared and administered by either, Pharmacist or Microbiologist who by profession are trained to

produce drugs both solid and liquid. Investigation revoke that the use of purgative as cure for various illnesses is common among Nigerians who believes that the concoction is highly medicinal and that it cures almost every illness ranging from headache, stomach ache, back ache, diabetes, malaria, cold, fever, diarrrhoea, infertility among others. Although many had argued that many plants used in preparing this purgatives contain active substances and on many occasions had been used in producing conventional medicines but the fact remains that the herbal medicine market is highly unregulated at the moment (Akande *et al.,* 2012).

2.2.8 Mal-absorption of food eaten

The main purpose of the gastrointestinal tract is to digest and absorb nutrients (fat, carbohydrate, protein and fiber), micronutrients (vitamins and trace minerals), water, and electrolyte (Bai, 1998). Digestion involves both mechanical and enzymatic breakdown of food. Mechanical processes include chewing, gastric churning, and the to-and-fro mixing in the small intestine. Enzymatic hydrolysis is initiated by intraluminal processes requiring gastric, pancreatic, and biliary secretions. The final products of digestion are absorbed through the intestinal epithelial cells. Malabsorption constitutes the pathological interference with the normal physiological sequence of digestion (intraluminal process), absorption (mucosal process) and transport (postmucosal events) of nutrients (Bai, 1998). Malabsorption may occur as a result of defective luminal digestion due to lack of pancreatobiliary enzymes, or from failure of absorption due to mucosal disease or structural disorders. Although there is generally a combination of fat, carbohydrate, protein, vitamin, and mineral deficiencies, a predominance of one or other of these may exist (Thomas *et al.,* 2003). Thus pancreatic exocrine insufficiency is the usual cause of severe and dominant steatorrhoea where faecal fat excretion exceeds 13 g/day (47mmol/day). This is rare in mucosal or structural disease although milder forms of steatorrhoea commonly occur. In comparison, carbohydrate malabsorption is predominantly associated with mucosal disease or dysfunction (Bai, 1998).

2.2.9 Functional (psychogenic or non-specific) cause of diarrhoea

Functional diarrhoea (FD) or non-specific chronic diarrhoea (NSCD) is considered as the most frequent cause of chronic diarrhoea without failure to thrive in toddlers. Functional diarrhoea as a clinical entity is defined as a daily painless, recurrent passage of three or more, large, unformed stools during a period of at least 4 weeks, with an absence of alarm signs such as failure to thrive if caloric intake is adequate, abdominal pain, or blood in the stool and emesis (Stefano and Haleh, 2010). The clinical history is the key point to identify patients with FD. Minimal blood and stool tests may help to differentiate between the causes of diarrhoea such as infection, celiac disease, and inflammatory conditions. The pathophysiology of FD is not well understood (Stefano and Haleh, 2010). This functional disorder not only occurs in toddlers or children but is also frequently present in the adult population. Generally the syndrome is associated with normal growth and intestinal absorption. The aetiology and pathophysiology is poorly understood, but it is believed to be secondary to abnormalities in the gastric and intestinal motility after ingestion of beverages containing fructose as a predominant carbohydrate. Recently an Italian prospective cohort study was performed to determine the prevalence of different

functional gastrointestinal disorders (FGID). A total of 9660 patients aged from birth to 12 years were enrolled by 13 primary care pediatricians from the Campania region of the Italian National Health Service. A total of 2% met the strict criteria for at least one functional gastrointestinal disorder. Of these 194 children, 3.6% fulfilled the criteria for functional diarrhoea resulting in a total prevalence of 0.07% (Miele *et al.,* 2004). The prevalence of FD in children has not been established among different races and regions in the world. A recent study among Afro-American children living in the United States revealed a prevalence of FGID of 19%; however, data concerning FD were lacking in this study (Stefano and Haleh, 2010).

2.2.10. Infectious agents

Infectious diarrhoea is the most common cause of diarrhoea worldwide and is the leading cause of death in childhood (Casburn-Jones and Farthing, 2004). Gastrointestinal infections have their major impact in the developing world (WHO, 2009). In the developed world, despite improvements in public health and economic wealth, the incidence of intestinal infection remains high and continues to be an important clinical problem (Casburn-Jones and Farthing, 2004). Reports from different parts of the world have implicated various pathogens such as bacteria e.g. *Enterotoxigenic E. coli (*ETEC*), Enteropathogenic E. coli (*EPEC*), Enteroaggregative E. coli (*EAggEC*), Enteroinavsive E. coli (*EIEC*), Enterohaemorraghic E. coli (*EHEC*), Shigella* spp*., Salmonella* spp*., Campylobacter* spp*., Yersinia* spp*., Clostridium difficile, Mycobacterium tuberculosis, cholera and other vibrio*. Others are *Yersinia enterocolitica, Bacillus cereus, Aeromonas hydrophiliae,* viruses e.g. Rotavirus , Enteric adenovirus (types 40 and 41), Calicivirus, Astrovirus, Cytomegalovirus, HIV with outbreak of diarrhoeal disease in children, Chlamydial e.g. Lympho-granuloma venereum, Fungal e.g. *Histoplasma duboisii* Protozoa e.g *Giardia intestinalis, Cryptosporidium parvum, Microsporidia, Isospora belli, Cyclospora cayetanensis, Entamoeba histolytica, Balantidium coli,* Helminths e.g *Strongyloides stercoralis, Schistosoma* (Casburn-Jones and Farthing, 2004).

However in the developing countries including Nigeria, infantile diarrhoea disease is grossly under-reported and the incidence under-estimated, this is attributed to poverty and ignorance among affected group who usually constitute up to 80% of the population of the area (Olowe *et al.,* 2003). In Nigeria, available reports indicate that more than 315,000 deaths of preschool age children are recorded annually as a result of diarrhoea disease (Ifeanyi *et al.,* 2010). Nevertheless, despite the public health implications and enormous burden imposed on the primary health care delivery system by infantile diarrhoea illness in the country, there is still paucity of information on the epidemiology and aetiology of infantile diarrhoea in many regions including the North-western Nigeria.

2.3 Severity of diarrhoeal infection

The clinical evaluation of diarrhoeic patient involve the determination of severity of infection which is determine by the size and number of stools passed within a period of time (Casburn-Jones and Farthing 2004).

Mild diarrhoea diarrhoea means having a few diarrhoea stools in a day and is caused by dietary factors or extra-gastrointestinal infections (Mei, 2001). Gastrointestinal symptoms associated are that stools become frequent but usually no more than ten times a day, gruel-like or watery, yellow or greenish yellow in color, smell sour, vomiting is less common and abdominal pain is mild. There is no obvious systemic symptom. Infants may be restless or irritable; temperature is normal or slightly high. There is no dehydration, electrolyte and acid- base disturbance (Mei, 2001).

Moderate diarrhoea is associated with 5-10% water loss, poor elasticity of the skin turgor, restlessness, and dry sunken eye socket, tears decreased and poor peripheral circulation. Lassitude, lethargy, coma or irritability with a deep rapid respiration (Kussmauls breathing) is other forms of obvious sign of this form of diarrhoea (Mei, 2001).

Severe presentations of diarrhoea constitute one of the commonest challenges faced by the medical team in pediatric ambulatories and emergency rooms in the developing world (Casburn-Jones and Farthing 2004). Supportive anti-dehydration treatment is the cornerstone of therapy and must be promptly started. Severe diarrhoea is usually caused by intestinal infections while gastrointestinal symptoms was reported as stools becomes more frequent (>10 times daily), watery in consistency, yellow or greenish yellow, sometimes with mucus, pus and blood. Vomiting is severe even blood in vomitus (Mei, 2001). Other symptoms include: anorexia, nausea, abdominal pain and abdominal distention. Obvious systemic symptoms are infants may be very irritable, lethargy even coma. The temperature may be high or low, while water and electrolyte balance usually present moderate or even severe dehydration, acidosis and electrolyte disturbances. Signs usually used as criteria include: dryness of lips, skin and mucous membranes, poor skin turgor (elasticity), depressed anterior fontanel, lack of tears, sunken eyes socket and poor peripheral circulation. The patient may show tachycardia, thin and thready pulse, a low or falling blood pressure, pallor, cool extremities, delayed capillary refilling, hypothermia, oliguria, excessive loss of water and electrolytes due to diarrhoea and vomiting (Mei, 2001). According to the osmolarity of body fluid following dehydration, the dehydration can be divided into three types: Isotonic, hypotonic, and hypertonic dehydration. Isotonic dehydration is caused by diarrhoea associated with vomiting and poor intake and detected by serum sodium level of 130-150 mmol/L. Hypotonic dehydration is caused by diarrhoea associated with malnutrition and characterized by serum sodium level less than 130 mmol/L. And the hypertonic dehydration is characterized by excessive sweating during diarrhoeal episode with a serum sodium level greater than 150 mmol/L (Mei, 2001).

2.4 Forms of diarrhoea

Classification of various forms of diarrhoea facilitates the approach to management of childhood diarrhoea (Hall, 2010). Issues related to the aetiology, clinical assessment, treatment, and prevention of different types of diarrhoea are better analyzed when classified. Diarrhoea can be classified based on aetiological factor, duration of each diarrhoeal episode, pathophisiological mechanism and systemic diseases contributing to diarrhoea. This distinction, supported by the World Health Organization (WHO, 2003), has implications not only for classification and epidemiological studies but also from a practical

standpoint because protracted diarrhoea often has a different set of causes, poses different problems of management, and has a different prognosis. Clinically, diarrhoea can be classified into many ways; based on the aetiologic epidemiology (community-acquired, hospital-acquired, traveler's diarrhoea), time course (acute, persistent and chronic), volume (large, small), pathophysiology (secretory, osmotic and functional), or stool characteristics (watery and fatty). However, there is considerable overlap amongst these classifications (Stefano and Haleh, 2010).

2.4.1 Infectious diarrhoea

Infectious diarrhoea can occur in various epidemiological settings including community acquired, hospital acquired, and during travel (traveler's diarrhoea). Understanding of the aetiology and epidemiological settings in which diarrhoea occurs directs the approach to diagnosis and treatment (Hall, 2010).

2.4.1.1 Community-acquired infectious diarrhoea

In developed nations, viral-induced diarrhoeas are the most common of community-acquired diarrhoeas and account for at least 30–40% of acute episodes with noroviruses and rotaviruses reported as the most common cause of diarrhoea (Musher and Musher, 2004). Rotavirus predominantly affects children below 5 years and is also the leading cause of diarrhoea-associated death in children below 5 years worldwide (Parashar *et al.,* 2003). It is responsible for childhood diarrhoea in 35% of hospitalized and 10–30% of community-based cases. Although rotavirus predominantly affects children, adults can also be affected as immunity wanes off and outbreaks tend to occur in close settings where chances of person-to person transmission are higher such as day care centers, long-term care facilities, and schools (Rodriguez *et al.,* 1987). In developing countries, bacterial and protozoal infections are more common causes of infectious diarrhoea than in developed countries (Feldman *et al.,* 2006). Poor sanitation predisposes to community-acquired infection with enteric bacterial pathogens and protozoa including *Vibrio cholerae*, enterotoxigenic, enteropathogenic and enteroinvasive *E. coli* (ETEC, EPEC and EIEC, respectively), amoeba, giardia, and intestinal parasites. The most common bacterial causes of diarrhoea in children include enterohemorrhagic *Escherichia coli* (EHEC), non typhoidal *Salmonella*, *Shigella*, and *Campylobacter* (Feldman *et al.,* 2006). *Campylobacter* infection is transmitted predominantly from infected animals and their food products and most human infections (50–70%) appear to be related to consumption of improperly cooked, contaminated chicken (Feldman *et al.,* 2006). Nontyphoidal salmonellosis is seen worldwide and one of the most common causes of food poisoning and diarrhoea. Transmission to humans appears to occur most commonly from infected animals and their food products. Most human infections are related to consumption of improperly cooked or contaminated poultry, although a variety of vehicles can transmit salmonellosis (Feldman *et al.,* 2006). Shigellosis is common and accounts for 10–20% of enteric infections throughout the world. It is seen commonly in children below 5 years of age, although adults of all ages are also susceptible (Feldman *et al.,* 2006). It can survive in acidic conditions and a very small inoculum of the organism (less than 100 organisms) is sufficient to produce disease (Feldman *et al.,* 2006). EHEC is one of the commonest causes of bloody diarrhoea. Infection usually occurs primarily by ingestion of undercooked hamburgers and meat patties

(Bell *et al.,* 1994). Enteroaggregative *E. coli* (EAEC) is a recently recognized cause of community-acquired diarrhoea and affects children and adults of both developing and developed countries (Bell *et al.,* 1994). EAEC has been shown to cause acute and/or persistent diarrhoea in 10–44% of patients with HIV infection and childhood diarrhoeas in developing countries (Bell *et al.,* 1994). *Clostridium difficile* infection (CDI) is also recently reported as a cause of community-acquired diarrhoea. Approximately 22–44% of patients who developed community-acquired CDI lacked the traditional risk factors like recent hospitalization, young age, being elderly or having an underlying health condition (Noren *et al.,* 2004). Worldwide, cholera is one of the most common causes of infectious diarrhoea and is seen predominantly in the Indian subcontinent, Southeast Asia, Africa, and South America (Morris and Black, 1985). Intestinal protozoa are important causes of diarrhoea in the developing world (Petri *et al.,* 2000). *Entamoeba histolytica* is one of the most important causes of diarrhoea/dysentery worldwide with 34–50 million symptomatic (amoebic colitis or abscess) infections leading to 40,000–100,000 deaths annually (Petri *et al.,* 2000). In the USA, *Cryptosporidium* and *Giardia lamblia* are the most commonly implicated intestinal protozoa to cause diarrhoea associated with 50.8 and 40.6% of waterborne outbreaks, respectively (Petri *et al.,* 2000). *Giardia* infection is commonly seen in children in day care facilities, men who have sex with men, as well as in the normal host (Kappus *et al.,* 1994). A longitudinal study in a United State day care center reported *Giardia* cysts at some time in the stool of more than 30% of children over the course of a year (Peters, *et al.,* 1986). Worldwide, *Giardia* infects infants more commonly than adults and in highly endemic regions, recurrent infections in childhood can result in malnutrition (Stefano and Haleh, 2010). *Cryptosporidium* infection while commonly seen in HIV-infected individuals can also cause self-limited diarrhoea in immunocompetent persons and severe diarrhoea resulting in malnutrition in children and elderly as well (Stefano and Haleh, 2010). It is mostly associated with outbreaks caused by contaminated water sources. The duration and severity of the infection however is directly related to the CD4 count. Other protozoal and parasitic infections that are reported include *Blastocystis, microsporidia (Enterocytozoon* spp*., Encephalitozoon* spp.*), Isospora, Cyclospora, Schistosoma,* and *Strongyloides* (Stefano and Haleh, 2010).

2.4.1.2 Traveler's diarrhoea

Traveler's diarrhoea is defined as the passage of more than three unformed stools that occur within a 24 hrs period, accompanied by one other symptom or sign of enteric infection, including abdominal cramps or pain, excessive gas, nausea, fever, blood or mucous in stools, tenesmus, and vomiting (Steffen, 2005). A variety of host, genetic, and environmental factors predispose to traveler's diarrhoea. Genetic factors in the form of polymorphisms in the lactoferrin and steoprotegerin gene, young age, length of stay, immunosuppression, and low gastric acidity can predispose to traveler's diarrhoea (Stefano and Haleh, 2010). Traveler's diarrhoea can present either as an acute gastroenteritis with vomiting, watery diarrhoea, dysenteric diarrhoea, or as a persistent diarrhoea and post-infectious irritable bowel syndrome (DuPont, 2009). The single commonest cause of traveler's diarrhoea is ETEC, while EAEC and diffusely adherent *E. coli* (DAEC) are also common (Stefano and Haleh, 2010). The most common form of transmission is food borne rather than water borne (DuPont, 2009). Around 20–40% of patients with traveler's diarrhoea do not have an identifiable etiology even after an extensive microbiological evaluation, although they may respond to antibiotics (Stefano and Haleh, 2010).

Noroviruses are responsible for up to 15% of patients and are the most common nonbacterial cause for traveler's diarrhoea. Rotavirus and astrovirus are also reported responsible for traveler's diarrhoea in a small proportion of travelers (DuPont, 2009).

2.4.1.3 Hospital-acquired infectious diarrhoea

Hospital-acquired diarrhoea is defined by the onset of diarrhoea 3 days after hospitalization and not incubating at the time of admission to the hospital (Pawlowski *et al.,* 2009). Distinguishing community-acquired and hospital acquired diarrhoea may be difficult as the possibility of the infection incubating at the time of admission to the hospital cannot be completely excluded. *Salmonella* has been implicated as a prominent cause of hospital acquired diarrhoea in developing countries along with other enteropathogens (Wall *et al.,* 1996). *Salmonella* infection occurs in a more severe form in immunocompromised patients. Similar to community acquired infection, contaminated food, person-to-person spread, and chemotherapy predispose to *Salmonella* infection (Alam *et al.,* 2005). Although protozoa are rarely implicated in the setting of hospital acquired diarrhoea and cryptosporidial infection remains the most common cause in the develop countries, there are also reports of co-infection with *C. difficile* and *Cryptosporidium* (Neil *et al.,* 1996). Among the viruses, norovirus and rotavirus may be responsible for hospital-acquired viral diarrhoeas both in the developing and the developed world (Kordidarian *et al.,* 2007).

2.4.2 Acute diarrhoea

This term refers to diarrhoea that begins acutely, lasts less than 14 days (most episodes last less than seven days), and involves the passage of frequent loose or watery stools without visible blood. Vomiting may occur and fever may be present (WHO, 2009). The most common causes of acute diarrhoea are infections, food poisoning, and medications. Medications are a frequent and often over-looked cause, especially antibiotics and antacids. Less often, various sugar free foods, which sometimes contain poorly absorbable materials, cause diarrhoea (Hall, 2010). Acute watery diarrhoea causes dehydration; when food intake is reduced it also contributes to malnutrition. When death occurs, it is usually due to acute dehydration. Severe presentations of acute diarrhoea constitute one of the commonest challenges faced by the medical team in pediatric ambulatories and emergency rooms in the developing world. This phenomenon is most likely caused by an infectious agent, such as bacterial, parasitic or viral invasion, or by a non-infectious agent such as dietary indiscretion or a new medication (Amerine and Keirsey, 2006). The most important causes of acute watery diarrhoea in young children in developing countries are rotavirus, enterotoxigenic *Escherichia coli, Shigella, Campylobacter jejuni,* and *Cryptosporodium*. In some part of Nigeria *Vibrio cholerae* 01, *Salmonella* and enteropathogenic *E. coli* are reported as important agent of this form of diarrhoea (Olowe *et al.,* 2003). Acute diarrhoea is typically self limiting and resolves quickly with no lasting sequelae (Amerine and Keirsey, 2006). Infectious agents are one of the factors associated with acute diarrhoea. Some of these pathogens can cause an inflammatory response in the gut where the epithelial lining is damaged either by a toxin produced by the organism or by an organism invading the mucosa (Bliss *et al.,* 2006). Some organisms that cause an inflammatory response are "Cytomegalovirus, Herpes simplex virus, *Shigella, Salmonella,*

Chlamydia, Nisseria gonorrheae, Campylobacter jejuni, Clostridium difficile, Escherichia coli O157:H5, *Entamoeba histolytic"* (Bliss *et al.,* 2006). Symptoms of acute inflammatory diarrhoea include fever (higher than 38.5^{o}C), lethargy, and a stool that contains pus, blood, leukocytes and/or mucus (Bliss *et al.,* 2006). There are organisms that cause acute diarrhoea that do not produce an inflammatory response although the person may have a low grade fever, malaise, nausea and vomiting as well as diarrhoea. These causative organisms include; Norwalk virus, Rotovirus, *Staphylococcus aureus, Clostridum perfringens, Vibrio cholerae* and enterotoxigenic *Escherichia coli* (Bliss *et al.,* 2006). Less commonly, protozoa such *Giardia* or *Cryptosporidium* may be the causative factor (Armerine and Keirsey, 2006). Norovirus, formerly known as the Norwalk-like virus, is one of the prime causes of outbreaks of viral gastroenteritis and is usually the culprit in outbreaks aboard cruise ships (CDC, 2007). Norovirus has been implicated in over 23 million cases of gastroenteritis and results in 23,000 hospitalizations of children under five yearly in the US (CDC, 2010). Rotavirus was the primary cause of severe, acute gastroenteritis in the very young population worldwide prior to 2006 (WHO, 2010).

Worldwide 3-5 billion cases of acute diarrhoea occur per year (WHO, 2010). The annual mortality in children less than five years old in developing countries has decreased during the last 20 years, due mainly to the introduction of oral rehydration therapy (ORT), from 4.5 million deaths to 1.8 million deaths, but the morbidity remains high (Liu *et al.*, 2012). It was estimated that there were 527,000 deaths of children under the age of five attributable to acute diarrhoeal disease in 2004 only (WHO, 2010). Two thirds of the mortality still occurs in developing countries, including Nigeria (Iwalokun *et al*., 2001). In Nigeria, diarrhoea was the third cause of mortality in 2010, with 24% of deaths per year in children under five years of age. The microbial agents causing these deaths are not known, because the aetiologies of acute gastroenteritis in Nigeria have not been much studied (Ifeanyi *et al.*, 2010).

2.4.3 Persistent diarrhoea

This is diarrhoea that begins acutely but is of unusually long duration lasting from 2 to 4 weeks (at least 14 days) (Stefano and Haleh, 2010). The episode may begin either as watery diarrhoea or as dysentery with marked weight loss often frequent (Pawlowski *et al.,* 2009). Diarrhoeal stool volume may also be great, with a risk of dehydration (Stefano and Haleh, 2010). There is no single microbial cause for persistent diarrhoea; infectious aetiologies predominate as a cause of persistent diarrhoea similar to those of acute diarrhoeas (Pawlowski *et al.,* 2009). The aetiology varies depending on the region (developing or developed), recent travel history, and the immune function of the underlying host. Persistent diarrhoea can be associated with significant morbidity due to the associated nutrient malabsorption that may often accompany the diarrhoea (Pawlowski *et al.,* 2009). In developing countries, EPEC and EAEC are the most commonly responsible bacterial pathogens in persistent diarrhoea, whereas *Campylobacter* and *Salmonella* are rare. In developed countries, viruses including Norovirus and rotavirus can contribute to persistent diarrhoea, particularly among children (Kosek *et al.,* 2003). ETEC, EHEC, and *Shigella* are predominant causes of acute diarrhoea and do not cause persistent diarrhoea. Intestinal protozoa are another common cause of persistent diarrhoea (Vernacchio *et al.,* 2006). *Giardia* and *Cryptosporidium* are most often responsible followed by *Entamoeba* and *Isospora*, particularly in HIV-positive patients (Gupta *et al.,* 2008). In the USA, protozoan infections are the most

common cause of persistent diarrhoea in immunocompromised patients including HIV and the elderly with *Giardia* and *Cryptosporidium* as commonly encountered (Pawlowski *et al.,* 2009).

2.4.4 Chronic diarrhoea

Chronic diarrhoea is a termed applied to diarrhoea episodes which are of long duration (more than 4 weeks), for which no known cause can be found and which does not respond to specific or non specific form of treatment (Binder, 2006). Chronic diarrhoea is defined as stool volume of more than 10 grams/kg/day in infants and toddlers, or more than 200 grams/day in older children for more than 14 days. This typically translates to persistent loose or watery stools occurring at least three times a day, where the change in stool consistency is more important than stool frequency. Some authors make a distinction between chronic diarrhoea, which they define as having a gradual onset, from persistent diarrhoea, which they define as having a sudden onset (Fine *et al.,* 1997, Binder, 2006, Stefano and Haleh, 2010). Chronic diarrhoea is a common diarrhoeic condition with diarrhoea lasting more than two to four weeks occurring in up to 3 to 5 percent of the population worldwide (WHO, 2010). It is generally more frequent in males, with a male-to-female ratio of 1.2 to 2.6:1 in the age range of 6 to 24 months. The etiologies of chronic diarrhoea vary depending on the region and the socio-economic status. In developed countries including the USA, irritable bowel syndrome, inflammatory bowel disease, malabsorption syndrome, and chronic infections predominate, whereas in developing countries, chronic bacterial, mycobacterial, and parasitic infections are the most common causes of chronic diarrhoea (Kotwal *et al.,* 1978, Manatsathit *et al.,* 1985 and Bytzer *et al.,* 1990).

2.4.5 Secretory Diarrhoea.

Secretory diarrhoea occurs when there is an increase in the amount of fluid being drawn into the lumen of the bowel such that the ability of the intestines to reabsorb is overwhelmed (Bliss *et al.,* 2006). Typically, infectious agents are the cause of secretory diarrhoea but any substance (secretagogue) that causes fluid to be pulled into the bowel can be the culprit (Strasinger and Di Lorenzo, 2008). Infectious secretagogues include *Vibrio cholerae, E. coli, Camylobacter jejuni, Salmonella, Shigella,* and *Clostridium difficile* (Farthing, 2006). These pathogens secrete toxins that bind with the structures within the gut, altering, sometimes irreversibly, the amount of fluid secreted into the bowel (Bliss *et al.,* 2006). As an example, the toxin excreted by the pathogen cholera causes massive secretory diarrhoea which, during its acute phase, can be as much as 24 liters in 24 hours (Farthing, 2006). Non-infectious include chemicals produced by certain types of cancer, prostaglandins produced in patients with bowel inflammation and substances not well absorbed such as fatty acids and bile acid (Bliss *et al.,*2006). Persons with secretory diarrhoea will typically have stool volume of more than one liter daily, with neutral pH and have no change in the amount of stool produced with fasting (Bliss *et al.,* 2006). Secretory diarrhoea occurs when there is active secretion of water into the gut lumen. This type of diarrhoea is often caused by a secretagogue, such as cholera toxin, binding to a receptor on the surface epithelium of the bowel and thereby stimulating intracellular accumulation of cyclic adenosine monophosphate or cyclic guanosine monophosphate. Certain substances, such as bile acids, fatty acids,

and laxatives, also can produce secretory diarrhoea, as can congenital problems (e.g., congenital chloride diarrhoea). Diarrhoea not associated with an exogenous secretagogue may also have a secretory component (congenital microvillus inclusion disease). Secretory diarrhoea tends to be watery and of large volume; the osmolality of the stool can be accounted for by the presence of electrolytes. Secretory diarrhoea generally persists even when no feedings are given by mouth (Schiller, 1999).

2.4.6 Osmotic Diarrhoea.

Osmotic diarrhoea occurs when there is a dysfunction in the ability of the intestine to reabsorb fluid as it flows through the lumen (Stefano and Haleh, 2010). This may be caused by incomplete breakdown or malabsorption of nutrients in the small intestine allowing a larger and more liquid mass to enter the colon (Strasinger and Di Lorenzo, 2008). This fecal matter then creates a negative osmotic gradient causing leakage of more fluid into the gut increasing the stool volume (Bliss *et al.,* 2006). The causes of this type of osmotic diarrhoea are varied but can be broken down into decreased enzymatic availability (lactose intolerance), a genetic abnormality that decreases or eliminates the ability of the body to absorb certain nutrients (celiac sprue), sugars that are poorly absorbed (sorbitol, mannitol or lactose), laxatives, magnesium containing antacids, and antibiotic administration as well as malabsorption of certain fats (Strasinger and Di Lorenzo, 2008). Other causes have more to do with changes within the bowel that decrease the ability to reabsorb fluid and nutrients as the stool is propelled through the lumen (Binder, 2006). Malnutrition, especially protein-calorie malnutrition causes "reversible atrophy of the villi and brush border," the structures within the intestine responsible for absorption. Resection of parts of the bowel, especially the terminal ileum, will mechanically decrease the body's ability to absorb due to decreased length of intestine available (Bliss *et al.,* 2006). Inflammation of the bowel due to infection or disease processes (Crohn's disease) can be another cause of osmotic diarrhoea. Typically, osmotic diarrhoea responds with decreased stooling when the individual fasts (Binder, 2006). A person with osmotic diarrhoea will have stool volume under one liter per day, the stool will be acidic and more potassium will be lost than sodium (Bliss *et al.,* 2006).

2.4.7 Childhood diarrhoea

In children, the strict definition of diarrhoea is excessive daily stool volume, more than the upper limit of around 10 g/kg/day (Gracey, 1985). It is certainly possible to have diarrhoea by this definition with stools that are at least partially formed, or to not have diarrhoea even with liquid bowel movements. As a practical matter, it is seldom possible for a physician to determine exactly how many grams per day of stool a child is having. The history would usually provide most of the information required to classify the diarrhoea by type and to consider the diagnostic approach (Gracey, 1985). In the recent years the spectrum of infectious diarrhoea in childhood has expanded, owing to the emergence of new conditions, such as the acquired immunodeficiency syndrome (AIDS), the advancement in therapy and survival of patients transplanted and treated with immunosuppressive drugs, and the improvement in endoscopic and microbiological techniques (Kosek *et al.,* 2003). The most common infectious causes of childhood diarrhoea are, viral, bacterial, protozoal and, more rarely, fungal (Stefano, 2004). Pathogens affecting the small intestine are usually non-invasive organisms but patients may

present with high-volume watery stools and in some cases malabsorption, frequently leading to dehydration and often have periumbilical pain and cramping (Stefano, 2004). Most pathogens that cause childhood diarrhoea share a similar mode of transmission – from the stool of one person to the mouth of another. This is known as faecal-oral transmission. There may be differences, however, in the number of organisms needed to cause clinical illness, or in the route the pathogen takes while travelling between individuals (for example, from the stool to food or water, which is then ingested). Childhood diarrhoea is thus an enormous problem both in developing and in industrialized countries, but with two distinct consequences. In the former, enteric infections are extremely frequent, as the incidence of diarrhoea is estimated at 3.8 episodes per child per year in infants and 2.1 episodes per child per year in children 1–4 years of age (WHO, 2010). A cumulative incidence of 2.5–3 million deaths each year has been estimated, corresponding to 25% of all deaths in childhood up to 5 years of age (WHO, 2010). In the developing world, one out of 40 children will die because of diarrhoea (Zimmermann *et al.,* 2001). In industrialized countries, on the other hand, the incidence of diarrhoea is approximately 1–2 episodes/child per year in subjects younger than 3 years. The case/fatality ratio is far from the figures in poor countries, but not negligible. In the USA, 150–300 infants or younger children die each year because of acute diarrhoea, and substantial resources are needed for hospitalizations and medical visits. Diarrhoea was associated with an annual average of 35 hospitalizations per 10000 children younger than 5 years, corresponding to 4% of all hospitalizations (Zimmermann *et al.,* 2001). It was estimated that 1 in 57 children would be hospitalized by 5 years of age for diarrhoea-associated illness. The rate of outpatient visits was 943/10000 children, corresponding to 2% of all visits (Stefano, 2004). The child history may provide valuable clinical information. Diarrhoeal onset may be abrupt or progressive. The child's age, admission to a daycare center, the time of year, exposure to diarrhoeal contacts, previous antibiotic courses, and ingestion of contaminated food such as eggs or water should be evaluated to define the origin of diarrhoea. Risk factors for severe childhood diarrhoea include malnutrition, immune derangement, AIDS, and history of repeated episodes of diarrhoea. Recent weaning from breast milk or introduction of feedings other than milk may also be associated with a more severe course of the diarrhoeal disease (WHO, 2010).

2.5 Aetiologic agents of childhood diarrhoea

During the past three decades, there has been a dramatic increase in the number of newly recognized aetiological agents of gastroenteritis. Before 1980, a pathogen could be identified in fewer than 10% of patients hospitalized with diarrhoea; the remaining 90% of cases represented a 'diagnostic void' consisting of various idiopathic, poorly defined conditions. Since 1990, more than 20 different microorganisms – viruses, bacteria, and parasites have been recognized as aetiological agents, and most cases of gastroenteritis are now presumed to have an infectious aetiology (WHO, 2011).

2.5.1 Viral diarrhoea

Despite the magnitude of the problem of infectious diarrhoea, the search for important aetiologic agents viral, bacterial, or parasitic was unrewarding until the 1970s. This was especially disappointing for virologists, because with the advent of tissue culture technology, scores of enteric viruses were discovered but none was found to be an important aetiologic agent of infectious diarrhoea

(Yow *et al.,* 1970). This frustration ended with the discovery in 1972 of the 27-nm Norwalk virus and its association with epidemic viral gastroenteritis in older children and adults, followed by the discovery of the 70-nm human rotavirus and its association with severe endemic diarrhoea in infants and young children (Kapikian *et al.,* 1973). Both of these fastidious agents were discovered without the benefit of tissue culture technology; by necessity, their identification relied on direct visualization by electron microscopy (EM).

Viral infections of the gastrointestinal tract cause 2 billion cases of diarrhoea in children per year, resulting in 18 million hospitalizations and as many as 3 million deaths (Taterka, 1992). Although numerous viruses have been identified in fecal samples of patients with diarrhoea, causal relationships have been determined for relatively few (Stefano, 2004). Most children are infected with viruses belonging to four distinct families: rotaviruses, caliciviruses, astroviruses and enteric adenoviruses. Other viruses such as the toroviruses, picornaviruses (Aichi virus), and enterovirus play a minor epidemiological role. Finally, selected viruses induce diarrhoea only in children at risk. These include cytomegalovirus, Epstein–Barr virus and picobirnaviruses. Enteric viral infections are usually associated with diarrhoea and, less frequently, with vomiting (Taterka, 1992). Diarrhoea usually lasts a few days and resolves spontaneously without any major problem. However, in selected cases it may be severe, leading to dehydration, requiring hospital admission and possibly even resulting in fatal outcome. The impact of viral diarrhoea is related to the specific strain, but occasionally also to the epidemiological setting, the host baseline features and the efficacy of early medical intervention. Viral diarrhoea may have a major impact in closed communities such as day-care centers and hospitals. It may be severe in malnourished or immunocompromized children, but also in children not belonging to groups at risk. Diarrhoea may become severe if rehydration is not initiated in the initial phases of the disease. Viral diarrhoea was originally believed to be caused by cell invasion and epithelial destruction by enteropathogenic agents, therefore being the result of endoluminal fluid accumulation osmotically driven by non-absorbed nutrients. It is now known that several mechanisms are responsible for diarrhoea, depending on the specific agents and the host features. In addition, selected viruses possess multiple virulence pathways that act synergistically to induce diarrhoea (Taterka, 1992).

2.5.1.1 Group A rotavirus

Rotavirus is a double stranded ribonucleic acid (RNA) virus measuring about 70nm, and considered as a common cause of diarrhoea in children (Maharjan *et al.,* 2007). Rotavirus is a double-stranded RNA virus belonging to the Reoviridae family. The virion is composed of a three-layered protein capsid that encloses 11 distinct segments of genomic RNA, each coding for a different capsid or non-structural protein (Stefano, 2004). The internal core contains viral proteins (VP) 1, 2 and 3; the inner capsid contains VP4; the two outer capsid proteins encoded by genes 4 and 7, namely VP4 and VP7, represent the only established neutralization antigens of the virus. The protective role of antibodies directed at these proteins has been confirmed both in experimental animal models and in humans. A

possible role has been suggested for antibodies directed at the inner capsid protein VP6, which is not associated with *in vitro* neutralization. The non-structural proteins NSP1, NSP2 and NSP4 are virulence factors in mice (Stefano, 2004). Rotavirus groups A–F have been described, but only groups A, B and C have been identified in humans. Most human infections are caused by group A rotaviruses that are classified into serotypes by a dual classification system based on neutralizing antigens on two outer capsid proteins, VP7 (G serotype) and VP4 (P serotype). There is great genetic diversity within each G and P type, as shown by gel electrophoretic analysis of gene patterns (electropherotypes) (Stefano, 2004).

Rotavirus is a contagious virus and, among children, is the leading cause of severe diarrhoea. In Some infants and children, diarrhoea may be so severe that they become dehydrated and may require emergency care or hospitalization. Transmission of rotavirus most frequently occur through faecal-oral contact. Usually, this occurs from poor hand washing or from ingestion of contaminated food or drinking water. The virus may also be transmitted through the respiratory tract or by other body fluids, but these routes are less common. The virus may live on inanimate surfaces, such as doorknobs, toys and hard surfaces, for quite some time. This may cause out breaks in child care centers and households where things are shared (WHO, 2010). Therefore hospitalized children need to be separated from the rest and quarantined. Oral administration of rotavirus-positive stool material induces diarrhoeal illness in volunteers (Kapikian *et al.,* 1983). Nevertheless, speculation continues as to whether rotaviruses are transmitted also by the respiratory route. Evidence for this is circumstantial and there have been scattered reports of detection of rotavirus in the upper respiratory tract; however, the predominance of data from other studies suggests that this is not the usual mode of transmission (Albert *et al.,* 2001).

The most obvious symptom of rotavirus ranges from mild to severe dehydration. Similarly, other common symptoms are nausea, vomiting, fever, abdominal pain, diarrhoea and dehydration (Moon, 1994). Information concerning the pathogenesis of rotavirus infection has been obtained from experimentally infected animals that developed diarrhoeal illness following administration of human rotavirus. In one study, the pathogenesis of human rotavirus strain was studied in newborn, gnotobiotic, colostrum-deprived calves that developed illness following intraduodenal administration of virus. Morphologic changes proceeded in a cephalocaudad direction in the small intestine. Within half an hour of experimentally induced diarrhoea, morphologic changes such as denuding of villi and flattening of epithelial cells were observed in the upper small intestine, but rotaviral antigens were not detected by immunofluorescence (IF). At this time, the lower small intestine was intact, but rotavirus antigens were abundant in swollen epithelial cells. Seven hours after onset of diarrhoea, the lower small intestine showed morphologic changes similar to those seen earlier in the upper small intestine; however, rotavirus antigens were not detectable by IF. Forty hours after onset of diarrhoea, the intestine appeared relatively normal (Mebus *et al.,* 1977). Rotaviruses have a rather limited tissue tropism, in most cases only infecting the villus epithelium of the small intestine. Previously, it was thought that the diarrhoeal disease resulting from infection is a consequence of a cytocidal infection. Increasing evidence indicates that disease may also result from the actions of viral proteins (e.g., the enterotoxin) as well as other proteins produced by infected epithelial cells (Holmes, 1996).

Epidemiological and molecular studies in many countries show complex changes from year to year in the rotavirus serotypes and electropherotypes that cause diarrhoea in children from the same geographical areas. But the toll from diarrhoeal diseases due to rotavirus in developing countries is staggering (Stefano, 2004). It is estimated that 1 billion diarrhoeal illnesses and 3.3 million deaths from diarrhoea occur annually in the under-5-year age group in developing countries of Africa, Latin America, and Asia (excluding China) (Bern *et al.,* 1992). Rotavirus is the leading cause of diarrhoea hospitalization among children worldwide (Parashar *et al.,* 2006). In 2000-2003, six causes accounted for 73% of the 10.6 million yearly deaths in children younger than age 5 years; of which diarrhoea was responsible for 18% of total deaths (Bryce *et al.,* 2005) and as of 31 March 2006, the World Health Organization estimated that globally 527,000 (475,000-580,000) child deaths occurred during 2004 due to rotavirus infection. National estimates of rotavirus attributable deaths among children less than five years of age ranged from 122,270 in Nigeria to fewer than 5 deaths in about 58 other countries in the world (WHO, 2011). Twenty-three percent of all rotavirus deaths less than five years of age occurred in six countries (India, Nigeria, the Democratic Republic of the Congo, Ethiopia, China and Pakistan) and accounted for more than half of all Rotavirus diarrhoeal deaths under age five in 2004 (WHO, 2004). Vaccination against rotavirus, which causes 40% of hospital admissions from diarrhoea in children less than 5 years worldwide, has recently been recommended for inclusion in all national immunization programmes (WHO, 2011).

2.5.1.2 Enteric adenovirus

Enteric adenoviruses are detected in 1.5–4% of children with diarrhoea and are isolated throughout the year with no specific seasonal distribution (Soares *et al.*, 2002). Transmission is fecal–oral while the incubation lasts from 3 to 10 days. Adenovirus diarrhoea lasts for 6–9 days and may be associated with vomiting and fever (Soares *et al.*, 2002). Adenoviruses were first isolated and characterized as distinct viral agents by two groups who were searching for the aetiologic agents of acute respiratory infections (Thomas, 2001). It soon became clear, however, that adenoviruses are not the aetiologic agents of the common cold; they are responsible for only a small portion of acute respiratory morbidity in the general population and for about 5% to 10% of respiratory illness in children (Thomas, 2001). Besides respiratory disease, adenoviruses cause epidemic conjunctivitis, and they have been associated with a variety of additional clinical syndromes—perhaps most notably, infantile gastroenteritis (Yolken *et al.*, 1982). Today, more than 100 members of the adenovirus group have been identified that infect a wide range of mammalian and avian hosts (Thomas, 2001). All of these viruses contain a linear, double-stranded DNA genome encapsidated in an icosahedral protein shell measuring 70 to 100 nm in diameter (Thomas, 2001). The particles (virions) contain DNA (13% of mass), protein (87% of mass), no membrane or lipid, and trace amounts of carbohydrate because the virion fiber protein is modified by addition of glucosamine (Thomas, 2001).

Adenoviruses are divided into 6 genogroups (A-F), further classified in more than 50 subtypes or serotypes (Aminu *et al.*, 2007). Of these, subtypes 40-41 belonging to genogroup E and F have been associated to diarrhoea. Two different enteric adenoviruses, EAd40 and EAd41, have been associated

with diarrhoea (DeJong *et al.*, 1983 and Wigand *et al.*, 1983). Epidemiologic studies to assess the importance of these agents have been completed for several population groups. A report of 14 enteric adenovirus–related cases of diarrhoea in 27 hospitalized patients studied during a 12-week period suggested that these viruses may be an important cause of acute gastrointestinal disease in hospitalized young children and may be nosocomially transmitted; this report also suggested that respiratory symptoms may be a prominent part of the clinical manifestations (Yolken *et al.*, 1982). The incidence of adenovirus-related gastroenteritis differs considerably in the various studies and locations reported by many authors. In general, it is not as prevalent as rotavirus diarrhoea, occurs most often in children younger than 4 years of age, and is not easily distinguished on clinical grounds from rotavirus infection (Reina *et al.*, 1994). In Bangladesh, it was responsible for 2.8% of the cases of diarrhoea in 4,409 specimens but reached 12.3% in some months as diagnosed by a monoclonal antibody specific for Ad40 and Ad41 (Jarecki-Khan *et al.*, 1993). In day care centers in Houston, weekly stool specimen collections from children 6 to 24 months of age were used to demonstrate that 38% of 249 children present during 10 separate outbreaks associated with enteric adenoviruses had this agent in their stool specimens. However, 46% of these samples came from patients who were asymptomatic, demonstrating that even during epidemics of enteric adenoviruses; many infected children do not develop gastroenteritis (Van *et al.*, 1992). In another study of children in day care reported from the Centers for Disease Control and Prevention in Atlanta, the prevalence of all adenoviruses in stool was 8%, and EAd40 and EAd41 were 2% in both 565 patients with diarrhoea and 129 controls (Lew *et al.*, 1991). The detection rates in North-western Nigeria have been found to be 5-10% in children with diarrhoea (Aminu *et al.*, 2007).

2.5.1.3 Norovirus

Norovirus is an important cause of viral gastroenteritis in adults as well as children (Swierczewski *et al.*, 2013). Its impact appears to be greater in developed countries, but several recent reports indicate that it is a common cause of gastroenteritis in children less than five years of age in developing countries (Dey *et al.*, 2007, Meng *et al.*, 2011 and Swierczewski *et al.*, 2013). On the other hand, norovirus infections in children in developing countries are frequently asymptomatic (Ayukekbong *et al.*, 2013). Noroviruses are highly contagious and they affect individuals of all age groups. They are transmitted by faecaly contaminated food and water, by person-to-person contact, or via aerosols (Moreno-Espinosa *et al.*, 2004). Norovirus induced diarrhoea is typically limited in time, with symptoms lasting a few days in most cases. However, severe forms have been reported in individuals at high risk: young children, the elderly, malnourished and immunocompromised persons (Bok and Green, 2012). It is not well known whether human norovirus infections induce any lasting protective immunity (Simmons *et al.*, 2013), or to what extent immunity protects against exposure to different strains. This is important because noroviruses are highly genetically and antigenetically diverse, a complexity that is a big challenge for the development of an efficient norovirus vaccine (Rackoff *et al.*, 2013). Noroviruses belongs to the family *Caliciviridae* which is composed of small (27 to 40 nm), nonenveloped, icosahedral viruses that possess a linear, positive-sense, single-stranded RNA genome. They are classified in five genogroups (GI –GV), three of which (GI, GII, and GIV) infect humans, with genogroup II being most strongly associated with diarrhoea illness in all age groups (Moreno-Espinosa *et al.*, 2004). The

genogroups are further subdivided into genotypes: 8 in GI, 17 in G2, 2 in G3, and 1 each in G4 and G5 (Zheng *et al.*, 2006). The development of norovirus infection depends on the presence of histo-blood group antigen (HBGA). These antigens are present on the surface of red blood cells, as well as intestinal epithelium, in a manner dependent of the expression of genes coding for enzymes involved in glycosylation. Persons with certain mutations in the gene coding for one of these enzymes, fucosyl transferase 2 (FUT2), so called non-secretors, have a natural resistance to infections with norovirus GII.4, which is the most common norovirus type in humans. Because synthesis of AB0 blood group antigens involves fucosyl transferases, susceptibility of norovirus infections can be predicted from the blood group, with blood group 0 individuals (who are "secretors") being susceptible, whereas blood group B are less likely to be infected (Rydell *et al.*, 2011).

2.5.1.4 Astrovirus

Astrovirus is a non-enveloped RNA virus that was identified in 1975 by electron microscopy in children with diarrhoea (Suzanne *et al.*, 2011). The virus is nonenveloped with a positive sense, single-stranded RNA, and belongs to the Astroviridae family. Several studies have shown that astrovirus is a relatively common cause of gastroenteritis in children, with most cases presenting in children below 4 years of age (Suzanne *et al.*, 2011). Its role as cause of diarrhoea in African countries is still insufficiently studied, but one study detected astrovirus in 4.5% of children with diarrhoea, as compared with 1.6% of controls (Reither *et al.*, 2007), indicating that this virus might be an important aetiology. In Australia, astrovirus was found to be the second most common cause of gastroenteritis in children, after rotavirus (Palombo and Bishop, 1996). Infection with serotype 1 predominated, particularly in the winter months when gastroenteritis is common. A biennial winter peak of astrovirus infection among hospitalized children was observed. Serotype 4 strains were associated with infection in summer and autumn months (Palombo and Bishop, 1996, Gaggero *et al.*, 1998 and McIver *et al.*, 2000). Among hospitalized patients, the incidence of astrovirus infection was 4.2%, far lower than rotavirus (63.2%), equivalent to all bacterial infections, and slightly higher than adenovirus (3.7%), suggesting that severe infection with astrovirus is seen in a subgroup of infected patients. In a population of Mayan infants, astrovirus was the most common enteric pathogen identified in stool samples collected during a prospective study of oral poliovirus immunogenicity (Maldonado *et al.*, 1998). The prevalence of astrovirus was compared with that of rotavirus, adenovirus 40/41, *Salmonella*, *Shigella*, and *Campylobacter* species. In 61% of the infants, astrovirus was detected by EIA from at least one stool sample over the 18-week period each infant was followed. Twenty-six percent of 305 diarrhoeal episodes reported at study visits were associated with astrovirus. The EIA results were confirmed by Realtime-PCR in a subset of EIA positive and negative samples. The reason for the high prevalence of astrovirus infection and the low rate of rotavirus infection (4% detected by a commercial EIA) in this semiclosed community is not known, but it may be related to temperature, rainfall, humidity, or other environmental factors (Maldonado *et al.*, 1998). In a group of child care setting, astroviruses were shown to be an important cause of outbreaks of diarrhoea. Children up to 36 months old attending child care centers were at greatest risk of developing diarrhoea (Lew *et al.*, 1991 and Mitchell *et al.*, 1993). An investigation of eight outbreaks of astrovirus diarrhoea (five human astrovirus serotype 1 and three serotype 2) in six child care centers

showed that 20% of the children with diarrhoea shed astrovirus. Although each outbreak was associated with a single astrovirus serotype, two distinct serotypes were identified in sequential outbreaks during one winter diarrhoea season (Mitchell *et al.*, 1999).

2.5.1.5 Cytomegalovirus

The cytomegaloviruses (CMVs) are a distinct, widely distributed subgroup of herpesviruses that share common growth characteristics and induce a cytopathology involving characteristic nuclear and cytoplasmic inclusions (Edward *et al.*, 2001). Viruses in this group were generally known as salivary gland viruses until the common name cytomegalovirus was proposed to reflect both virus-induced cytopathic effects and the virus's role in congenitally acquired cytomegalic inclusion disease. In most areas of the world, human CMV spreads at an early age and infects a large majority of the population. This pattern has been altered by increased hygiene in developed countries, where this virus may reach only 40% to 60% of the population. Thus, in the developed world, a large proportion of the adult population remains susceptible to primary infection, increasing the risk of congenital transmission and subsequent disease (Edward *et al.*, 2001). The importance of human CMV as a pathogen has also risen over the past three decades with the increase in organ allografting and immunosuppressive post transplant therapies and the increase in acquired immunodeficiency syndrome (AIDS). These conditions predispose individuals to a primary CMV infection or to reactivation of latent infection, which may lead to fulminant, life-threatening disease that can be difficult to treat despite available antiviral drugs (Hutto, 1996). CMV can affect any part of the gastrointestinal tract in immunocompromised hosts, especially those with advanced human deficiency virus (HIV) (Dieterich and Rahmin, 1991). Only CMV enteritis and colitis cause diarrhoea, with colonic disease predominating. Symptoms of colitis include explosive watery diarrhoea, low-grade fever, weight loss, anorexia, malaise, abdominal pain, and bleeding (Stefano and Haleh, 2010).

Cytomegalovirus infection is important in patients with HIV infection because it is arguably the leading opportunistic infection among patients with advanced AIDS, and some investigators have found that CMV infection is associated with clinical progression of HIV infection that may result into diarrhoea. Cytomegalovirus has been suspected of being a cofactor in the development of AIDS because clinical studies link CMV and progression of HIV-1 infection, and laboratory studies provide a biologic framework for CMV enhancement of HIV-1 infection (Edward *et al.*, 2001).

2.5.1.6 HIV diarrhoea

Persistent and recurrent diarrhoeas are amongst the most frequent manifestations of HIV/AIDS in both children and adults, especially in developing countries, where diarrhoea is associated with growth failure, weight loss and death (Stefano, 2004). In Zaire, 85% of adults admitted with persistent diarrhoea had HIV infection (Colebunders *et al.*, 1987). Among children presenting to a primary health care facility in South Africa, a history of persistent diarrhoea in the preceding 3 months strongly predicted HIV infection (odds ratio 4.8; CI 2.5–9.3 and positive predictive value of 63%). In South Africa, the profile of children admitted with diarrhoeal diseases has changed, with increasing prevalence of

persistent diarrhoea and loss of seasonal peaks of acute diarrhoea (Chintu, 1998). HIV infected children admitted to hospital with diarrhoea have more severe symptoms than children uninfected by HIV; they frequently have severe co-infections such as pneumonia, pseudomonal skin sepsis and tuberculosis (TB). These result in longer periods of admission and case fatality rates in the order of 27%. In Zaire, children with HIV and persistent diarrhoea had an 11-fold increased risk of death compared with uninfected controls (Chintu, 1998). Hence, in developing countries, HIV-related diarrhoeal diseases represent a major burden on both caregivers and health resources. The availability of antiretroviral drugs in North America and Europe has dramatically altered the natural history of HIV infection and has reduced the incidence of gastrointestinal disease due to opportunistic infections (Monkemuller *et al*., 2000). Although there is considerable knowledge of the pathophysiology and management of diarrhoea in adults with AIDS, less is known about this problem in children (Stefano, 2004). Opportunistic infections and fat malabsorption are common causes of prolonged and recurrent diarrhoea in infected adults and among children with HIV, carbohydrate intolerance is common and may follow acute infections with rotavirus or other common enteropathogens (Yolken *et al.,* 1991).

2.5.2 Bacterial Diarrhoea

The majority of infectious diarrhoeal illnesses in both children and adults in developing countries are due to bacteria (Petronella and Marie, 2001). The risk factors for transmission of these pathogens are higher in these countries, owing to increased exposure to the organisms (by contact with feces, water, food and fomites) and increased susceptibility of the host due to malnutrition. Bacterial infections exact a heavy toll on human populations, particularly among children. Despite the explosion of knowledge on the pathogenesis of enteric diseases experienced during the past decade, the number of diarrhoeal episodes and childhood deaths reported worldwide remains of apocalyptic dimensions. In the next 15 seconds, a child somewhere in the world will die from bacterial diarrhoea (WHO, 2009). It is estimated that 6–60 billion cases of gastrointestinal illness occur annually, the vast majority being severe enough and affecting so many unprivileged populations to pose a serious global health burden. In overpopulated developing countries, poor sanitation and hygiene, unsafe water supplies and limited education contribute to the propagation of diarrhoeal diseases. In industrialized nations, diarrhoea had been thought of as more of an inconvenience rather than a serious cause of illness, but recent attention to food contamination by pathogens such as *Salmonella* and *Escherichia coli*, in combination with a growing population of immunosuppressed patients, has led to increased recognition of their impact. The situation is further complicated by the recent escalation of international terrorism that is raising the risk of epidemics of enteric pathogens beyond the boundaries of natural endemic areas. Diarrhoea due to bacterial infections is an important cause of morbidity and mortality in infants and young children in most developing countries including Nigeria (Adegunloye, 2005). Clarification of the enteropathogens involved in diarrhoeal disease in the country is an essential step towards the implementation of effective primary health care activities against the disease (Olowe, *et al.,* 2003).

2.5.2.1 *Escherichia coli* pathovars

Theodore Escherich (1885) first described *Escherichia coli* as *Bacterium coli commune*, which he isolated from the feaces of newborns. It was later renamed *Escherichia coli* and for many years the bacterium was simply considered to be a commensal organism of the large intestine. It was not until 1935 that a strain of *Escherichia coli* was shown to be the cause of an outbreak of diarrhoea among infants (Todar, 2008). *Escherichia coli* is the bacterial species most commonly recovered in the clinical laboratories and has been incriminated in infectious diseases involving virtually every human tissue and organ system (Koneman, 1992).

Escherichia coli is the head of large bacterial family, *Enterobacteriacea*, the enteric bacteria (Abbott *et al*., 2003). They are Gram-negative, oxidase negative, non-spore-forming, rod-shaped bacteria, facultative anaerobes, which are often motile by peritrichously arranged flagella. They are capable of fermenting a wide variety of carbohydrates with production of both acid and gas, although anaerogenic biotypes exist. Rapid fermentation of lactose is a characteristic feature of many strains, particularly those of *E. coli*, whilst other *Escherichia* species, including strains of enteroinvasive *E. coli* (EIEC), as well as some *E. coli* (metabolically inactive) strains ferment it slowly or fail to utilise this substrate at all. Other genera within the family Enterobacteriaceae (*Klebsiella*, *Enterobacter*, *Serratia* and *Citrobacter*), which share this ability to ferment lactose rapidly (typically within 24 h), are collectively termed coliform bacteria. Besides *E. coli*, other species belonging to the genus *Escherichia* are *Escherichia blattae*, *Escherichia fergusonii*, *Escherichia hermannii* and *Escherichia vulneris*. The species *Escherichia adecarboxylata* has since been assigned to the genus *Lecercia*, and a new species, *Escherichia albertii*, has been described (Abbott *et al*., 2003).

Although most *E. coli* strains are harmless, there are others that cause disease in humans and animals that have evolved to become important pathogens in their own right (Stephen and Peter, 2006). Clinically, two distinct types of pathogenic *E. coli* are recognized. One group commonly called extraintestinal pathogenic *E. coli* (ExPEC) includes those *E. coli* associated with newborn meningitis (NBM) or sepsis and urinary tract infections (UTIs). The second group termed intestinal pathogenic *E. coli* (IPEC) includes *E. coli* responsible for a range of distinct classes of diarrhoeal disease (Christopher *et al.,* 2006). *Escherichia coli* is a genetically diverse species that includes many intestinal and extraintestinal pathotypes. Many of these have highly efficient and specialized mechanisms of colonisation and pathogenicity, developed through the acquisition of virulence-associated genes and adaptation to their changing surroundings aided by mutations and natural selection. Many virulence-associated genes are localized on mobile genetic elements such as plasmids, bacteriophages and pathogenicity islands (PAIs) (Christopher *et al.,* 2006). Several groups of *E. coli* pathotypes cause diarrhoea, and are termed as intestinal pathogenic *E. coli* (IPEC) (Stefano, 2004).

Six distinct groups have been defined within IPEC commonly associated with intestinal disease (Christopher *et al.,* 2006): ETEC (Enterotoxigenic *E. coli*): Causes watery (secretory) diarrhoea due to the production of plasmid mediated toxins (LT, ST) in infants and adults, particularly in developing countries. It is often referred to as traveller's diarrhoea. Many serogroups are involved (Cheesbrough, 2000). EPEC (Enteropathogenic *E. coli*): Causes vomiting, fever, and prolonged diarrhoea mainly in infants (less than 2 years), due to bacteria adhering to epithelial cells, multiplying and causing lesions. Many serogroups are

involved. EPEC is a major problem in developing countries. EIEC (Enteroinvasive *E. coli*): Causes dysentery (similar to shigellosis), fever and colitis, with blood, mucus, and many pus cells in faecal specimens, due to bacteria invading and multiplying in epithelial cells. Many serogroups are involved. EHEC (Enterohaemorrhagic *E. coli*): Causes life-threatening haemorrhagic diarrhoea (colitis) in all ages, without pus cells, and often without fever. It can progress to haemolytic uraemic syndrome with renal failure. EHEC has been reported mainly from Europe and North America. Outbreaks have also occurred in refugee camps in Mozambique, Swaziland and Malawi (Christopher *et al.,* 2006). EHEC is due to cytotoxins damaging vascular endothelial cells, and is mainly associated with the serogroup 0157:H7. It is sometimes referred to as VTEC (verocytotoxin-producing *E. coli*, because it is toxic to vero monkey cells in culture) (Stefano, 2004). Infection occurs by ingesting contaminated meat products, unpasteurized milk and dairy products (Cheesbrough, 2000). EaggEC (Enteroaggregative *E. coli*): Causes chronic watery diarrhoea and vomiting, mainly in children, due to the bacteria adhering to tissue cells often in stacks (aggregates). The DAEC (Diffusely adherent *E. coli)*: causes watery diarrhoea, usually without blood, due to its ability to adhere to HEp-2 cells in a diffuse pattern (Nataro and Steiner, 2002).

2.5.2.1.1 Enteropathogenic *E. coli*

This was the first group of *E. coli* serotype shown to be pathogens for humans and has been responsible for devastating outbreaks of nosocomial neonatal diarrhoea and infant diarrhoea in virtually every corner of the globe (Alessio, 2004). Strains of enteropathogenic *E. coli* (EPEC) are distinguished from other *E. coli* strains by their ability to induce a characteristic attaching and effacing lesion in the small intestinal enterocytes and by their inability to produce Shiga toxins (Cheesbrough, 2000). Between the 1940s and the 1960s, EPEC was associated with infant diarrhoea in summertime and nursery outbreaks of diarrhoea in the USA and other industrialized countries. Since then, it has become extremely uncommon in industrialized countries, although it is occasionally reported in child-care settings (Paulozzi *et al.,* 1986). In Nigeria, the epidemiology of diarrhoeagenic bacteria and the virulence of the various *E. coli* pathotypes have not been well studied because most of the currently published data were from the south and western axis leaving the north unstudied (Nweze, 2009).

2.5.2.1.2 Enterotoxigenic *E. coli*

Strains of enterotoxigenic *E. coli* (ETEC) are an important cause of diarrhoeal disease in humans and animals worldwide (Alessio, 2004). The clinical importance of these micro-organsims was first outlined in the 1970s by epidemiological studies in India that identified them as a major cause of endemic diarrhoea (Gorbach, 1971). Their pathogenicity is related to the elaboration of one or more enterotoxins that are either heat stable (ST) or heat labile (LT) without invading or damaging intestinal epithelial cells. Together with rotavirus, ETEC is the leading cause of dehydrating diarrhoeal disease among weaning infants in the developing world (Alessio, 2004). These children experience 2–3 episodes of ETEC diarrhoea in each of the first 2 years of life. This represents over 25% of all diarrhoeal illness and results in an estimated 700000 deaths each year (Black *et al.,* 1981). In industrialized countries, ETEC does not contribute to endemic disease, but is notorious for being the leading agent of travelers' diarrhoea, accounting for about half of all episodes (Sack, 1990). Transmission occurs by ingestion of

contaminated food and water, with peaks during the warm, wet season. Like EPEC, ETEC requires relatively high inoculums (40 Cfu) and a short incubation period (14–30 hrs) (Cheesbrough, 2000). The cardinal symptom is watery diarrhoea, sometimes with associated fever, abdominal cramps and vomiting (Alessio, 2004). In its most severe form, ETEC can cause cholera like purging, even in adults. The illness is typically self-limited, lasting for less than 5 days and with few cases persisting beyond 3 weeks. Infection with ETEC has also been associated with short and long-term adverse nutritional consequences in infants and children. Most diarrhoeal illnesses due to ETEC are selflimited and do not require specific antimicrobial therapy. Empirical therapy is reserved for those whose diarrhoea is moderate to severe despite rehydration and supportive measures. Antibiotic regimens that have been efficacious in clinical trials, shortening the duration of illness by 1–2 days, include doxycycline, ciprofloxacin, quinolones, and furazolidone (DuPont and Ericsson, 1993).

The development of vaccines against ETEC has received a great deal of attention because of its disease burden. Oral vaccines for ETEC are being developed by five different strategies, including killed whole cells, toxoids, purified fimbriae, living attenuated strains and live carrier strains elaborating ETEC antigens. A killed whole-cell *Vibrio cholerae* vaccine given with cholera toxin B (CTB) provided 67% protection against LT-producing ETEC diarrhoea for 3 months. A formalin-inactivated whole-cell oral vaccine consisting of ETEC strains bearing colonization factor antigens (CFAs) in combination with CTB has entered field trial (Svennerholm *et al.,* 1997).

2.5.2.1.3 Enteroinvasive *E. coli*

This group consists of invasive *E. coli* strains that are genetically, biochemically and clinically nearly identical to *Shigella* (Alessio, 2004). Strains of enteroinvasive *E. coli* (EIEC) are endemic in developing countries, where they exhibit similar epidemiology to *Shigella* and cause an estimated 1–5% of diarrhoeal episodes among patients visiting treatment centers. The occurrence of EIEC in industrialized countries is limited to rare food-borne outbreaks (Macdonald *et al.,* 1985).

Both *Shigella* and EIEC are responsible for bacillary dysentery, a disease that has had a major impact on society throughout history (Christopher *et al.,* 2006). Transmission is via the faecal–oral route, with contaminated food and water being the main sources of infection, but person-to-person transmission can also occur. The syndrome produced by EIEC infection can be indistinguishable from *Shigella* infection. In both cases the site of infection is predominantly the colon. The most common symptom is watery diarrhoea which may precede dysenteric stools containing mucus and blood. In severe cases the bacteria may attack the colonic mucosa, invading epithelial cells, multiplying and causing ulceration of the bowel (Christopher *et al.,* 2006). In EIEC, genes necessary for invasiveness are carried on a 140-MDa plasmid (pINV). These genes encoding a range of invasion plasmid antigens (IpaA to IpaD) are necessary for strains to be fully pathogenic. The rare episodes for which treatment is desired are treated with antibiotics recommended for shigellosis. The same general preventive measures used for *Shigella* infections apply to EIEC-associated diarrhoea (Christopher *et al.,* 2006).

2.5.2.1.4 Enterohaemorrhagic *E. coli*

Enterohaemorrhagic *E. coli* (EHEC) have emerged as one of the most important threats to human health (Kaper *et al*., 2004). Strains belonging to serotype O157:H7 are now regarded as model EHEC based on their phylogenetic profiles and histopathology (Christopher *et al.,* 2006). Since it was first identified in 1983 (Riley *et al*., 1983) as the cause of two outbreaks of gastroenteritis in the United States, the previously 'rare serotype' *E. coli* O157:H7 has become responsible for an increasing number of large food and waterborne outbreaks of haemorrhagic colitis throughout the world including the United States, Canada, United Kingdom and Japan (Kaper and O'Brien, 1998). The term EHEC was originally used to describe strains causing distinct clinical manifestations such as haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) in humans. EHECs are characterized by the production of a distinct group of prophage encoded toxins, their ability to cause lesions on epithelial cells using mechanisms similar to those found in EPEC and the possession of a plasmid carrying other virulence determinants that may contribute to pathogenesis (Christopher *et al.,* 2006). Besides O157:H7 and O157:H, *E. coli* strains traditionally belonging to common EPEC serogroups, e.g. O111 and O26 strains, which have acquired the genes and ability to produce these toxins now also belong to the EHEC group. Genetically, it has been proposed that *E. coli* O157:H7 originally evolved in a step-wise manner from an ancestral strain of EPEC O55:H7 (Feng *et al*., 1998). The close relationship between O157:H7 and O55:H7 is supported by numerous studies showing genetic similarities between these serotypes (Perna *et al*., 1998, Shaikh and Tarr, 2003), including evidence that the H7 *fliC* genes of O55:H7 and O157:H7 are almost identical (Reid, *et al*., 1998). However, *E. coli* O157:H7 and O157:H strains appear to belong to a unique clonal lineage with various phenotypic characteristics that distinguish these strains from other *E. coli*. These include the lack of β-glucuronidase (GUD) activity and the inability of most strains to ferment sorbitol in 24 hrs, which have been used as diagnostic features to aid methods for their detection and isolation. Carriage of the toxin genes is however, evident in over 200 serotypes of *E. coli*, some of which do not possess the additional virulence genes found in EHEC and have not yet been associated with human infection. Other serotypes have been responsible for sporadic cases of infection and outbreaks (Goldwater and Bettelheim, 1994). Therefore, EHECs can be regarded as a subgroup of a larger group of toxin-producing *E. coli* which is collectively termed VTEC or Stx producing *E. coli* (STEC). These bacteria are present in the intestinal tract of a wide range of domestic and wild animals, but farm animals, particularly cattle and sheep, are major reservoirs of these pathogens (Chapman *et al*., 1993 and Blanco *et al*., 2001). They have also been isolated from a range of non-ruminant animals, including birds, horses, dogs and cats (Beutin, 1999). Persistent carriage and host associations between particular serotypes and certain animals exist, but others appear to be carried transiently. VTEC infections have been linked to a diverse range of food types, although many are linked directly or indirectly to animal reservoirs, e.g. undercooked beef, unpasteurised milk and cheese. Infection can also occur via contaminated water, contact with animals and their faeces and secondary person-to-person transmission. The ability of some *E. coli* strains to produce this range of toxins with potent cytotoxicity against Vero cells was first described in 1977 (Konowalchuk, *et al*., 1977). These toxins, termed Verotoxins or Verocytotoxins (VT), were later shown to be structurally and functionally similar to the Stx produced by *S. dysenteriae* (O'Brien and Holmes, 1987).

EHEC is now recognized as a global health problem; in 1996, an outbreak in Japan linked to eating radish sprouts affected over 6000 persons (Centers for Disease Control and Prevention (CDC),

1997). One the most severe EHEC outbreaks in the USA took place in New York State in 1999, with more than 1000 ascertained cases, two hemolytic uremic syndrome (HUS), related casualties, and eight children in dialysis because of renal failure. The predominant mode of transmission is ingestion of contaminated, undercooked ground beef. However, the spectrum of vehicles is widening to include raw fruits (including apple juice) and vegetables, raw milk, processed meats, and drinking or swimming in contaminated water (Christopher *et al.,* 2006). The uncooked food vehicles are usually contaminated with manure from infected animals during growth or processing. Person-to-person transmission is the mode of spread in day-care outbreaks, where secondary transmission rates of 22% have been reported (Belongia, *et al.,* 1993). EHEC also causes sporadic diarrhoea (Christopher *et al.,* 2006). Isolation from stools of unselected patients is low (<1%), but isolation from stools of patients with bloody diarrhoea may be as high as 20–30% (Slutsker, *et al.,* 1997). Illness with EHEC follows 3–9 days after ingestion of as few as 100 organisms. Crampy abdominal pain and non-bloody diarrhoea are the first symptoms, sometimes associated with vomiting. By the 2nd or 3rd day of illness, diarrhoea becomes bloody in about 90% of cases, and abdominal pain worsens.64 Bloody diarrhoea lasts for 1–22 days (median 4 days). Unlike other infectious causes of bloody diarrhoea, fever is usually absent or remains low-grade. Younger children appear to excrete the organisms for longer (median 3 weeks) than older children and adults (Christopher *et al.,* 2006).

2.5.2.1.5 Enteroaggregative *E. coli*

In certain developing countries, EAggEC (or EAEC) are a major cause of chronic infantile diarrhoea and they have also emerged as a cause of diarrhoeal disease in adults and children in developed countries (Alessio, 2004). Currently, EAggEC are defined as *E. coli* strains that do not secrete enterotoxins LT or ST and which adhere to HEp-2 cells in a distinct pattern termed aggregative adherence (AA). This is characterized by the layering of the bacteria in a stacked-brick configuration on the surface of the host cells, which is associated with the carriage of a 60-MDa plasmid (Baudry *et al*., 1990). It has been hypothesized that the aggregating pattern of adherence may be a result of non-specific, possibly hydrophobic interaction, and therefore, not all organisms meeting the definition of EAggEC may be pathogenic in humans. Moreover, since epidemiological studies have not uniformly implicated EAggEC as pathogenic, some investigators have questioned the virulence of all EAggEC isolates. Volunteer studies performed to address both of these questions confirmed that at least some EAggEC strains are genuine human pathogens but that virulence is not uniform among isolates (Baqui, *et al.,* 1992). More recently, EAggEC pathogenicity has also been proven in several outbreaks. From the earliest epidemiological reports, EAggEC was most prominently associated with persistent cases of pediatric diarrhoea (i.e. lasting ≥ 14 days), a condition that represents a disproportionate share of diarrhoeal mortality (Henry, *et al*., 1996). On the Indian subcontinent, several independent studies have demonstrated the importance of EAggEC in pediatric diarrhoea. These studies include hospitalized patients with persistent diarrhoea, outpatients visiting health clinics, and cases of sporadic diarrhoea detected by household surveillance (Bhatnagar, *et al*., 1993). In Fortaleza, Brazil, study has demonstrated a consistent association between EAggEC and persistent diarrhoea, and EAggEC accounts for more cases of persistent diarrhoea than all other causes combined (Fang *et al*., 1995). EaggEC have been implicated as a cause of sporadic diarrhoea in other developing countries (including Mexico, Chile,

Bangladesh, Congo and Iran) as well as in industrialized countries such as Germany and England (Nataro and Steiner, 2002). In Nigeria EAEC has been implicated in acute and persistent diarrhoea, and most strains were reported to harbor a member of a partially conserved plasmid family (called pAA), when children aged <5 years were recruited to elucidate the roles of plasmid and chromosomal EAEC loci in Southwest Nigeria (Iruka, *et al*., 2000).

2.5.2.1.6 Diffusely adhering *E. coli*

Until recently, diffusely adhering *E. coli* (DAEC) was considered a non-pathogenic *E. coli*, since early studies failed to find an association between this micro-organism and diarrhoeal disease (Bhan, *et al*., 1989). However, more recent studies have demonstrated such an association, particularly in children older than 2 years of age (Christopher *et al.,* 2006). A community-based case–control study in southern Mexico revealed that DAEC was significantly associated with diarrhoea in children less than 6 years of age (Giron, *et al*., 1991). Prospective cohort studies in Chile and Bangladesh also demonstrated a diarrhoeagenic role for DAEC that peaked in the 48–60-month age group (Baqui *et al*., 1992 and Levine, *et al*., 1993). This micro-organism was more frequently isolated from cases of prolonged diarrhoea, and it showed a seasonal pattern similar to that of ETEC, occurring more frequently in the warm season (Levine, *et al*., 1993). The gastrointestinal symptoms that characterize DAEC infection are practically indistinguishable from those caused by ETEC, with self-limiting watery diarrhoea rarely associated with vomiting and abdominal pain. The diagnosis is mainly based on the DNA probe technique and on the pattern of adherence of the micro-organism to HEp-2 cells (Christopher *et al.,* 2006).

2.5.2.2 *Salmonella*

Salmonella enterica are Gram-negative, motile bacilli that colonize only humans and mammals (Alessio, 2006). Therefore, disease is acquired through close personal contact or through the ingestion of water or food contaminated with human excrement (Claire and Stephen, 2006). Infection with organisms of the *Salmonella enterica* is an important public health problem throughout the world (Alessio, 2006). *Salmonella* are fermentative facultatively anaerobic, oxidase-negative Gram-negative rods. They are generally motile, aergenic, nonlactose fermenting, urease negative, and citrate utilizing and acetyl methyl carbinol negative (Cheesbrough, 2000). Historically, the classification of *Salmonella* was based on host specificity, serotyping the lipopolysaccharide (LPS) and flagella antigens and phage typing, resulting in more than 2500 different, often imaginatively named, serovars (Kauffmann, 1957). However, molecular methods, such as DNA hybridization, protein isozyme analysis, and DNA sequence similarity, show that *Salmonella* serovars are sufficiently similar at the genomic level to be regarded as the same species (Claire and Stephen, 2006). The genus *Salmonella* consists of two species, *Salmonella enterica* and *S*. *bongori*. Within the species *S. enterica* there are six subspecies differentiated by biochemical variations, namely *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica* (Barrow and Feltham, 1992, Old and Threlfall, 1998). Serovars within the subspecies other than subsp. *enterica* are designated by their antigenic formulae, for example, *S. enterica* subsp. *arizonae* 61: k: 1, 5, 7, (Claire and Stephen, 2006).

Infection with organisms of the genus *Salmonella* results in three main syndromes: infection localized to the intestine, often known as salmonellosis, enteric fever and invasive or bacteraemic disease (Alessio, 2006). Symptoms of salmonellosis can develop in as little as 6 hrs and usually appear within 24 hrs after ingestion of the organisms. Patients complain of nausea, vomiting and diarrhoea. Fever and abdominal pain are also common, but the degree of each of these symptoms varies considerably between patients. In salmonellosis, patients complain of symptoms related to enteritidis and dysentery; blood in the stool is not uncommon. Abdominal pain is usually mild or moderate but can be sufficiently severe to mimic an acute abdomen in some cases. After symptoms subside patients continue to excrete organisms in their stools for up to 3 months. Approximately 1–3% may excrete organisms for more than a year (Claire and Stephen, 2006). In community outbreaks the majority of patients will suffer no serious effects but a small proportion, usually the elderly, will suffer invasive disease with a significant mortality (Old and Threlfall, 1998). Enteric fever caused by *Salmonella enterica* subspecie *typhi* and serovars Paratyphi A, B and *C enterica* (Willey *et al.,* 2008) is an endemic disease in the tropic and sub-tropic and has become a major public health problem in developing countries of the world with an estimated annual incidence of 540 per 100,000 (Ibekwe *et al*., 2008). Although *S. typhi and paratyphi* can cause systemic disease in the human host, infection is usually localized in the intestinal epithelium (Ohl and Miller, 2001). *Salmonella typhi* is not typically associated with acute diarrhoea, suggesting that the initial interaction between this serovar and the human gut is less inflammatory. After ingestion, *S. typhi* must pass through the stomach, which provides a considerable barrier to infection. Organisms cannot be cultured from the stomach 30 min after ingestion, and antacids and hydrogen antagonists reduce the number of organisms required to initiate infection. Bacteria will multiply in the intestine, but stools are only intermittently positive to culture during the first week and even after experimental infection with 10^5–10^7 Cfu (colony forming unit), the presence of organisms in the stools during the first 5 days did not necessarily indicate that illness would develop (Hornick *et al*., 1970). They are transported from the small intestine to the mesenteric lymph nodes via the M-cells of the Peyer's patch and then disseminated widely via the lymphatics and bloodstream to grow intracellularly within the cells of the reticuloendothelial system. This time corresponds roughly to the incubation period. After approximately 1 week there is secondary bacteraemia, and typhoid bacilli reinvade the gut via the liver and gall bladder, settling in Peyer's patches and causing inflammation, ulceration and necrosis. This may lead to the complications of perforation or haemorrhage (Claire and Stephen, 2006).

The definitive diagnosis of salmonellosis or enteric fever requires the isolation of *S. typhi* or *S. paratyphi* from the patient (Claire and Stephen, 2006). Cultures of blood, stool, urine, bone marrow and gastric and enteric secretions may all be useful in establishing the diagnosis (Cheesbrough, 2000). Chloramphenicol has been the treatment of choice since its introduction, given its low costs and its high efficiency after oral administration. Treatment with chloramphenicol reduced typhoid fever mortality from approximately 20 to 1% and reduced the duration of the fever from 14–28 days to 3–5 days while the most effective attenuated vaccine for typhoid fever currently available, Ty21a, has proved to be free of adverse reactions in large-scale efficacy field trials involving almost 600,000 pediatric subjects (Levine *et al*., 1990). When administered as a liquid suspension, Ty21a protected both young (82% vaccine efficacy) and older children (69% vaccine efficacy) (Levine *et al*., 1990). The incidence of typhoid is falling

worldwide due to improvements in public health, such as provision of clean water and good sewage systems, but it still remains a major threat to human health in developing world (WHO, 2003). The World Health Organization estimates there are 17 million typhoid cases annually and that these infections are associated with about 600, 000 deaths (Pang *et al*., 1998).

2.5.2.3 *Shigella*

The genus *Shigella* is part of the Enterobacteriaceae, which have been classified together on the basis of the disease they cause (Christopher *et al.,* 2006). The shigellae have been shown to be closely related to *E. coli* by DNA hybridisation, isoenzyme analysis and the presence of Shiga-like toxin (Brenner *et al*., 1969, Crosa *et al*., 1973 and Goullet, 1980). Recently, the full genome of *Shigella flexneri* was sequenced and comparisons with *E. coli* K12 showed that *Shigella* were phylogenetically indistinguishable from *E. coli* (Jin *et al*., 2002 and Wei *et al*., 2003). It has been suggested that *Shigella* spp. should be reclassified and new nomenclature introduced to clarify the relationship between these two species as most closely related, which also are capable of producing a dysenteric illness (Lan and Reeves, 2002). The genus *Shigella* contains four species: *S. dysenteriae*, *S. flexneri*, *Shigella boydii* and *S. sonnei* (Christopher *et al.,* 2006). The species are differentiated on the basis of simple biochemical tests and serology. Shigellae are Gram-negative, non-lactose fermenting, non-motile bacilli, with *S. sonnei* the main type found in industrialized countries, and *S. flexneri* and *S. dysenteriae* predominating in underdeveloped countries (Alessio, 2006). Humans are the only natural hosts and transmission occurs by fecal–oral contact (Alessio, 2006). The low infectious inoculum (as few as ten organisms) renders shigellae highly contagious (DuPont, *et al*., 1989). *Shigella* causes 250 million cases of diarrhoea and 650 000 deaths worldwide (Institute of Medicine, 1986). After an incubation of 1–4 days, shigellosis begins with fever, headache, malaise, anorexia and occasional vomiting and watery diarrhoea with progression to dysentery within hours to days (Christopher *et al.,* 2006). Unusual extraintestinal manifestations may occur, including hemolytic uremic syndrome (HUS) in children and thrombotic thrombocytopenic purpura in adults. Most episodes of shigellosis in otherwise healthy individuals resolve within 7 days (Christopher *et al.,* 2006). It is estimated that there are 164.7 million cases of bacillary dysentery annually, of which 163.2 million are in developed countries and 1.5 million in industrialised countries (Kotloff *et al*., 1999). Approximately 1.1 million people die from shigellosis each year, 61% of these are children under 5 years old (Kotloff *et al*., 1999). Infection is spread by the faecal–oral route with great ease, as the infective dose is low (10^2–10^3 cfu). *Shigella* spp. are obligate human pathogens and do not infect other hosts, although experimental infection can be achieved in primates. Most cases of bacillary dysentery spread from person to person, and this may occur rapidly, especially in closed communities and when individuals are brought together in large numbers and sanitary arrangements are inadequate (Christopher *et al.,* 2006). Dysentery is a disease of poverty, and the incidence of infection can be correlated with poor housing and sanitary facilities and when large populations are suddenly displaced through war or natural disaster. Epidemics of disease may be both water and food borne and can be associated with faecally contaminated wells. Shigellosis is a disease of children under 5 years old in developing countries, reaching a peak at between 18 and 23 months (Henry, 1991). Disease is more severe in children who are malnourished (Bennish, 1991). In one study, all of the fatal cases were severely malnourished (Thapa *et al*., 1995). Patients suffered convulsions, bacteraemia, renal failure,

intestinal perforation and toxic megacolon (Thapa *et al*., 1995). *Shigella* dysentery has a significant effect on childhood mortality and on growth in excess of dysenteric illnesses of other aetiologies (Bennish, 1991 and Henry, 1991). In developing countries, *S. flexneri* and *S. dysenteriae* are most frequently isolated, whereas *S. sonnei* and *S. flexneri* predominate in developed countries (Kotloff *et al*., 1999). The change in pattern of infection occurred in industrialised countries more than 50 years ago and resulted in a fall in mortality from dysentery as the more virulent forms became less common (Christopher *et al.,* 2006). A study of infectious intestinal disease among cases in the community in England, carried out between August 1993 and January 1996, showed that *Shigella* spp. was isolated in 0.8% of faecal samples from cases and none of the controls (Tompkins *et al*., 1999). However, in many industrialised countries the scale of bacillary dysentery is not clearly established as many cases do not come to medical attention or are neither investigated bacteriologically nor notified to public health authorities. *Shigella* is reported as important cause of travellers' diarrhoea, especially in individuals returned from developing countries (Vila *et al*., 1994). Data from the Health Protection Agency on *Shigella* isolates received between April and June 2004 showed that 24% of all reports of *Shigella* infection recorded recent travel abroad, 63% of which specified travel to the Indian subcontinent, 19 of those were due to *S. sonnei* (Health Protection Agency, 2004). In Nigeria, shigellosis is still the major cause of dysentery in children aged 0-9 years, and many older children are hospitalized almost immediately after the onset of the disease (Niemogha *et al*., 1995 and Iwalokun *et al*., 2001). Shigellae are extremely fastidious and are best isolated from fresh stool rapidly inoculated onto selective culture plates incubated immediately at 37^0C. Appropriate antibiotics given for 5 days significantly decrease the duration of fever, diarrhoea, intestinal protein loss and pathogen excretion (Christopher *et al.,* 2006). However, *Shigella* strains that are resistant to one or both drugs have been identified. Several promising *Shigella* mutants with deletions in virulence genes have entered clinical trials as oral vaccine candidates

2.5.2.4 *Campylobacter*

These organisms are small, microaerophilic, spiral-shaped Gram-negative organisms that enjoy a widespread reservoir in the intestines of both wild and domestic animals (Blaser, and Reller, 1981). It is the most frequently identified bacterial cause of diarrhoea in the USA (Diane and Monika, 2004). Common vehicles are poultry, unpasteurized milk and contaminated water. After an incubation of 3–6 days, enteritis begins abruptly with cramps and watery diarrhoea, which may progress to blood-containing stools. The abdominal pain may mimic appendicitis. Diarrhoea usually lasts 4–5 days, with the highest duration of fecal excretion of 1 month (Cheesbrough, 2000). Although the bacteria of the genus *Campylobacter* spp. are the most common bacterial cause of enteritis worldwide, they are largely unfamiliar to the general public although their importance to human health is as great, or greater, than the well-known *Salmonella*, *Shigella* and verotoxin-producing *Escherichia coli* (Diane and Monika, 2004). Campylobacteria first identified with human illness were referred to 'related vibrios' to distinguish them from species of *Vibrio*, e.g. *Vibrio cholerae* (King, 1957 and 1962). A convincing association of these organisms with human diarrhoea was demonstrated a decade later (Dekeyser *et al*., 1972 and Butzler *et al*., 1973). Their significance to human health, however, was not established until a medium was designed for routine isolation of the organisms (Skirrow, 1977). Campylobacteraceae can be conveniently separated into three groups on the basis of phenotypic characteristics. The first and most

important group to human health consists of five thermophilic enteropathogenic species (Diane and Monika, 2004). In this group are *C. jejuni* with two subspecies, *C. coli*, *C. lari*, *C. upsaliensis* and *C. helveticus*. A second group includes four species well known to veterinary microbiologists because of their frequent occurrence as pathogens or commensals in farm livestock, although some species have been shown to be pathogenic for humans. Included is *C. fetus* with two subspecies, *C. hyointestinalis* with two subspecies, *C. sputorum* with three biovars and *C. mucosalis* (Diane and Monika, 2004). A third group, found in human periodontal disease, consists of *C. concisus*, *C. curvas*, *C. rectus*, *C. showae* and *C. gracilis*. Two new species, *C. lanienae* spp. nov. and *C. hominus* spp. nov., have been grouped according to their similarity with other *Campylobacter* species (Diane and Monika, 2004). Since *C. jejuni* subsp. *jejuni* genome has been sequenced (Parkhill *et al*., 2000), it has become possible to decipher some of the functions of the bacteria by examining the content and expression of the genome (Dorrell *et al*., 2001). A total of 1,300 *C. jejuni* core genes present are associated with metabolic, biosynthetic, cellular and regulatory processes, as well as many virulence determinants (Diane and Monika, 2004). The factors determining the clinical outcome of infection with *C. jejuni* are likely dependent upon the infecting strain as well as on the susceptibility of the host. Colonization of human intestine with *C. jejuni* results in the expression of several putative virulence factors, yet the mechanisms of pathogenesis are unclear (Van Vliet and Ketley, 2001). Colonization may be influenced by the expression of lipopolysaccharides (LPS) on the bacterial cell surface. A mutation in the *galE* gene involved in the synthesis of LPS reduced the ability of *C. jejuni* to adhere and invade intestinal cells, suggesting that LPS is a virulence factor for *C. jejuni* infection, although the *C. jejuni* was still able to colonize chickens (Frye *et al*., 2002). Flagella are an important determinant in virulence since colonization of intestinal tracts does not occur with non-motile mutants of *C. jejuni* (Wassenaar, *et al*., 1995). Internalization of *C. jejuni* into mammalian cells requires the secretion of invasion proteins (Konkel *et al*., 1999). *Campylobacter jejuni* also produces cytolethal distending toxin which interferes with the cell cycle (Whitehouse *et al*., 1998). The presence of plasmids in *C. jejuni* may play an important role in the pathogenesis associated with infection. A transmissible plasmid encodes resistance to tetracycline via the Tet (O) determinant in *C. jejuni* (Taylor *et al*., 1981 and Connell *et al*., 2003). *Campylobacter jejuni* subsp. *jejuni* is recognized as the most important cause of *Campylobacter* enteritis (campylobacteriosis) in humans occurring in 89–93% of the cases. *Campylobacter coli* is most closely related to *C. jejuni* and is the cause of 7–10% of the human cases, while *C. lari* is the cause of only 0.1–0.2% (Healing, *et al*., 1992). The frequency of infections in the human population due to these three species is difficult to estimate precisely, but incidences ranging from 6 to 7 per 100 000 to 87 per 100 000 have been reported in the USA and the UK (Skirrow and Blaser, 1992). Most infections are due to sporadic cases: exposure to raw meat, a pet with diarrhoea and drinking untreated water, family outbreaks and larger community outbreaks arising from contaminated food, water or unpasteurized milk (Sopwith *et al*., 2003). It has also been implicated in travellers' diarrhoea (Adak *et al*., 1995). In developing countries it is the paediatric population in which the largest number of cases occurs. Incidences as high as 40 000 per 100 000 have been reported and most of these occur in children 2 years old or younger. The adult population in the underdeveloped world is much less affected, presumably owing to the development of immunity during childhood exposure. Isolates of these three thermophilic species are rarely recovered from healthy humans in developed countries, but they can be obtained almost as frequently from healthy children as from children with diarrhoea in underdeveloped countries (Taylor, 1992). The campylobacteriosis produced in humans by these three

thermophilic species varies from mild short-term diarrhoea of 2 days to a severe form that may last a week or longer (Diane and Monika, 2004). The diarrhoea is watery and may contain blood. Vomiting only occurs in a small percentage of cases. The diarrhoea may be accompanied by severe pain, leading to suspicions of acute appendicitis, cholecystitis or peritonitis. Complications are infrequent but include septicaemia, reactive arthritis, Guillain–Barré syndrome, erythema nodosum and meningitis (Diane and Monika, 2004). In addition to its role as a major cause of human enteritis, *C. jejuni* subsp. *jejuni* also causes diarrhoea in cattle and has been isolated from dogs, cats and non-human primates with diarrhoea. This species has also been implicated as the cause of mastitis in cattle and abortion in sheep. In chickens, turkeys, pigeons, crows and gulls and other wild birds, *C. jejuni* subsp. *jejuni* constitutes part of normal intestinal flora (Diane and Monika, 2004). Other sources of isolates include hamsters, flies and mushrooms. Since there are such a large number of potential contributors to the contamination of natural waters, it is not surprising that large outbreaks have been traced to un-chlorinated water supplies (Diane and Monika, 2004). Unpasteurized milk has also been implicated in a number of milk-borne outbreaks. However, the majority of the human cases can be traced to poultry, particularly to the consumption of chickens that have been incompletely cooked or to cookware, utensils and cutting boards that have been contaminated during the preparation of the chicken. These cases are, for the most part, sporadic but they constitute the majority of the human cases. Transmission of *C. jejuni* subsp. *jejuni* from cats and dogs has been reported to be the cause of sporadic cases, usually limited to a small number of associated individuals often in the same family (Tauxe, 1992). Occasionally complications of *C. jejuni* infection result in the developmentof more severe neurological and autoimmune illnesses such as Guillain–Barré, Miller–Fisher and Reiter's syndromes (Nachamkin *et al*., 1998). Differentiation of *C. jejuni* subsp. *jejuni* from *C. coli* is commonly achieved with the hippurate hydrolysis test, in which *C. coli* is negative (Diane and Monika, 2004). Difficulty arises when some *C. jejuni* strains are also unable to hydrolyse hippurate (Totten *et al*., 1987). Additional tests such as growth on a minimal media are useful (Diane and Monika, 2004). Epidemiological investigations have confirmed that campylobacteriosis is a zoonotic disease. The use of both phenotyping and genotyping methods is required to achieve greater discrimination among subtype isolates of *C. jejuni* from humans and animals (Wassenaar and Newell, 2000). Tracing infections to animal sources has been accomplished mostly through the use of serotyping systems used mainly by reference laboratories (Penner, 1988). One system distinguishes strains on the basis of differences in specificities in thermostable antigens that are known to be lipopolysaccharide (Penner system) and the other employs differences in thermolabile antigens to distinguish between strains (Lior system) (Diane and Monika, 2004). Serotyping of heat-stable antigens is widely accepted as a basis for reference typing of strains (Frost *et al*., 1998).

Bacteria of the genus *Campylobacter* have a characteristic morphology and a darting type of motility that permits their identification by direct examination of broth suspensions of faeces (Diane and Monika, 2004). However, *C. jejuni* subsp. *jejuni* cannot be distinguished from *C. coli* by this procedure and the test is considerably less sensitive than isolation by culture (Chessbrough, 2000). A method used in early studies utilized the supernatant from a saline suspension of faeces which is drawn up in a syringe and then forced through a 0.65 µm filter. The filtered fluid, which contains the filter-passing Campylobacteria, is inoculated on a solid isolation medium (Dekeyser *et al*., 1972). It has been replaced by direct plating on a selective isolation medium, the first of which was described in 1977 (Skirrow,

1977). The Skirrow medium consists of a blood agar base in which is incorporated lysed horse blood, vancomycin, trimethoprim and polymyxin B. Blood contains catalase, peroxidase and superoxide dismutase which are believed to remove toxic oxygen derivatives that inhibit Campylobacteria, while the three antibiotics inhibit the growth of other enteric bacteria. The Skirrow medium is immensely successful in isolating *C. jejuni* subsp. *jejuni* but some faecal flora is not inhibited and some species of *Campylobacter* are inhibited (Diane and Monika, 2004). Accurate identification of *Campylobacter* species is necessary to collect useful surveillance data for subsequent risk assessment studies and development of effective interventions to prevent the spread of Campylobacteria. Unfortunately, the biochemical inertness of Campylobacteria prevents effective discrimination of the species by phenotypic methods, such as tolerance to different temperatures and to agents that inhibit growth. However, tests for oxidase, catalase, hippuricase, urease, indoxyl acetate hydrolysis, nitrate and nitrite reductases, hydrogen sulfide production, susceptibility to nalidixic acid and cephalothin and growth at 15, 25 and 42^0C are the most reliable for species differentiation (Diane and Monika, 2004). Tests for tolerance to 1% glycine, 3.5% sodium chloride and 0.1% trimethylamine *N*-oxide are less reproducible. Colonies produced by *C. jejuni* subsp. *jejuni* are grey, moist, flat and spreading after 42 hrs of incubation at 42^0C. They are often found to spread along the lines of inoculation. *Campylobacter coli* colonies tend to be creamy-grey, moist and more discrete than those of *C. jejuni* subsp. *jejuni* (Nachamkin *et al*., 2000). The ability of many *C. jejuni* to hydrolyse hippurate allows its differentiation from *C. coli*, which lacks this ability (Totten *et al*., 1987).

2.5.2.5 *Yersinia*

The genus *Yersinia* contains three species that are well-established human pathogens (Prentice, 2006). *Yersinia pestis* is responsible for bubonic plague, an often-fulminant systemic zoonosis. *Yersinia pseudotuberculosis* and *Y. enterocolitica* cause yersiniosis, a self-limiting gastrointestinal illness that may have serious complications in special circumstances. As for many other bacterial pathogens, genome-sequencing projects are now revealing the genetic basis for these phenotypic differences (Prentice, 2006). *Yersinia pestis* was isolated for the first time by Alexandre Yersin in 1894 in Hong Kong (Yersin, 1894), following the spread of infection from mainland China. *Yersinia pseudotuberculosis* was first reported in 1883 from caseating lesions in rodents and was for many years classified with *Yersinia pestis* in the genus *Pasteurella* (Bercovier and Mollaret, 1984). *Yersinia enterocolitica* was first reported as a *Y. pseudotuberculosis* like organism isolated from enteric specimens and skin lesions of patients in the United States in 1939 (Schleifstein and Coleman, 1939) and later named *Bacterium enterocoliticum*. In the 1960s European microbiologists identified another heterogeneous group of bacteria resembling but distinct from *Yersinia* (then *Pasteurella*) *pseudotuberculosis* and termed them *Pasteurella* X. In 1964 Frederiksen pointed out similarities between *Pasteurella* X and *Bacterium enterocoliticum* and proposed the new species name *Yersinia enterocolitica* to cover this biochemically disparate group (Frederiksen, 1964). *Yersinia intermedia*, *Y. frederiksenii*, *Y. kristensenii*, *Y. aldovae Y. mollaretti* and *Y. bercovieri* have subsequently been split off from *Y. enterocolitica sensu stricto* within the group of *Y. enterocolitica*-like organisms on biochemical and genetic grounds. They are of doubtful pathogenicity and little clinical significance (Prentice, 2006).

Yersinia enterocolitica and *Y. pseudotuberculosis* are two important human enteropathogens distributed widely in the environment, with swine serving as the major reservoir. The incubation period is 3–7 days, with food-borne transmission the suspected route for most infections. *Yersinia*'s preference for cool temperatures makes this pathogen more common in Northern Europe, Scandinavia, Canada, the USA and Japan, where it is responsible for up to 8% of sporadic diarrhoea episodes. *Yersinia* enterocolitis occurs most often in children younger than 5 years25 and is characterized by fever, vomiting, exudative pharangitis, cervical adenitis, abdominal pain and watery diarrhoea, which may contain blood (Marks *et al.,* 1980). Diarrhoea typically lasts for 14–22 days but fecal excretion may persist for 7 weeks or longer (Alessio, 2006). Abdominal complications include appendicitis, pseudoappendicitis, diffuse ulceration of the intestine and colon, intestinal perforation, peritonitis, ileocecal intussusception, toxic megacolon, cholangitis and mesenteric vein thrombosis. Bacteremic spread may result in abscess formation and granulomatous lesions in the liver, spleen, lungs, kidneys and bone, as well as mycotic aneurysms, meningitis and septic arthritis. Infection can also be associated with immunopathological sequela including reactive arthritis, uveitis, Reiter's syndrome and erythema nodosum (Cover *et al*., 1995). It may be isolated from stool or pharyngeal exudates on commonly used selective media, and appears as gram-negative colonies after 48h of growth at 25–28^{0}C. Most uncomplicated cases resolve without treatment, which is reserved for patients with severe symptoms, extraintestinal infections and immunocompromised hosts. Production of β-lactamases generally renders all but third generation cephalosporins, aztreonam and imipenem ineffective. Treatment is generally 2–6 weeks, with an initial intravenous antibiotic (third-generation cephalosporin often in combination with aminoglycosides), followed by an oral agent to whom the clinical isolate is sensitive. In a study in Nigeria, one hundred and fifty stool samples from diarrhoeic children and adults seeking for medical attention (including hospitalized patients) in Vom Christain Hospital (VCH), Mandela Clinic (MC) Vom and Dagott Family Health Clinic (DFHC) Vom were screened for *Yersinia enterocolitica* infection between August 2005 and August 2006. The isolation methods adopted were direct plating on MacConkey Agar (MCA), Deoxycholate Citrate Agar (DCA) and cold enrichment method using phosphate buffered saline prior to subculture onto selective solid culture media (Cefsulodin Irgasan Novobiocin [CIN] agar). Out of the 150 samples screened, 6 (15%) were positive. The incidence of the infection was highest among those aged 1 - 10 years 3 (7.5%), followed by 21 - 31 years 2 (5%) and 11 - 20 years 1 (2.5%). Serotyped and biotyped, pathogenic *Y. enterocolitica* (2/O: 9. 4/O: 9) were susceptible to ciprofloxin, floxavid, streptomycin and tetracycline (Okwori, *et al*., 2007).

2.5.2.6 *Vibrio cholerae*

Vibrio cholerae is responsible for the most rapidly fatal diarrhoeal disease in humans. Although cholera is rare in developed countries, it remains a major cause of diarrhoeal morbidity and mortality in many parts of the developing world. However, with the occurrence of both natural (e.g. earthquakes) and human generated calamities (such as ethnic wars), the spreading of cholera infection in refugee

camps, where sanitary conditions resemble those in cholera endemic areas, represents a significant threat worldwide (Alessio, 2004).

Vibrios are fermentative, Gram-negative bacteria that are oxidase positive. They are part of a widespread group of organisms, many of which are common in the environment (Prentice, 2006). In clinical bacteriology we focus our attention mainly upon *Vibrio cholerae*, the cause of cholera, and *V. parahaemolyticus*, a major cause of food poisoning. However, it is important to remember that they are not the only vibrios that can cause human disease and indeed there are very many other vibrios that are not associated with human disease. The genus as currently understood is comparatively well characterized and consists of oxidase positive fermentative organisms. All members are facultative anaerobes and grow well on artificial culture media. Historically, taxonomically and medically the genus *Vibrio* focuses on the organisms that cause cholera. These are largely members of the species *V. cholerae*; other species may occasionally cause clinically similar illness. Advances in molecular biology, immunology and bacterial taxonomy mean that *V. cholerae* is now among the best understood of bacterial species. The taxonomy of the organisms, the virulence mechanisms by means of which it causes disease and the means of treating apparent cholera have all been thoroughly investigated (Tom, 2006). *Vibrio cholerae* enters the intestine and attaches to the mucosal surface. When attached, it produces cholera toxin (CT), which attaches to the intestinal mucosal cells. An important function of mucosal cells is the control of ion transport; under normal conditions the net flow of ions is from the lumen to the tissue, which results in a net uptake of water. CT disrupts ion fluxes without causing apparent damage to the mucosa (Gangarosa *et al*., 1960). CT decreases the net flow of sodium into the tissue. This produces a net flow of chloride and water out of the tissue into the lumen, causing the massive diarrhoea and electrolyte imbalance that is associated with this infection (Tom, 2006).

Developed countries have an almost zero incidence of cholera because they have widespread water treatment plants, food-preparation facilities that usually practice sanitation protocols, and most people have access to toilets and hand-washing facilities. Apart from the recent outbreak of cholera in Nigeria that killed three in Lagos and some others in Zamfara, at least 352 people have been killed by this infection in a space of three months and more than 6,400 cases reported majorly in the North. Doctors are now monitoring outbreaks in 12 of Nigeria's 36 states. Nigeria had the first series of cholera outbreak between 1970- 1990. Despite this long experience with cholera, an understanding of the epidemiology of the disease is still lacking (Ajoke *et al*., 2012).

Both killed whole-cell and live attenuated cholera vaccines have been proposed as a preventive intervention for cholera (Alessio, 2004). A large double-blind field trial of the killed vaccine showed 85% efficacy for a period of 4–6 months, dropping to 50% over 3 years of follow-up (Clemens *et al*., 1986). A locally produced killed vaccine in Vietnam provided a 66% protection against El Tor cholera during an outbreak occurring 8–10 months after vaccination (Trach *et al*., 1997). A genetically engineered attenuated cholera vaccine (CVD 103-HgR), obtained by deleting the active subunit of cholera toxin from a *V. cholerae* O1 classic biotype, was well tolerated when administered to volunteers. This vaccine elicited a high level of protection (82–100%) against homologous challenge with a strain of the same biotype (Levine *et al*., 1990). Protection across biotypes was also observed, albeit to a lesser extent,

lasting for at least 6 months after a single oral dose. Live attenuated *V. cholerae* O139 vaccines have been developed, with promising preliminary results (Alessio, 2004).

2.5.3 Intestinal parasites

The World Health Organization (WHO, 2009), estimates that some 3.5 billion people worldwide are infected by intestinal parasitic and protozoan infections, including 350 million with morbidity from ascariasis, 220 million from trichuris and 150 million from hookworm infections. Intestinal parasitic infections had long been recognized as an important health problem especially among Nigerian children, with diarrhoea and/or failure to thrive from anorexia and malabsorption being the most common symptoms (Graitcher and Gentry, 1998). The protozoa *Giardia lamblia* and *Cryptosporidium parvum* are common causes of diarrhoea in children, but other protozoa such as *Cyclospora cayetanensis, Entamoeba histolytica,* and *Microsporidia* species may also cause diarrhoea, and these include *Isospora belli* in immunodeficient (HIV-seropositive) patients (David, 2004). Only five helminthic parasites are associated with diarrhoeal disease: *Strongyloides stercoralis*, *Trichuris trichiura*, *Schistosoma mansoni*, *Trichinella spiralis* and *Capillaria philippinensis* while strictly intraluminal worms (e.g. *Ascaris lumbricoides*) only rarely affect intestinal function adversely when the intensity of infection is high. Infections such as giardiasis, cryptosporidiosis and severe trichuriasis may contribute to growth failure via malabsorption, so their resolution or treatment should lead to catch-up growth. Malnutrition appears to predispose children to an acute diarrhoeal syndrome in strongyloidiasis, which may then exacerbate the nutritional status through anorexia and enteropathy (David, 2004).

2.5.3.1 Giardiasis

Giardia lamblia (also called *Giardia intestinalis* or *Giardia duodenale*) is a flagellate protozoan with two stages: motile trophozoites and cysts (David, 2004). Infection may result from ingesting cysts, which release trophozoites in the upper small intestine, attaching to the mucosa by a ventral disc. *Giardia* trophozoites undergo significant biological changes to survive outside the host by differentiating into infective cysts, which is promoted by exposure to conjugated bile salts and low levels of cholesterol (Gillin *et al*., 1987). The incubation period is 1–2 weeks (David, 2004). *Giardia lamblia* is one of the most common parasitic infections in humans with a prevalence of 2–5% in industrialized countries, and high transmission rates in day-care center outbreaks among 12–36-month old children who are not toilet trained (Thompson, 1994). In developing countries, prevalence of 20–30% occurs with up to 60% of children infected at some time during childhood (WHO, 2009). *Giardia* accounts for about 5% of travelers' diarrhoea and is more common in children with immunodeficiency, although not HIV infection (David, 2004). The main routes of transmission are water, food and fecal–oral (Molbak *et al*., 1994). Infection may occur after the ingestion of only 10–100 cysts and unfiltered water has been a common source of outbreaks in developed countries (David, 2004). A Brazilian 4 year cohort study of 157 children from an urban slum documented substantial morbidity from giardiasis, with a similar frequency between diarrhoeal and non-diarrhoeal cases (9.7% vs. 7.4%), an association with persistent diarrhoea (20.6% of episodes) and recurrent or relapsing infections (46%) and poorer nutritional status for those

with symptomatic infections (Newman *et al*., 2001). A study of a rural Egyptian cohort of 152 infants documented 4.5 episodes per child-year with a mean duration of excretion of 7.2 weeks, and reduced risk in breast-fed infants, particularly for symptomatic illness (Mahmoud *et al*., 2001). A Kenyan cohort study of 84 rural children aged 10–28 months reported 44.7% prevalence, an estimated incidence of 2.8 episodes per year and duration of 75.3 days per child for giardiasis (Chunge *et al*., 1991). In Nigeria prevalence of 7·9 % was recorded for *Giardia intestinalis amongst children* in the age-group 1–5 years (Oyerinde *et al*., 1977). In a more recent study that utilised ELISA coproantigen to determine the prevalence of *Giardia lamblia* in stool samples of children in day-care centres, nursery and primary schools in two Local Government Areas, Sabon-Gari and Zaria, in Kaduna State, Nigeria, 41.45% were recorded positive for Giardia *lamblia.* The prevalence of giardiasis was higher in males (50.3%) than females (49.7%). There was no significant difference in the level of infection in both sexes. The highest prevalence of asymptomatic giardiasis was in the age group 3-5 years (32.9%) while the lowest was in the 0-2 years (11.6%). The study also indicated that there was no significant difference in the prevalence of the disease between the age groups (Inabo *et al*., 2011).

2.5.3.2 Cryptosporidiosis

The protozoa *Cryptosporidium parvum*, *Isospora belli*, *Cyclospora cayetanensis* and *Sarcocystis hominis* all belong to the group of intestinal coccidial infections, which cause diarrhoea (David, 2004). They have come into prominence in recent years due to causing severe and protracted diarrhoea in AIDS or cell-mediated immune deficiency and infecting piped water supplies due to the chlorine resistance of oocysts (WHO, 1987). The opportunistic protozoal infections which affect HIV-infected patients include *Cryptosporidium, Microsporidia, Cyclospora* and *Isospora belli. Cryptosporidium* also causes persistent diarrhoea and proximal small-intestinal enteropathy in children with normal immune function (Phillips *et al*., 1992). Cryptosporidiosis is transmitted by a 3–6 µm acidfast thick-walled oocyst shed in stool which encysts in the lumen of the small bowel after ingestion and produces four sporozoites (David, 2004). These penetrate the microvillus border and develop into trophozoites, which cause disease and can reproduce asexually, releasing eight merozoites, which invade nearby cells and redevelop into trophozoites to continue the infection. The life cycle is completed by asexual reproduction of trophozoites to schizonts, which develop progressively into male microgametes or female macrogametes, combining to form zygotes and then oocysts, which pass into the stool (David, 2004). After ingestion of oocysts, encystation takes place in the upper small intestine and sporozoites invade the absorptive epithelial cells causing inflammation, partial villus atrophy, malabsorption and diarrhoea. About 20% of oocysts are thin-walled and can autoinfect the host, which explains how a small number of ingested oocysts can cause severe disease, especially in immunocompromised patients. Fecal oocysts can survive for at least 2 days, and the incubation period from cyst ingestion to diarrhoea in humans is 2–14 days (David, 2004). The major difference from *Plasmodium* species (malaria) is that *Cryptosporidium* completes its life cycle in a single host without the mosquito vector. *Cryptosporidium* causes diarrhoeal disease in children of developing countries, in travelers and in immunocompromised patients; there have been waterborne outbreaks in developed countries. Transmission is from person to person and from animals to people by ingestion of fecally contaminated food or water. *C. parvum* infects numerous mammals, including man and cattle, and although there is a human-specific form,

animal forms may also cause disease in humans. Oocysts are highly infectious, while person-to-person transmission is common, occurring in 19% of infected children and is especially common in day-care center settings (David, 2004). Oocysts are relatively resistant to conventional chlorination and filtering, but 1 µm filters, heating (72 ^{0}C for a minute) and freezing are effective. *Cryptosporium* has been reported to occur in 6.1% of diarrhoeal cases vs. 1.5% of controls in developing world subjects compared to 2.2% vs. 0.2%, respectively, in the developed world. For AIDS patients, these rates increase to 24% vs. 5%, respectively, in the developing world and 14% vs. 0% in the developed world (Phillips *et al*., 1992). A Peruvian periurban study found a peak cryptosporidiosis incidence of 0.42 episodes/year in 1 year old (mean childhood incidence of 0.2), with the wet season and lack of toilets as risk factors (Bern *et al*., 2002). In Zambian urban children, cryptosporidial infection appeared to be predominantly waterborne with prevalence among diarrhoeal cases of 18%, and no association with home animals, nutritional status or parental education (Nchito *et al*., 1998). In community studies of children in Guinea- Bissau, *Cryptosporidium* was found in 5.8–7.4% of diarrhoeal episodes and 2.0% without diarrhoea, with peak prevalences in the early wet season (17.6% in May), in young children (12.6% in infants < 6 months) and with persistent diarrhoea of 15% (Molbak *et al*., 1994). In Kuwait, *C. parvum* oocysts were found in 10% of childhood diarrhoea, but older children were affected (73% in > 2 years old) (Iqbal *et al*., 2001). A Gambian study of 1200 children documented *Cryptosporidium* in 8.8% of diarrhoeal cases vs. 2.8% of controls ($P < 0.001$), with peak incidence during the wet season and among infants (6–11 months) (Adegbola *et al*., 1994). A Brazilian case–control study showed that children who acquired symptomatic cryptosporidiosis before 1 year of age had a higher burden of subsequent diarrhoeal disease (Agnew *et al*., 1998). Cryptosporidial infection causes watery diarrhoea with low-grade fever, vomiting and often cramps, severe dehydration and hypokalemia (David, 2004). A retrospective hospital study of 109 Cryptosporidial cases in London, showed that 92% had watery and offensive stools with blood and mucus only occasionally, 50% had persistent diarrhoea (> 14 days, and 33% > 21 days), 51% had vomiting, 21% had significant abdominal pain and 23% were underweight (Phillips *et al*., 1992). *Cryptosporidium* causes more prolonged and severe diarrhoea, which may be fulminant, in immunocompromised patients. In an Italian study of HIV-infected children with cryptosporidiosis, the median duration of diarrhoea was 32 days compared to only 4 days for other causes (Guarino *et al*., 1997). It was also associated with loss of 5–30% of body weight.

Cryptosporidiosis is diagnosed by finding oocysts in stool using an acid-fast stain (David, 2004). This is sensitive with more than 10000 cysts/g in diarrhoeal cases, but 50 times less sensitive without diarrhoea. Immunofluorescent and ELISA techniques are more sensitive, and PCR may be even more sensitive for detecting low numbers of oocysts in stool specimens (Javier *et al*., 1997). Serological testing for cryptosporidial antibodies is useful for epidemiological surveys, but not for clinical diagnosis in endemic areas (Xiao *et al*., 2001).

2.5.3.3 *Entamoeba* amoebiasis

Two distinct species of *Entamoeba* that are morphologically identical were recently identifyied by molecular and immunological techniques; *E. histolytica* and *E. dispar* (David, 2004). *E. histolytica* is pathogenic, causing symptomatic disease in 10% of infections whereas *E. dispar* causes only

asymptomatic colonization. In addition to the *E. histolytica* strain, other risk factors for invasive disease are interaction with bacterial flora, host genetic susceptibility, malnutrition, male sex, young age and immunodeficiency. *Entamoeba coli* and *E. hartmanni* are also nonpathogenic morphologically distinct members of the genus. The *E. histolytica* life cycle consists of an infective cyst (10–15μm in diameter) and an invasive trophozoite (10–60μm in diameter). Cysts are resistant to chlorination, gastric acidity and desiccation and can survive in a moist environment for several weeks. For transmission of *E. histolytica*, cysts are ingested from fecally contaminated food and water and encystation occurs in the intestine, transforming the cyst into eight trophozoites. Mucosal invasion by trophozoites leads to colonic ulcers with migration of parasites to the liver via the portal vein (David, 2004).

It is estimated that 500 million people are infected with *E. histolytica* in the world with 50 million per year developing invasive disease and 50000–100000 deaths. The case/fatality rate is 1 per 500–1000 diagnosed cases, but in a hospital setting, the case/fatality ratio in children is higher, with 9% mortality and 27% morbidity. Liver abscess and extra-abdominal amoebiasis has a much higher mortality rate of 10–40% or up to 90% for cerebral amoebiasis (WHO, 2010). The epidemiology of amoebiasis has been complicated by the difficulty of identifying two genetically identical but morphologically distinct species, *E. histolytica* and *E. dispar*. It is estimated that 10% of the population is colonized by *Entamoeba* but 90% of these are with nonpathogenic *E. dispar*. Although *E. dispar* distribution is worldwide, *E. histolytica* infection occurs predominantly in Central and South America, Africa and the Indian subcontinent (WHO, 2010). Studies based on stool parasites measure predominantly *E. dispar*, whereas serological surveys reflect the incidence of *E. histolytica*, since *E. dispar* does not cause seroconversion (David, 2004). A Bangladeshi study of urban children found the prevalence of *E. histolytica* to be 4.2% compared to 6.5% for *E. dispar* with the highest prevalence of *E*. *histolytica* infection in children with diarrhoea aged 6–14 years (Haque *et al*., 1997). A Mexican national survey reported 8.4% seropositivity to *E. histolytica*, peaking at 11% in the 5–9 year old age group; with 4.2% of diarrheal cases due to *E. histolytica* infection, and 6.5% with *E. dispar* (Heckendorn *et al*., 2002). Children 6–14 years of age with diarrhea had the highest incidence of *E. histolytica* infection (8%), whereas rural asymptomatic children had a 1% prevalence of *E. histolytica* and 7% prevalence of *E. dispar*. A West African study in school children found a ratio of *E. histolytica*: *E.dispar* of 1: 46, suggesting that the vast majority of *Entamoeba* infections in this region are not pathogenic (Heckendorn *et al*., 2002). A Brazilian study documented a 10.6% colonization rate for *E. histolytica* (excluding *E. dispar*), affecting predominantly those of poor socioeconomic status, hygiene and nutritional status, but most infections were asymptomatic and self-limited (clearing within 30–45 days) and reinfection rates were low (Braga *et al*., 2001).

Amoebic disease occurs when trophozoites invade colonic tissue, which is initiated by prior adherence to mucins lining the surface of the large bowel followed by enzymatic destruction of the basement membrane and underlying tissue. The resulting inflammatory response contributes further to tissue destruction (David, 2004). The stages of amoebic infection include: adherence to bacteria (especially *Escherichia coli*) and intestinal epithelial cells via galactose-binding lectin, which protects the parasite by blocking complement; activation of virulence factors in parasites such as cysteine proteinases; stimulation of intestinal epithelial cells to produce cytokines and inflammatory mediators by activation of NF-κB; the resulting release of chemokines leading to neutrophil influx; mediators

greatly contributing to the destruction of host tissues by lytic necrosis and apoptosis, enabling amoebic invasion (Stanley, 2001). The diagnosis of amoebiasis should be considered in any child with blood or mucus in the stools, particularly if associated with abdominal pain or distension. Stool microscopy will yield cysts in less than 30% of infected individuals, owing to the intermittent nature of cyst shedding, although multiple examinations increase the diagnostic yield to 60–70% with amoebic colitis but to under half of amoebic hepatic abscesses (David, 2004). Serology can be helpful with invasive amoebiasis since asymptomatic infections with *E. dispar* do not usually elicit a serological response. The gold standard remains culture or iso-enzyme analysis, but stool antigen detection tests are now commercially available which are more reliable than microscopy. A PCR-based amplification technique is now available for stool samples; infecting isolates are genetically diverse, with differences between those associated with intestinal and hepatic disease (Ayeh-Kumi, *et al*., 2001). The major problem with serological tests is that they remain positive for years after an episode of amoebiasis, so they may not be useful in endemic areas. Wet preparations of material aspirated or scraped from the base of ulcers on colonoscopy can be examined for motile trophozoites and tested for *E. histolytica* antigen. The appearance of amoebic colitis resembles inflammatory bowel disease with a granular, friable and diffusely ulcerated mucosa. It is preferable for biopsy specimens to be taken from the edge of ulcers, but the sensitivity of this method of diagnosis appears variable (David, 2004).

2.6 Laboratory diagnosis of childhood diarrhoea

Laboratory clues to identification of aetiologic agents of infectious diarrhoea can be found by evaluating the incubation period, history of diarrhoeal episode, unusual food or eating circumstances, risks (malnutrition, poor hygiene, history of travel, etc), recent use of antimicrobials, stool analysis and culture for bacterial pathogens (Thielman and Guerrant, 2004). Accuracy can be achieved as well costs can be reduced by improving the selection and testing of the specimens submitted on the basis of interpreting the case information— such as patient history, clinical aspects, visual stool inspection, and estimated incubation period. Timely and accurate identification of suspected pathogens that are present in the stool specimens from patients with sign and symptoms of diarrhoea is still a challenge in the laboratory (Thielman and Guerrant, 2004). Most medical microbiology laboratories use conventional diagnostic procedures, which involve culture and microscopy of stool samples, for routine detection of enteric pathogens. These culture procedures include enrichment steps, use of selective culture media, biochemical identification, serotyping, and resistance profiling. The method still convincing and termed ‘gold standard’ for the identification of enteropathogenic bacteria, but the procedures are time consuming (require 3–5 days), laborious intensive and also required an experienced clinical microbiologist (Pawlowski *et al.,* 2009). Microscopic examination is conventionally required for parasite identification and presence of any abnormal components (e.g. mucus or blood) (Chessbrough, 2000, Vandepitte *et al.,* 2003, Ochei and Kolhatkar 2003). Examination for parasitic ova and cyst or cellular exudates of diarrhoeal stools requires a trained technologist, but however in some instances microscopic examination is limited in distinguishing species of organisms. For example *E. histolytica* (pathogenic species) and other non-pathogenic species example *E. dispar* can hardly be distinguishable because of morphological similarities (Sherry *et al*., 2003).

Examination by electron microscopy (EM) can be used for a range of viruses (adenoviruses, noroviruses, rotaviruses, etc) and bacteria, but the method is cumbersome and requires significant expertise. EM was actually initially the only method for viral diagnosis, but its use has been limited owing to its low sensitivity and specificity. Various stool antigens test have also been available for the detection of different diarrhoeagenic pathogens (Henchal *et al*., 2001).

Immunodiagnostic or immunodetection assays for detecting proteins and other non-nucleic acid targets, including antigens, antibodies, carbohydrates, and other organic molecules, were conducted using antibodies produced in appropriate host animals. In reality, numerous other nonantibody molecules, including aptamers, peptides, and engineered antibody fragments, are now being used in affinity-based detection technologies. Immunodiagnostic methods an integrated approach to agent detection and identification, which is essential for a complete and accurate disease diagnosis, provides the most reliable laboratory data and play a key role in diagnosing disease by detection of agent-specific antigens and/or antibodies present in clinical samples (Henchal *et al*., 2001). Although most antigen tests are simple and rapid, they usually lack sensitivity and specificity. Several comparison studies have shown that non-molecular tests, such as the glutamate dehydrogenase (GDH) antigen test, enzymes immunoassay (EIA) and Enzyme-Linked Immunosorbent Assay (ELISA), for viruses and bacterial toxins, are reported less sensitive and or less specific when compared with molecular testing for microbial infection detection (Sherry *et al*., 2003).

2.6.1 Molecular assay for detecting bacterial pathogen causing diarrhoea

Molecular diagnostics are recent complex advancement adopted by clinical laboratories for detection and identification of all existing microbial. These recent advancements provides a rapid function and correlation, high sensitivity and specificity as well as multiple answer for both qualitative and quantitative detection and identification of pathogens and commensals in a clinical sample. In the past few years, commercially available genomic test have been implemented in routine diagnostic use. The assay use molecular technology whereby polymerase chain reaction (PCR) technique is used to rapidly increase the amount of either a specific segment or total DNA in a sample is amplified as DNA target and detected with a molecular beacon. This approach were divided into monoplex and multiplex genomic test, with the first test use for detecting a single pathogen through a single reaction perfumed on a patient sample and the later on a panel of pathogens (Sherry *et al*., 2003).

2.6.1.2 Polymerase Chain Reaction (PCR)

Originally conceived in 1983 by Mullis, polymerase chain reaction (PCR) became a reality only 2 years later with the publication by Saiki *et al*, of its first practical application. The description of PCR marked the beginning of the field now known as molecular diagnostics (Sherry *et al*., 2003). PCR is a simple, in-vitro chemical reaction that permits the synthesis of almost limitless quantities of a targeted nucleic acid sequence. At its simplest, the PCR consists of target DNA (also called template DNA), two oligonucleotide primers that flank the target DNA sequence to be amplified, a heat-stable DNA polymerase, a defined solution of salts, and an equimolar mixture of deoxyribonucleotide triphosphates

(dNTPs). The mixture is then subjected to repeated cycles of defined temperature changes that help to facilitate denaturation of the template DNA, annealing of the primers to the target DNA, and extension of the primers so that the target DNA sequence is replicated. A typical PCR protocol comprises 30 to 50 thermal cycles. Each time a cycle is completed; there is a theoretical doubling of the target sequence. Therefore, under ideal conditions, a single copy of a nucleic acid target can be multiplied over a billion-fold after 30 cycles. The whole procedure is carried out in a programmable thermal cycler that precisely controls the temperature at which the steps occur, the length of time the reaction is held at the different temperatures, and the number of cycles. The PCR products are typically visualized as bands on Screen tape system or on an agarose gel after electrophoresis and staining with a DNA intercalating dye such as ethidium bromide or Sybr green (Loh *et al*., 2008). In multiplex PCR, two or more sets of primers specific for different targets are included in the same reaction mixture, allowing for multiple target sequences to be amplified simultaneously (Chamberlain *et al.*, 1988). The primers used in multiplexed reactions must be carefully designed to have similar annealing temperatures and lack complementarity. Multiplex PCR assays have played a larger role in human and cancer genetics than in the detection of infectious organisms, where they have proven more complicated to develop and often result in lower sensitivity than PCR assays using single primer sets (Sherry *et al*., 2003).

2.6.1.3 Real-Time PCR

By far the most important development in rapid identification of biological agents has been the development of “real-time” PCR methods (Sherry *et al*., 2003). Although traditional PCR was a powerful analytical tool that launched a revolution in molecular biology, it was difficult to use in clinical and field laboratories. As originally conceived, gene amplification assays could take more than 5 to 6 hours to complete, not including the sample processing required before amplification. The improvement of assay throughput came with the development of assay chemistries that allowed the PCR reaction to be monitored during the exponential amplification phase on fast thermocyclers. Lee *et al*. (1993) and Livak *et al*. (1995) demonstrated assays based on the detection and quantification of fluorescent reporters that increased in the direct proportion to the amount of PCR product in a reaction. By recording the amount of fluorescence emission at each cycle, it is possible to monitor the PCR reaction during the exponential phase, in which the first significant increase in the amount of PCR product correlates to the initial amount of target template. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed. A significant increase in fluorescence above the baseline value measured during cycles 3 through 15 indicates the detection of accumulated PCR product. There are three main probe-based fluorescence-monitoring systems for DNA amplification: (1) hydrolysis probes, (2) hybridization probes, and (3) DNA-binding agents. Hydrolysis probes most exemplified by TaqMan (Applied Biosystems, Foster City, Calif) chemistries have been the most successful for rapidly identifying pathogens. Probe hydrolysis assays use the fluorogenic 5’ exonuclease activity of QIAamp DNA stool Minikit (Qiagen GmbH, Hilden, Germany) multiplex realtime–PCR system for the detection of Human diarrheal viruses and bacteria that included astrovirus, Group A rotavirus, enteric adenovirus, norovirus (GI/GII), *Vibrio spp, Clostridium difficile toxin B, Salmonella spp, Shigella spp, Campylobacter spp, Clostridium perfringens, Yersinia enterocolitica, E.coli* O157:H7, VTEC and

Aeromonas spp (Josiane *et al.,* 1997, *et al.,* 2011, Inglis and Kalischuk, 2003, Persson and Olsen, 2005 and Islam and Lindberg, 1992).

2.7 Malnutrition and diarrhoea

Nutrition is the process by which dietary constituents are converted into, maintain and sustain the body in health (Michael, 2004). Nutrition and genetic coding are the determinants of the body's development and composition; they modulate and control its function and enable it to resist disease. Nutrition is the major variable that determines the quality of the body – the 'soil' in which the 'seeds' of disease germinate (Michael, 2004). The battle between the nutritional 'soil' and the aetiological 'seed' determines the course of most diseases. Both nutritional state and genetic endowment should be viewed separately from the agents of disease. They determine the internal environment in which the seeds of disease flourish or fail. Primary malnutrition is a condition of the dependent and vulnerable that relies on others for nourishment. It is seen most frequently in the young child, the elderly and groups such as prisoners and the mentally ill or disabled. Secondary malnutrition accompanies any disease that disturbs appetite, digestion, absorption or utilization of nutrients. In poor, technologically backward countries, malnutrition-associated disease is the major cause of death. More than half of all deaths have anthropometric malnutrition as the underlying cause; many more are compromised by micronutrient malnutrition, not associated with weight loss (Pelletier, 1994). Malnutrition stunts the physical and mental development of the majority of the surviving population. Undernourished children are at higher risk of suffering more severe, prolonged and often more frequent episodes of diarrhoea (WHO, 2009). Repeated bouts of diarrhoea also place children at a greater risk of worsening nutritional status due to decreased food intake and reduced nutrient absorption, combined with the child's increased nutritional requirements during repeated episodes. Diarrhoea often leads to stunting in children due to its association with poor nutrient absorption and appetite loss. The risk of stunting in young children has been shown to increase significantly with each episode of diarrhoea and diarrhoea control, particularly in the first six months of life, may help to reduce stunting prevalence among children (Checkley, 2003). The relationship between diarrhoea and malnutrition is bidirectional: diarrhoea leads to malnutrition while malnutrition aggravates the course of diarrhoea. On the one hand, severe and prolonged episodes of diarrhoea cause malnutrition in individual patients; on the other hand, malnourished children are more likely to develop complications with diarrhoea (Nel, 2010). Many studies, addressing the relationship between diarrhoea and malnutrition, have demonstrated a detrimental effect of diarrhoea on nutritional status. Some of the first evidence of the association between diarrhoea and malnutrition was the graphical representation of the interaction between growth and infection in children in South America. Mata *et al.* (1971) observed cohorts of infants born in Santa Maria Cauquè in Gautemala, intestinal as well as other infections and growth parameters were recorded from birth to three years. Episodes of diarrhoea, upper and lower respiratory tract infection, measles and other infections were associated with progressive growth faltering: children with poor growth (growth in the lower quartile of the study) had more infections than those who grew the best (growth in the upper quartile). Especially dysentery and bronchopneumonia occurred more frequently in the lower growth quartile. Subsequent studies assessed the impact of diarrhoea on the growth of large groups of children. The contribution of diarrhoea to growth failure in children in developing countries

was estimated to be as high as 25–30% (Nel, 2010). The same effect is, however, not seen in children in developed countries. This lack of effect may be due to the lower burden of disease in developed countries, the better nutrition of children in these countries, and bias in measuring episodes of morbidity (Montrel *et al*., 1975). Growth and episodes of infection in children in eastern Guatemala was measured from shortly after birth to seven years. Diarrhoea was associated with reduced growth rate while there was no significant association between fever or respiratory illness and growth. Children with a low prevalence of diarrhoea (less than or equal to 5% of time with diarrhoea) grew 6,3% more in length and 11% more in weight than those with a high prevalence of diarrhoea (more than 5% of time with diarrhoea). This effect occurred independently of the type of nutritional supplement that the children were receiving (some were on a high and others on a low energy supplement) (Montrel *et al*., 1975). Diarrhoea had a similar effect on the growth of children in the Gambia, Rowland, *et al*. (1997), found that there was a negative correlation between weight and height gain and diarrhoea prevalence in children younger than three years and malaria was the only other disease for which there was also a significant association in this study. The regression coefficient for the diarrhoea versus weight gain was found to be -95 +/-85 g/kg body weight per month. A longitudinal study from Egypt found that diarrhoea was unlikely to have a major impact on the prevalence of malnutrition. Diarrhoea was associated with a small increase in the risk of low height-forage and children showed some catch-up growth in the following observation period. There was no effect on the weight for age or weight for height. On the other hand, children with a low weight for height (as well as those with poor sanitation and previous episodes of diarrhoea) had an increased risk of diarrhoea. Few children in this study however had persistent diarrhoea (1%) and the prevalence of malnutrition in this population was lower than in other populations that have been studied. The results of this study may thus not be applicable to poorer populations (Wierzba *et al*., 2001).

2.8 Poor hygiene and diarrhoea

Lack of access to safe, clean drinking-water and basic sanitation, as well as poor hygiene cause nearly 90% of all deaths from diarrhoea, mainly in children (WHO, 2008). While 87% of the world's populations now have access to improved water sources, 39% still lack access to improved sanitation (WHO, 2010). Moreover, in developing countries 1.1 billion people still defecate in the open, and hand washing with soap is practiced, on average, only after 17% of toilet uses (Lutter, 1992). Diarrhoea most often results from the ingestion of pathogens from faeces that have not been disposed of properly, or from the lack of hygiene. Primary prevention of diarrhoea through water, sanitation and hygiene interventions is based on reducing the faecal-oral transmission of pathogens, and includes the provision of an improved water supply, water safety planning, household water treatment and safe storage, improved sanitation facilities, and hygiene education. Improved water supplies refer to technologies such as piped household water connections, public taps, standpipes, or protected dug wells, springs or rainwater collection. Improved sanitation facilities may include flush/pour flush toilets to a confined system, improved latrines (e.g. ventilated, with slab), or composting toilets (WHO, 2008). Water safety planning considers the management of water from the source to tap (Schmidt and Cairncross, 2009). Water treatment may be carried out at source or in the home, and safe water storage takes place in containers, preventing recontamination of water in the household. Hygiene education can address a

number of practices, including hand washing after toilet use and before the preparation of food (Waddington, 2009).

Reductions in diarrhoea incidence have reached 5% for water supply at source, 19% for water quality interventions (results after 12 months), 36% for sanitation interventions, and 47% for hand washing with soap (estimates from pooled analyses) (Cairncross and Valdmanis, 2006). Larger scale and possibly longer term interventions may have additional effects, due to a reduction in the circulation of pathogens across households or communities, and a sustained behaviour change. Due to the multiple and frequent infection opportunities in unsanitary environments, the effectiveness of some of these interventions may seem limited, yet they often are a prerequisite to reaching the next level of sustainable disease reduction. Water, sanitation and hygiene interventions also prevent intestinal parasitic infections alongside diarrhoea, and these infections also have synergistic effects with malnutrition (VanDerslice and Briscoe, 1995). Various studies have documented how access to safe water, sanitation and adequate hygiene can predict child growth and malnutrition (Merchant, 2003). Behavioural factors are important in determining the uptake and sustainable adoption of water, sanitation and hygiene technologies and practices. While water, sanitation and hygiene interventions are potentially highly efficient, their effectiveness in part depends on behaviour change and context. The installation and functioning of water and sanitation facilities need to be accompanied by the transfer of knowledge on how to use them, together with sustainable behaviour change. Maintenance and periodic replacement of existing services or facilities, and hygiene promotion are also necessary to achieve improvements (Bartram and Cairncross, 2010). Low-income countries are particularly affected by deficient water systems and services, poor sanitation and hygiene (WHO, 2008). Implementing water, sanitation and hygiene interventions among poor households would show its highest effectiveness during the first 50% coverage; however, wealthier households are also in need of such interventions.

2.9 Prevention and control of childhood diarrhoea

Reducing childhood diarrhoea requires interventions to make children healthier and less likely to develop infections that lead to diarrhoea; clean environments that are less likely to transmit disease; and the support of communities and caregivers in consistently reinforcing healthy behaviours and practices over time. Many well-known child survival interventions are critical to reducing child deaths from diarrhoea. They work in two ways: by either directly reducing a child's exposure to the pathogens that cause diarrhoea (through the provision of safe drinking water, for example) or by reducing a child's susceptibility to severe diarrhoea and dehydration (through improved nutrition and overall health) this include: disposing of human excreta in a sanitary manner, washing hands with soap, increasing access to safe water, improving water quality at the source, and treating household water and storing it safely. Improvements in sanitation reduce the transmission of pathogens that cause diarrhoea by preventing human faecal matter from contaminating environments. Improving sanitation facilities has been associated with an estimated median reduction in diarrhoea incidence of 36 per cent across reviewed studies (Jamison *et al.*, 2008). However, a major challenge in this regard is scaling up sanitation facilities to the point where they are used by an entire community (total sanitation). Use of such facilities by all

community members is necessary to significantly reduce diarrhoeal disease transmission (WHO, 2007). Washing one's hands with soap is another important barrier to transmission, and has been cited as one of the most cost-effective public health interventions (Sandy *et al*., 2010). A number of studies have shown that hand washing with soap can reduce the incidence of diarrhoeal disease by over 40 per cent (WHO, 2007). Accessible and plentiful water has also been shown to encourage better hygiene, although the extent to which access to improved water sources reduces diarrhoea rates often depends on the type of water source available (such as public taps or standpipes, protected dug wells or boreholes) (Curtis, and Cairncross, 2000). Interventions to improve water quality at the source, along with treatment of household water and safe storage systems, have been shown to reduce diarrhoea incidence by as much as 48 per cent. Proven and field-tested household water treatment options that are currently being promoted include filtration, boiling, combined flocculation, chlorination, disinfection, and solar disinfection. Household water treatment could potentially be scaled up quickly and inexpensively in both development and emergency situations. It has even become common practice in large cities where homes are connected to a municipal water supply, since water is often polluted between the source and the point of use (WHO, 2008).

Micronutrient supplementation such as Vitamin A supplement is a critical preventive measure, and studies have shown mortality reductions ranging from 19 per cent to 54 per cent in children receiving supplements (Barreto *et al*., 1994). This reduction is associated in large part with declines in deaths due to diarrhoeal diseases and measles. Vitamin A supplementation has also been shown to reduce the duration, severity and complications associated with diarrhoea (International Vitamin A Consultative Group, IVACG 1996). Adequate zinc intake among children is critical for normal growth and development. Recents supplementation trials have shown that adequate zinc leads to a substantial reduction in childhood diarrhoea cases (Lazzerini *et al*., 2008).

Immunizations help reduce deaths from diarrhoea in two ways: by helping prevent infections that cause diarrhoea directly, such as rotavirus, and by preventing infections that can lead to diarrhoea as a complication of an illness, such as measles. Undernourished children are more likely to suffer from diarrhoea and its consequences, which, in turn, increase their chances of worsening nutritional status. Today, 129 million children under the age of five in the developing world are underweight for their age. Together, Africa and South Asia account for more than 80 per cent of total underweight children (25 per cent and 57 per cent, respectively) (WHO, 2010).

Breastfeeding is also reported to be among the prevention package as breast milk contains the nutrients, antioxidants, hormones and antibodies needed by a child to survive and develop (Feachem *et al*., 1983). Infants who are exclusively breastfed for the first six months of life and continue to be breastfed until two years of age and beyond develop fewer infections and have less severe illnesses than those who are not, even among children whose mothers are HIV-positive. This protection has been shown to be higher where maternal literacy is lower and where sanitation is worse (Victora *et al*., 1987). Infants who are not breastfed have were reported to have a six fold greater risk of dying from infectious diseases in the first two months of life, including from diarrhoea, than those who are breastfed (Feachem *et al*., 1983). So over the past decade, there has been some progress in exclusive

breastfeeding rates among infants in the first six months of life across the developing world, and particularly in Africa. But despite these advances, overall levels remain low and only 37 per cent of infants in developing countries are exclusively breastfed for the first six months of life (WHO, 2009).

2.10 Treatment against childhood diarrhoea

Oral rehydration therapy (ORT) has been the cornerstone of treatment programmes to prevent life-threatening dehydration associated with diarrhoea (Integrated Management of Childhood Illness, IMCI, 2005). ORT is the administration of appropriate solutions by mouth to prevent or correct diarrhoeal dehydration. ORT is a cost-effective method of managing acute gastroenteritis and it reduces hospitalization requirements in both developed and developing countries. Oral rehydration salts (ORS), used in ORT, contain specific amounts of important salts that are lost in diarrhea stool. The new lower-osmolarity ORS (recommended by WHO and UNICEF) has reduced concentrations of sodium and glucose and is associated with less vomiting, less stool output, lesser chance of hypernatremia, and a reduced need for intravenous infusions in comparison with standard ORS. This formulation is recommended irrespective of age and the type of diarrhoea including cholera. ORT consists of: rehydration water and electrolytes to replace losses and maintenance fluid therapy, to take care of ongoing losses once rehydration is achieved (along with appropriate nutrition). Rice-based ORS is superior to standard ORS for adults and children with cholera or malnourish, and can be used to treat such patients wherever its preparation is convenient. It is not superior to standard ORS in the treatment of children with acute non cholera diarrhea, especially when food is given shortly after rehydration, as is recommended to prevent malnutrition (IMCI, 2005). The WHO (2010) and UNICEF (2010), recommend routine zinc therapy for children with diarrhea, irrespective of the types. Zinc deficiency is widespread among children in developing countries and routine zinc therapy, as an adjunct to ORT is useful in modest reduction of the severity but more importantly reduce diarrhea episodes in children in developing countries. The recommendation for all children with diarrhea is 20 mg of zinc per day for 10 days. Infants aged 2 months or younger should receive 10 mg per day for 10 days. Supplementation with zinc sulfate in recommended doses reduces the incidence of diarrhea during the following 3 months, and reduces nonaccidental deaths by as many as 50%. It is more important in the management of diarrhea in malnourished children and persistent diarrhea (WHO, 2010).

Antibiotics are reported not effective in treating most diarrhoeal episode and only given when there is severe dehydration, dehydration in cholera cases and cases of dysentery (Murray and Lopez, 1998). Antibiotics in cases of bacterial diarrhoeas rarely help and make some children sicker. Unnecessary use of antibiotics may increase the resistance of some pathogens. In addition, antibiotics are costly. Money is often wasted on ineffective treatment and therefore, is not given routinely (Guandalini, 2002). Moreover high rates of resistance to commonly used, inexpensive, oral antimicrobial agents such as ampicillin, tetracyclines, and trimethoprim-sulfamethaxosole were observed in isolates of enteroadherent *E. coli*, ETEC, EPEC, and *Shigella* spp. in a survey in Somalia (Murray and Lopez, 1998). Similarly, high overall rates of resistance, often to multiple drugs, were seen in isolates from children with acute diarrhea in Nigeria (Lamikanra *et al*., 1989). Unfortunately, detailed resistance patterns of each pathogen were not reported. High levels of resistance to commonly used antibiotics have also been

observed in *Shigella* spp. in Nigeria (Olukoya and Oni, 1990). Initial therapeutic measures are in some reported cases consistent and are derive from the folk perception of the cause and severity of the disease. For example the educational level of the mother may also influence decisions in the management of child diarrhoea by mothers. In a study of home management of diarrhea in Nigeria, Yoruba mothers with a higher level of education were more likely to use a combination of Western and traditional treatments, although very few mothers were inclined to use Western medicine alone (Brieger, 1990). Antimicrobial agents are in most study in Africa reported frequently the first-line treatment of diarrhea in the home, followed by herbal remedies and, last of all, ORS (Olango and Aboud, 1990, Oni *et al*., 1991 and Omotade *et al.,* 1994). One of the study also showed that antibiotics were administered on 54.5% of days with diarrhea, local herbs on 27.7%, and ORS was only given on 14.8% (Oni *et al*., 1991). Very young children and those with multiple episodes of diarrhea were more likely to receive combinations of treatment, especially antimicrobial drugs and ORS. Although the frequency of usage varies, antibiotics and herbal teas clearly play a central role in the home management of diarrhea in several Sub-Saharan countries, even though traditional treatment practices have been associated with a higher risk of death from diarrhea (Olango and Aboud 1990).

Treatment of Viral diarrhoea is based on symptomatic treatment using carbohydrate and electrolyte solution administered through the oral or parenteral route. Selected probiotics may be effective in reducing the duration of symptoms, whereas, in severe cases in which rotavirus is involved, oral administration of human serum immunoglobulin may be effective. Several studies have shown that oral administration of human serum immunoglobulin is associated with an effective antiviral effect. For children hospitalized with severe rotavirus diarrhea, passive immune treatment should be considered. Efforts towards vaccine development have been hampered by withdrawal of antirotavirus vaccine, because of its association with intussusceptions, but novel vaccines are currently under investigation (De Zoysa and Feachem, 1985).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Sokoto State is located at the extreme north-west of Nigeria, between longtitudes $4^0 8^1$and $6^0 54^1$ and latitude 12^0N and $13^0 58^1$N. The state shares borders with the Republic of Niger to the north and north-east, Kebbi state, Nigeria to the west and south-east, and Zamfara state, Nigeria to the east. The State covers a land area of 32,000 square kilometres. Based on the 2006 census, the State has a population of 3,696,999 (Sokoto Diary, 2008).

The State is located within the basin which is surrounded to the east and south by the Precambrian basement complex. The State is drained by the Sokoto and Rima rivers and their tributaries, most of which rise from the south-east. These rivers and their tributaries form one of the most extensive Fadama areas in Nigeria. The major indigenous tribes in the state are the Hausa and Fulani. Majority of the Hausa's are

farmers while the Fulani's are nomads and are engaged in animal rearing. The common sources of drinking water are tap and borehole in the metropolitan cities, boreholes, hand dug wells, ponds and stream water in the rural areas (Iman, 2006).

3.2 Sampling Design

Using the multistage (cluster) sampling method (Snedeoor and Cochran, 1976), six Local Governments areas (LGA) were covered by selecting two LGA through random balloting from each of the three senatorial zones of the State. After clustering the health-facilities from the six LGAs, health facilities were then randomly selected by facility type (general hospitals, Primary health care, and dispensaries) (Fig. 3.1). Instances of diarrhoeal diseases outbreak due to contamination of drinking water and food with microbes had been reported in several areas of Sokoto State (Nigeria Demographic and Health Survey (NDHS), 2008 and Raji *et al.,* 2010).

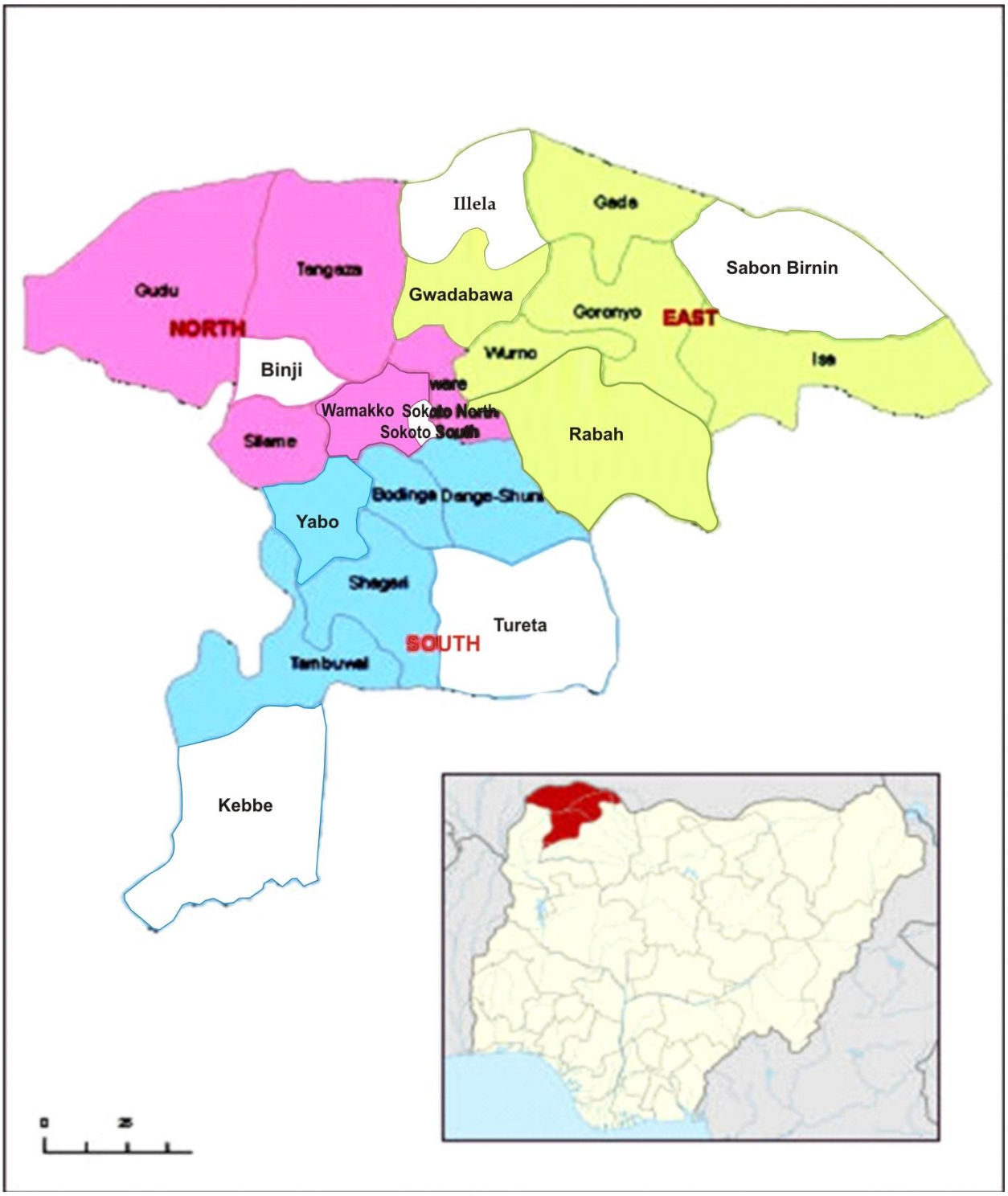


Figure 3.1: Map of Sokoto State showing the sampling LGAs

(Sokoto Diary, 2008).

3.3 Ethical approval

Ethical approval was obtained from the Ethical Committee in the state Ministry of Health and the targeted healthcare facilities.

3.4 Sampling Techniques:

3.4.1 Inclusion criteria:

Children of age ≤5 years who presented or admitted at clinics or hospitals for acute diarrhoeal illness in the selected LGAs (study area) were included for the study.

3.4.2 Exclusion criteria:

Any child presented for an illness other than diarrhoea and with no history of diarrhoea on the day of, or in the three weeks preceding sampling and of the age above five years.

3.4.3 Sample size:

The sample size was calculated using the following equation (Aroye, 2004):

$$N = \frac{(z)^2 pq}{(l)^2}$$

Where N=sample size

z= score for a given confidence interval usually set at 1.96 for 95% C.

p= prevalence value of 50% (0.5)

q= (l-p)=1- 0.5 = 0.5

l= permissible error of estimation which is taken as 0.05 (5%)

$$N = \frac{(1.96)^2 \times 0.5 \times 0.5}{(0.05)^2}$$

= 384

This value was increased to 420 samples of diarrhoeic stool collected for the study.

3.4.4 Sampling frame:

A total of seventy samples each were collected from health facilities at Binji, Illela, Kebbe, Sabon Birni, Sokoto South and Tureta, LGAs of Sokoto state.

3.4.5 Sample collection and storage:

At the respective health care facility, arrangements were made with a physician, matron or laboratory technician whereby the parent/caregiver of any child who satisfied the study inclusion criteria (age 0-5 years of both sexes and diarrhoeic) was requested to provide the child's stool specimen after consultation. Prior to collection of specimen, the parent/caregiver was interviewed using structured questionnaire (appendix III) designed to obtain basic demographic data, history of illness and clinical information concerning the child. Information on the clinical features and the dehydration status was also obtained from the doctor/matron on duty or from the hospital folder of each child.

Only one stool specimen was collected per child. The specimens were collected in sterile, labelled screw capped tubes pocked in iceboxes and was transported to the Medical Microbiology Laboratory, Usmanu Danfodiyo University, Sokoto within 2 hrs for preliminary isolation. The isolates in nutrient agar slant were transfered to Department of Microbiology, Ahmadu Bello University Zaria, within 6 hrs for further analysis.

3.5 Detection of Enteropathogenic Bacteria from Stool Specimens

3.5.1 Microscopic examination of stool specimens

The stool samples were examined macroscopically for the presence of any abnormal components (e.g. mucus or blood) and this was then recorded. When the specimen contains mucus, pus, or blood a drop of the specimen was mixed thoroughly in a drop of 0.05% methylene blue solution on a clean slide and then covered with a coverslip, avoiding the formation of air bubbles. After 2 to 3 minutes the slide was then examined under the microscope using the high-power objective. (Chessbrough, 2000, Vandepitte *et al.,* 2003, Ochei and Kolhatkar 2003). A dry and heat-fixed thin smear of the specimen was stained with 10 g/l basic fuchsin for 10 to 20 seconds then washed with water and allowed to dry in air. The preparation was then examined using oil immersion objective (Chessbrough, 2000).

3.5.2 Stool culture

Using a sterile nickel-chromiun wire loop of diameter 2 mm (0.002 ml), loopful of the faecal specimen was inoculated onto two solid media and one liquid selective medium. The media consist of Eosin Methylene blue agar (EMB), Columbia blood agar and Selenite F broth (Weimer *et al.,* 2011). The EMB plates and the broth were incubated at 37 ^{o}C in an aerobic incubator, while the Columbia blood agar plates were incubated at 42 ^{o}C in a microaerophilic atmosphere with 10% CO_2 for 48 hrs. Following incubation a subculture of the broth was made onto Salmonella Shigella agar (SSA) and incubated at 37 ^{o}C for 24 hrs (Cheesbrough, 2000).

For initial screening of isolates motility-indole–lysine medium (MIL) and Kligler's iron agar (KIA) were inoculated and incubated at 37 ^{o}C for 6 hours (Ochei and Kolhatkar 2003). All the media used were prepared based on manufacturer's instructions (Cheesbrough, 2000). All strains isolated were subcultured onto nutrient slant agar and stored at -60 ^{o}C (VT 307 A/S Vestfrost DK-6705 Esbjerg Ø, Denmark) for further testing.

3.5.3 Identification of Isolates using Biochemical Methods:

The isolates were further identified and characterized according to standard biochemical methods which included catalase, indole, methyl red, Voges-Proskauer, hydrogen sulphide production, citrate utilization, sugar utilization tests and microscopy of Gram stained isolates (Cheesbrough, 2000). Other identification test include carbohydrate fermentation, β-galactosidase activity, gelatine hydrolysis, amino acids and enzymes activity (coagulase, Oxidase, lysine, ornithine decarboxylase, phenylalanine deamination test) (Harley, 2002).

3.5.3.1 Gram staining

A colony from primary culture medium was picked using sterile wire loop and emulsified into sterile distilled water to make a thin smear on a clean glass slide. The smear was allowed to dry before fixing using gentle heat. The fixed smear was covered with crystal violet stain for I minute and washed with clean water. The mear was then covered with Lugol's iodine solution for 1 minute and washed with clean water. It was then decolourized rapidly with acetone-alcohol and washed with clean water. The smear was then covered with neutral red stain for 2 minutes before washing off with clean water. The slide was drained on rack and then examined under oil immersion objective (Koneman, *et al*., 1992, Cheesbrough, 2000, Ochei and Kolhatkar 2003).

3.5.3.2 Catalase test

A quantity (3 ml amount) of hydrogen peroxide solution was dispensed into a test tube. Using a sterile wooden stick pure colonies of the test organism were immersed in the hydrogen peroxide solution. Development of a rapid effervescence indicates production of molecular oxygen and a positive catalase test (Koneman *et al*., 1992, Cheesbrough, 2000, Ochei and Kolhatkar 2003).

3.5.3.3 Oxidase test

On a piece of filter paper in a clean petri dish 3 drop of freshly prepared oxidase reagent (tetramethyl-*p*-phenylenediamine dihydrochloride) was added. Using a sterile wooden stick, pure colonies of the test organism were removed and smeared onto the filter paper. Bacterial colonies having

a positive oxidase activity develops a blue-purple colour within a few seconds (Koneman, *et al*., 1992, Cheesbrough, 2000, Ochei and Kolhatkar 2003).

3.5.3.4 Indole test

Tryptone broth was inoculated with few pure colonies of the test organism and incubated. After incubation at 37 ^{o}C for 24 hrs, 15 drops of Kovac's reagent was added down the inner wall of the tube. The development of a bright red colour at the interface of the reagent and the broth within seconds after adding the reagent is indicative of the presence of indole and is a positive test (Koneman *et al*., 1992, Ochei and Kolhatkar 2003).

3.5.3.5 Methyl red test

Methyl red/ Voges-Proskauer (MR/VP) broth was inoculated with few pure colonies of the test organism and incubated at 37 ^{o}C for 48 hrs. Following incubation 5 drops of methyl red reagent was added directly to the broth. The developments of a red colour in the surface of the medium indicate a positive test (Koneman, *et al*., 1992, Ochei and Kolhatkar 2003).

3.5.3.6 Voges-Proskauer (VP) test

A MR/VP broth was inoculated with few colonies of the test organism from pure culture and incubated at 37 ^{o}C for 24 hrs. At the end of incubation time, 1 ml amount of the broth was removed and placed into a clean test tube. A quantity (0.6 ml amount) of 5% α-naphthol was added and then followed with addition of 40% Potassium hydroxide (0.2 ml amount). The mixture was shaken gently and allowed to remain undisturbed for 10 minutes. A positive VP test was represented by the development of a red colour (Koneman *et al*., 1992, Ochei and Kolhatkar 2003).

3.5.3.7 Citrate utilization test

Few colonies from pure culture medium were picked and inoculated as a single streak on slant surface of the citrate agar tube. The tube was incubated at 37 ^{o}C for 24 hrs. A positive test was represented by the development of a deep blue colour (Koneman, *et al*., 1992, Ochei and Kolhatkar 2003).

3.5.3.8 Urease production test

Few pure colonies of the test organisms previously recovered on primary isolation medium were picked and inoculated as a single streak on slant surface of the urea agar tube. The tube was incubated at 37 ^{o}C for 24 hrs. A positive test was represented by the development of a brick red colour throughout the medium (Koneman *et al*., 1992, Ochei and Kolhatkar 2003).

3.5.3.9 Orthonitrophenyl-β-D-Galactopyranoside (ONPG) test

Substrate solution of ONPG was prepared by dissolving ONPG tablet (Roche Diagnostics GmbH, Germany) in 1 ml amount sterile distilled water and mixed with a loopful of bacterial suspension in a clean test tube. The suspension was placed in a water bath at 37 ^{o}C for 10 minutes. The development of distinct yellow colour indicate positive test (Koneman, *et al*., 1992).

3.5.3.10 Gelatin hydrolysis test

Pure colonies of the test bacteria were picked with sterile straight wire and stabbed tube containing nutrient gelatin medium and incubated at 37 ^{o}C for 7 days. Tube along with uninoculated (control) medium were then refrigerated at 4 ^{o}C for 30 minutes and were observed for partial or total liquefaction (positive result) or complete solidification (negative result) (Koneman, *et al*., 1992, Cheesbrough, 2000, Ochei and Kolhatkar 2003).

3.5.3.11 Phenylalanine deamination test

Pure colonies of the test organism recovered from primary culture were inoculated into phynylalanine agar slant. After incubation at 37 ^{o}C for 24 hrs, 5 drops of ferric chloride reagent was added directly to the surface of the slant. Immediate appearance of intense green colour indicates the presence of phynyl-pyruvic acid and a positive test (Koneman, *et al*., 1992).

3.5.3.12 Decarboxylases test

From a pure colony of the test organism tubes of Moeller decarboxylase media containing the amino acid to be tested (lysine and ornithine) and a control devoid of amino acid were inoculated and incubated at 37 ^{o}C for 24 hrs. Change in colour of the tube containing the amino acid to a blue-purple colour indicates a posite test (Koneman, *et al*., 1992).

3.6 Antibiotic Susceptibility Test (Kirby-Bauer Disk Diffusion Method)

Antibiotics susceptibility test was carried out on all isolates using paper disc diffusion technique. A bacterial suspension corresponding to 0.5 McFarland turbidity standards was used as inoculum on Mueller Hinton agar. Antibiotic discs (Biotec Lab. Ltd. UK) used were; Amoxycillin-clavulanic acid (30 µg/disc), Amoxicillin (25 µg/disc), Erythromycin (15 µg/disc), Tetracycline (30 µg/disc), Cefuraxime (30 µg/disc), Gentamicin (10 µg/disc), Cotrimoxazole (25 µg/disc), Chloramphenicol (10 µg/disc), Ofloxacin (200 µg/disc), Ciprofloxacin (10 µg/disc), Streptomycin (15 µg/disc), Ceftriaxone (30 µg/disc). The plates with the antibiotic discs were incubated at 37 ^{o}C for 24hours (Harley, 2002). Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2012) guidelines. Multi resistance (non susceptibility to at least three families of antibiotics) and antibiotic resistance index was calculated against the tested bacteria (Hala *et al.,* 2007).

3.7 Bacteriological Examination of the Various Source of Water from the Study Areas.

3.7.1 Water sampling

Samples were collected in 1 liter capacity containers and labelled appropriately; samples from well water were collected by dragging clean thread tied to a sterile 250 ml capacity bottle. The bottles were filled with the water and capped carefully, labeled with sample code number and transported to the laboratory in ice cold box and analyzed within 6 hrs (Cheesbrough, 2000). A total of 90 well water samples, 54 borehole water, 60 satchet water (from vendors for each brand) and 6 pond water samples were collected from each sampling point.

3.7.2 Enumeration of total and faecal coliform from water samples (Membrane Filtration Technique)

The membrane filtration method was used to process all water samples. For each water sample, 100 ml was filtered in duplicate through 0.45 µm pore size nitrocellulose membranes. Briefly, 100 ml of the water samples were aseptically transferred into sterile filtration units fitted with sterile 0.45µm pore size nitrocellulose membrane filters. This unit was connected to a suction machine which enabled efficient and timely filtration process. After filtration used filters were aseptically transferred onto freshly prepared EMB ager plates and incubated at 37 ^{o}C (for total coliform count) and 44 ^{o}C (for faecal coliform count) for 24 hrs (Cheesbrough, 2000).

3.8 Detection of Enteropathogenic Bacteria Directly from Stool Sample by PCR.

3.8.1 DNA extraction (using feacal DNA MiniPrep kit)

The feacal DNA MiniPrep (Zymo research (ZR) California, USA) was used for DNA extraction and was performed in accordance with the manufacturer's instructions: A quantity (2 grams) of the stool sample was weighed into bashing bead lysis tube and mixed with 750 µl lysis solution (provided with the kit). The bead containing the solution was secured in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for 5 minutes. The ZR BashingBead™ Lysis Tube was centrifuged in a micro-centrifuge at 10000 x g for 1 minute. Exactly 400 µl of the supernatant was pipeted into a Zymo-Spin™ IV Spin Filter in a collection tube and centrifuged at 7,000 x g for 1 minute. This was followed by the addition of 1,200 µl of feacal DNA binding buffer into the filtrate in the collection tube. After this 800 µl of the mixture was transferred into a Zymo-Spin™ IIC column in a collection tube and centrifuge at 10000 x g for 1 minute. The flow through was discarded from the collection tube and the process was repeated to obtain the remaining products. Then 200 µl DNA pre-wash buffer was added into the Zymo-Spin™ IIC Column in a new collection tube and centrifuged at 10000 x g for 1 minute. This was followed by the addition of 500 µl feacal DNA Wash buffer into the Zymo-Spin™ IIC column and centrifuged at 10000 x g for 1 minute. The Zymo-Spin™ IIC column was transferred into a clean 1.5 ml micro-centrifuge tube and 100 µl of DNA Elution buffer was then added directly to the column matrix. This was centrifuged at 10000 x g for 30 seconds to elute the DNA. The Ultra-pure resulting filtrate (DNA) obtained was used as a template during the PCR assay (Islam and

Lindberg, 1992, Josiane *et al.,* 1997, Inglis and Kalischuk, 2003, Persson, and Olsen, 2005 and Weimer *et al.,* 2011).

3.8.2 Amplification of target DNA (utilizing Dual Priming Oligonucleotide [DPO])

Samples were simultaneously amplified in two separate reactions using Seeplex Diarrhoea-B1 and B2 ACE Detection kit and the GeneAmp PCR System 9700 thermocycler. Mastermix was prepared by mixing 4 µl of Primer mixture (DB1/DB2 PM), 3 µl of 8-methoxypsoralen (8-MOP) and 10 µl Multiplex Master Mix (contains DNA polymerase and buffer containing dNTPs; dATP, dCTP, dGTP and dTTP). The 17 µl reaction Mastermix was then dispensed into 0.2 ml PCR tubes and mixed with 3 µl of each sample's nucleic acid. Negative and positive controls were prepared by adding 3 µl of diarrhoea ACE negative control and ACE positive control respectively instead of sample's nucleic acid. The PCR thermocycling reactions start immediately using the following program; One cycle at 94 ^{o}C for 15 minutes followed by fourty cycle at 94 ^{o}C for 0.5 minute, 60 ^{o}C for 1.5 minute and 72 ^{o}C for 1.5 minute then one cycle at 72 ^{o}C for 10 minutes (Min-Chul *et al.,* 2010).

3.8.3 Detection of bacterial gene on Agarose Gel

The agarose gel used was prepared by dissolving 2 gm of molecular grade agarose (Denville Scientific, USA) in 100 ml of electrophoresis buffer. The mixture was then heated in a microwave oven (Sharp, Japan) until completely melted. Gels were stained by adding ethidium bromide at a concentration of 0.5 µg/ml to facilitate visualization. After cooling the solution to about 60^{o}C, it was poured into a casting tray containing a sample comb and allowed to solidify at ambient temperature. After the gel has solidified, the comb was removed, carefully not to rip the bottom of the wells. The gel, still in its plastic tray, was inserted horizontally into the electrophoresis chamber containing loading buffer. Of the amplified products 5 µl were transferred and allowed to separated in the 2% agarose gel electrophoresis in TAE buffer (40mM Tris-acetate, 2 mM EDTA [pH 8.3]). The lid was placed on the apparatus, and the current applied and confirm flowing by observing bubbles coming off the electrodes. According to the principle, DNA would migrate towards the positive electrode. The distance DNA has migrated in the gel was judged by visually monitoring migration of the Bromophenol blue tracking dyes. After the run at 70 Volt for 2.5 hours, stained gels were examined under ultra-violet (UV) transilluminator. A DNA ladder of 1 Kb (Fermenters USA) was used as a molecular weight marker. The electrophoretic profiles were observed visually to determine the presence or absence of amplification band in the sample lane and on the lane for standard molecular weight. The presence of amplification band corresponding to the expected band size in relation to the result of the negative control was considered as positive (Min-Chul *et al.,* 2010).

3.9 Data analysis

The data obtained were entered and analyzed using Analyse-it version 2.22 Excel 12+ statistical package. Data were summarized using frequency tables and bar charts. Risk factors for diarrhoea were analysed by contingency table and the strength of association was compared using Chi-square test at a 95% confidence level.

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence of bacterial diarrhoea among the study population.

The overall prevalence of bacterial pathogens recovered from diarrhoiec children in sokoto state is presented in Fig.4.1. Of the four hundred and twenty stool specimens processed one hundred and fourty were found with enteric bacteria accounting for percentage prevalence of 33.3%.

4.2 Prevalence of various bacterial agents of diarrhoea in Sokoto state.

The prevalence of various aetiologic agents of diarrhoea in the study population was presented in Table 4.1. Of the four hundred and twenty diarrhoeaic stools samples analyzed by the conventional culture techniques, *Campylobacter* species, *Escherichia coli*, *Salmonella enterica* and *Shigella* species were detected in 14 (3.3%), 79 (18.8%), 30 (7%) and 17 (4%) of diarrhoeal cases respectively thus indicating that, 140 (33.33%) of all childhood diarrhea in the study area are attributable to entero-pathogenic bacteria (namely *Escherichia coli, Campylobacter* species, *Salmonella enterica* and *Shigella* species).

4.3 Distribution of enteric pathogens from senatorial zones in Sokoto State by Gender.

The distribution of enteric pathogens from the three senatorial zones is presented in Table 4.2. Of the total number examine from all the three senatorial zones of the state, male diarrhoeic children were found to be more infected (31.0%) than the female (17.4%). The East senatorial zone was found to be less infected (43.6%) than children from the North (55.0%) and South (46.4%) senatorial zones. The Eastern senatorial zone had 49.3% and 35.4% infection prevalence for male and female respectively. The North senatorial zone had 71% infection prevalence for male against 39.4% recorded for female children. The Southern senatorial zone had 59.7% and 32.4% infection prevalence for male and female respectively.

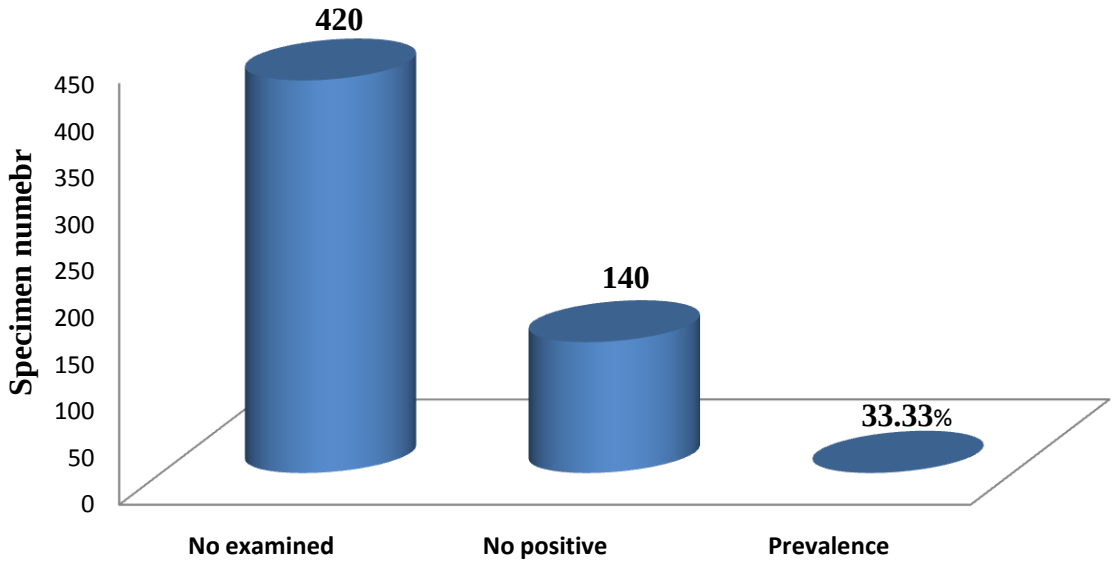


Figure 4.1 Prevalence of bacterial diarrhoea among the study population

No = number

Table 4.1: Prevalence of various aetiologic agents of diarrhoea in the study population (*n*= 420).

Organism	Number of isolate	% prevalence
Campylobacter species	14	3.3
Escherichia coli	79	18.8
Salmonella enterica	30	7.1
Shigella species.	17	4.1

Table 4.2: Distribution of enteric pathogens from senatorial zones in Sokoto State by gender.

Senatorial Zones	Sex	No. Examined	No. Pos. with orgs.	%prevalence
East (n=140)	Male	75	38	50.7
	Female	65	23	35.4
	Total	140	61	43.6 (χ^2=1.865, *P*=0.172)
North (n=140)	Male	69	39	71.0
	Female	71	38	39.4
	Total	140	77	55.0 (χ^2=6.344, *P*=0.012*)
South (n=140)	Male	72	43	59.7
	Female	68	22	32.4
	Total	140	65	46.4 (χ^2=5.641, *P*=0.018*)

χ^2 = chi square

* = significant at P ≤ 0.05

By conventional criteria the result obtained from the East geo-senatorial zone indicated that there was no statistical significant difference between the male and female infected diarrhoiec children within the zone. While from the North and South geo-political zones statistical analysis shows that there was significant difference between the number of infected male and female diarrhoiec children from the zones (P = ≤ 0.05). (ESZ; χ^2=1.865, *P*=0.172, NSZ; χ^2=6.344, *P*=0.012, SSZ; χ^2=5.641, *P*=0.018).

4.4 Distribution of diarrhoea among children in Sokoto State by age group

Of the total four hundred and twenty diarrhoeic children enrolled for this study, 88 were of age group 0-11 months (less than one year old), 78 were between 12 - 23 months (one year old). There were 83 recorded cases of age group 24 - 35 months (two years old). There were 61 cases of age group 36 - 47 months (three years old). Occurrences of diarrhoeal cases were recorded against the ages of four and five years old as 57 and 53 respectively (Table 4.3). The incidence of diarrhoea among children within 0 to 11 month and 4 to 5 years in the study area was found to be 21% and 12.6% respectively. It was observed that, the incidences of diarrhea among children of 0 to 5 years across the study area is higher (21- 19.8 %) among children within the 0 to 3 years age bracket compared with the 13 – 15% recorded for children of 4 -5 years age bracket. Also, a consistent trend of decreasing incidence with increasing age of the children was noted across the study area.

4.5 Occurrences of *Campylobacter* species among diarrhoeic children in Sokoto state.

The relative involvement of *Campylobacter* species by sex from the culture method is presented in Figure 4.2. Of the overall *Campylobacter* species (*n* = 14) isolated, 10 isolates (71.4%) were recovered from the male, 4 (28.6%) isolates were recovered from female who were less infected. It was also observed that from all the senatorial zones results indicated that diarrhoeagenic *Campylobacter* species affect more of the male than female.

Table 4.3: Distribution of diarrhoea among children in Sokoto State by age group.

Age group (months)	ESZ (n=140)	NSZ (n=140)	SSZ (n=140)	Total (%) (n=420)
0-11	20	30	38	88 (21)
12-23	26	25	27	78 (18.6)
24-35	29	28	26	83 (19.8)

36-47	22	20	19	61 (14.5)
48-59	22	19	16	57 (13.6)
≤60	21	18	14	53 (12.6)

ESZ = East senatorial zone

NSZ = North senatorial zone

SSZ = South senatorial zone

n = number of population

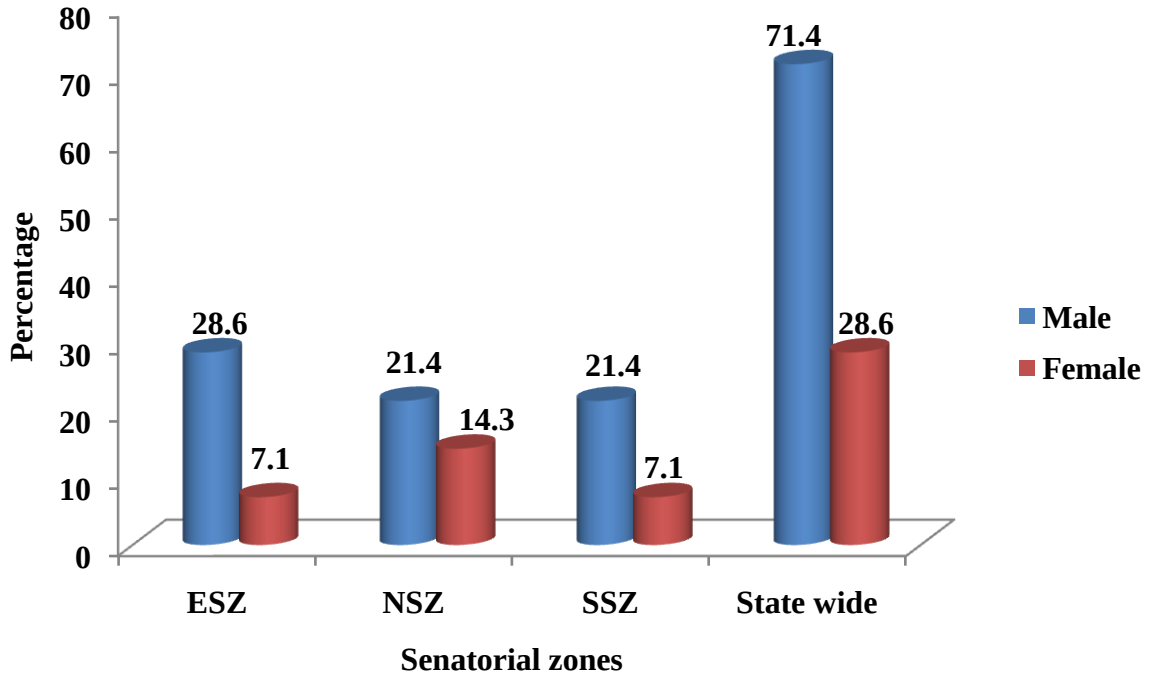


Figure 4.2: Occurrence of *Campylobacter* species among diarrhoeic children in Sokoto state according to sex.

ESZ = East senatorial zone

NSZ = North senatorial zone

SSZ = South senatorial zone

4.6 Occurrences of *Escherichia coli* among diarrhoeic children in Sokoto state.

Figure 4.3 shows the percentage involvement of *Escherichia coli* from culture and according to sex among the senatorial zones in the State. From the total stool specimen analysed by culture, 48(60.8%) and 31(39.2%) *Escherichia coli* isolates were recovered from male and female diarrhoeic children respectively. Furthermore the results shows that there were 11(14%), 18(22.8%) and 19(24.1%) associated *Escherichia coli* infection in the male stool samples compared to 7(8.8%), 15(19%) and 9(11.4%) in female from the South, East and North senatorial zones of the state respectively.

4.7 Occurrences of *Salmonella enterica* among diarrhoeic children in Sokoto state.

The relative occurrence of *Salmonella enterica* by gender across the three senatorial zones is presented in Figure 4.4. Of the total *Salmonella enterica* isolates (*n* = 30) recovered from the children diarrhoeic stool sample 19(63.3%) were recoverd from the male while 11(36.7%) were recovered from the female. It was also observed that male diarrhoeic children were more afflected by *Salmonella enterica* infection than the female from all the three senatorial zones of the state.

4.8 Occurrence of *Shigella* species among diarrhoeic children in Sokoto state.

Figure 4.5 shows the percentage involvement of *Shigella species* from the culture. There were high percentage occurrences among the male from the North (29.4%) and South (23.5%) than from the East (11.8%). The female were less infected from the North (11.8%) and South (6%) than from the East (17.6%).

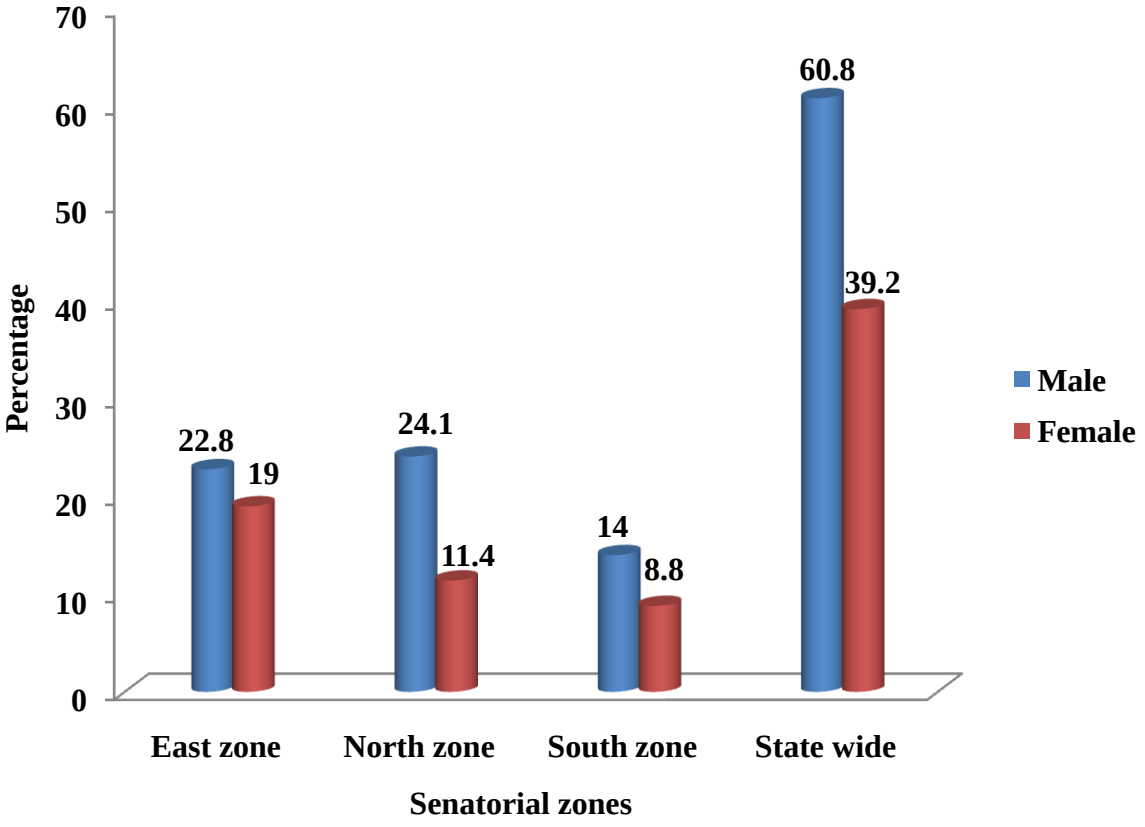


Figure 4.3: Occurrence of *Escherichia coli* among diarrhoeic children in Sokoto state according to sex.

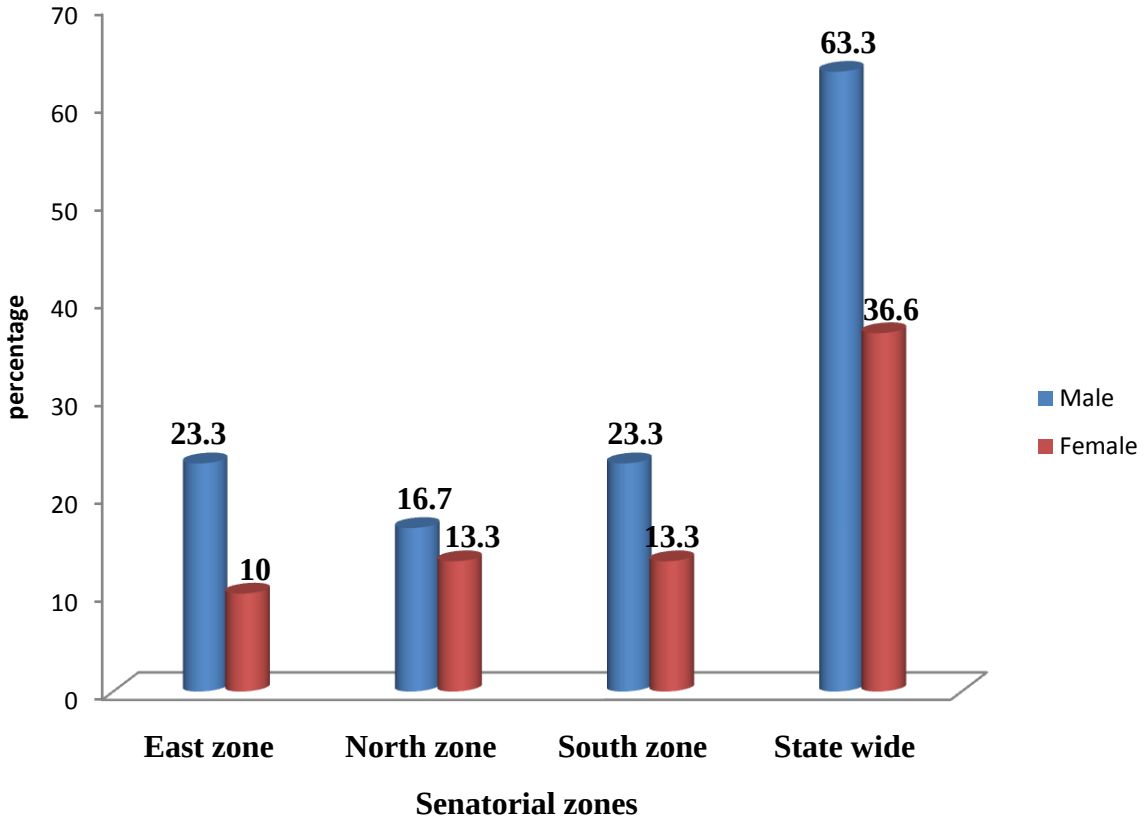


Figure 4.4: Occurrence of *Salmonella enterica* among diarrhoeic children in Sokoto state according to sex.

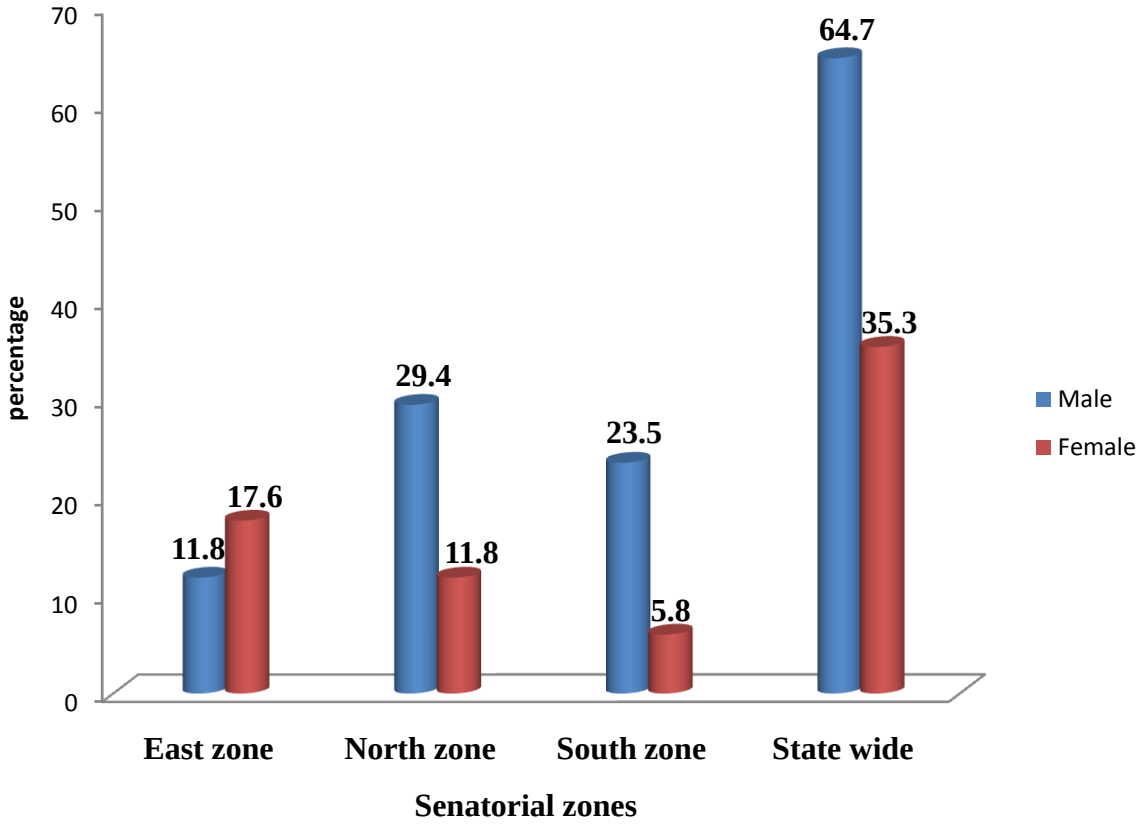


Figure 4.5: Occurrence of *Shigella* spp. among diarrhoeic children in Sokoto state according to sex.

4.9 Antibiotic susceptibility pattern of *Campylobacter* species.

The result of antimicrobial susceptibility testing of *Campylobacter* spp. against antibiotics is presented in Table 4.4. Of the fourteen *Campylobacter* spp. isolated 11 were sensitive to Ofloxacin, 9 were sensitive to Ciprofloxacin and Cefuroxime. However great resistance by the isolates against Amoxycillin-clavulanic acid and Cotrimoxazole was observed.

4.10 Antibiotic susceptibility pattern of *Escherichia coli.*

Table 4.5 represent the result of antimicrobial susceptibility testing on seventy nine *Escherichia coli* isolated from diarrhoeic children in Sokoto State. Of total *Escherichia coli* isolates tested, high sensitivity patterns were observed against Ofloxacin, Ciprofloxacin, Amoxycillin-clavulanic acid and Chloramphenicol. A total of 68 isolates were sensitive to quinolones group of antibiotics used (Ofloxacin and Ciprofloxacin), 50 were sensitive to Chloramphenicol while 48, 44 and 41 were found sensitive to Amoxycillin-clavulanic acid, Cotrimoxazole and Ceftriaxone respectively. The overall resistance of *Escherichia coli* was found to be high for Cloxacillin, Erythromycin and Streptomycin. But, relatively low resistance was observed against Cefuroxime and Cotrimoxazole.

4.11 Antibiotic susceptibility pattern of *Salmonella enterica.*

From the results obtained as shown in Table 4.6, *Salmonella enterica* were found to be sensitive to Ofloxacin, Ciprofloxacin, Ceftriaxone and Gentamicin. While Amoxycillin-clavulanic acid, Chloramphenicol, Tetracycline and Cefuroxime were less active. From the *Salmonella* isolates 27, 24, 22 and 17 were found sensitive to Ofloxacin, Ciprofloxacin, Ceftriaxone and Gentamicin respectively. In contrast, high rate of resistance was recorded against Cotrimoxazole, Erythromycin, Cloxacillin, and Streptomycin.

Table 4.4: Antibiotic responses of *Campylobacter* spp. (*n*=14), to tested antibiotic.

Antibiotic agent	Susceptibility		
	Sensitive	Intermediate	Resistance

Amoxycillin-clavulanic acid	3	0	11
Cefuroxime	9	2	3
Ceftriaxone	7	5	2
Ciprofloxacin	9	3	2
Chloramphenicol	8	3	3
Cloxacillin	7	1	6
Cotrimoxazole	4	0	10
Erythromycin	6	0	8
Gentamicin	6	1	7
Ofloxacin	11	1	2
Streptomycin	7	1	6
Tetracycline	7	0	7

Zone sizes are measured and interpreted using Clinical and Laboratory Standards Institute (CLSI, 2012) formally National Committee for Clinical Laboratory standards (NCCLS).

Table 4.5: Antibiotic responses of *Escherichia coli (n*= 79) to tested antibiotic.

Antibiotic agent	Susceptibility		
	Sensitive	Intermediate	Resistance
Amoxycillin-clavulanic acid	48	3	28
Cefuroxime	37	17	25
Ceftriaxone	41	33	5
Ciprofloxacin	68	10	1
Chloramphenicol	50	7	22
Cloxacillin	37	3	39
Cotrimoxazole	44	9	26
Erythromycin	38	2	39
Gentamicin	33	12	34
Ofloxacin	68	11	0
Streptomycin	39	3	37
Tetracycline	27	21	31

Zone sizes are measured and interpreted using Clinical and Laboratory Standards Institute (CLSI, 2012) formally National Committee for Clinical Laboratory standards (NCCLS).

Table 4.6: Antibiotic responses of *Salmonella enterica* (*n*= 30), to tested antibiotic.

Antibiotic agent	Susceptibility		
	Sensitive	Intermediate	Resistance
Amoxycillin-clavulanic acid	14	3	13

Cefuroxime	13	6	11
Ceftriaxone	22	3	5
Ciprofloxacin	24	5	1
Chloramphenicol	14	14	2
Cloxacillin	8	2	20
Cotrimoxazole	4	1	25
Erythromycin	6	4	20
Gentamicin	17	3	10
Ofloxacin	27	3	0
Streptomycin	9	2	19
Tetracycline	12	2	16

Zone sizes are measured and interpreted using Clinical and Laboratory Standards Institute (CLSI, 2012) formally National Committee for Clinical Laboratory standards (NCCLS).

4.12 Antibiotic susceptibility pattern of *Shigella* species.

As indicated in Table 4.7, sensitivity result of *Shigella* species against tested antibiotics shows high sensitivity pattern to Ofloxacin, Ciprofloxacin, Ceftriaxone and Cefuroxime. Of the 17 isolates, 15 were sensitive to Ofloxacin and Ceftriaxone, while 14, 11, and 9 were susceptible to Ciprofloxacin, Ceftriaxone and Gentamicin respectively. However 15, 12 and 8 isolates were found to be resistance against Cotrimoxazole, Cloxacillin and Amoxycillin-clavulanic acid respectively.

4.13 Multiple antibiotic resistance (MAR) indices of the isolates

The MAR Index analysis revealed that all the four isolates had a very high MAR index value (≥0.2%) (Fig. 4.6). The percentage frequency of MAR index value greater than 0.2% were recorded as 57.1% (8), 55.7% (44), 63.3% (19) and 58.8% (10) against *Campylobacter* species, *Escherichia coli*, *Salmonella* and *Shigella* species isolates respectively.

4.14: Assessment of risk factors associated with diarrhoea in children from Sokoto

The potential risk factors associated with diarrhoeal disease were registered; type of toilet, type of water supply or main source of drinking water. Records (Table 4.8) from the three senatorial zone of the state indicated that 208 (49.5%) and majority used open field to defecate out of which 66.5% were infected with bacterial pathogens. Of the 175 (41.6%) of cases that uses pit latrine system in their houses for defecation 66 (32.5%) were infected. Whereas the modern water closet system of toilet type was reported to be less commonly used as only 37(8.8%) claimed to use it as the only type of toilet in their houses. The assessment indicated that those that used open field to defecate were more infected (66.5%) than those that uses pit latrines (32.5%) in their household. Infection was found to be minimal among patient that claimed to use water system type of toilets. However the difference observed is considered to be statistically significant ($\chi^2 = 30.116$, $P = 0.0001$).

Table 4.7: Antibiotic responses of *Shigella* species (*n*= 17), to tested antibiotic.

Antibiotic agent	Susceptibility		
	Sensitive	Intermediate	Resistance
Amoxycillin-clavulanic acid	7	2	8
Cefuroxime	11	3	3
Ceftriaxone	15	1	1
Ciprofloxacin	14	3	0
Chloramphenicol	4	6	7
Cloxacillin	2	3	12

Cotrimoxazole	1	1	15
Erythromycin	6	2	9
Gentamicin	9	0	8
Ofloxacin	15	2	0
Streptomycin	5	0	12
Tetracycline	7	2	8

Zone sizes are measured and interpreted using Clinical and Laboratory Standards Institute (CLSI, 2012) formally National Committee for Clinical Laboratory standards (NCCLS).

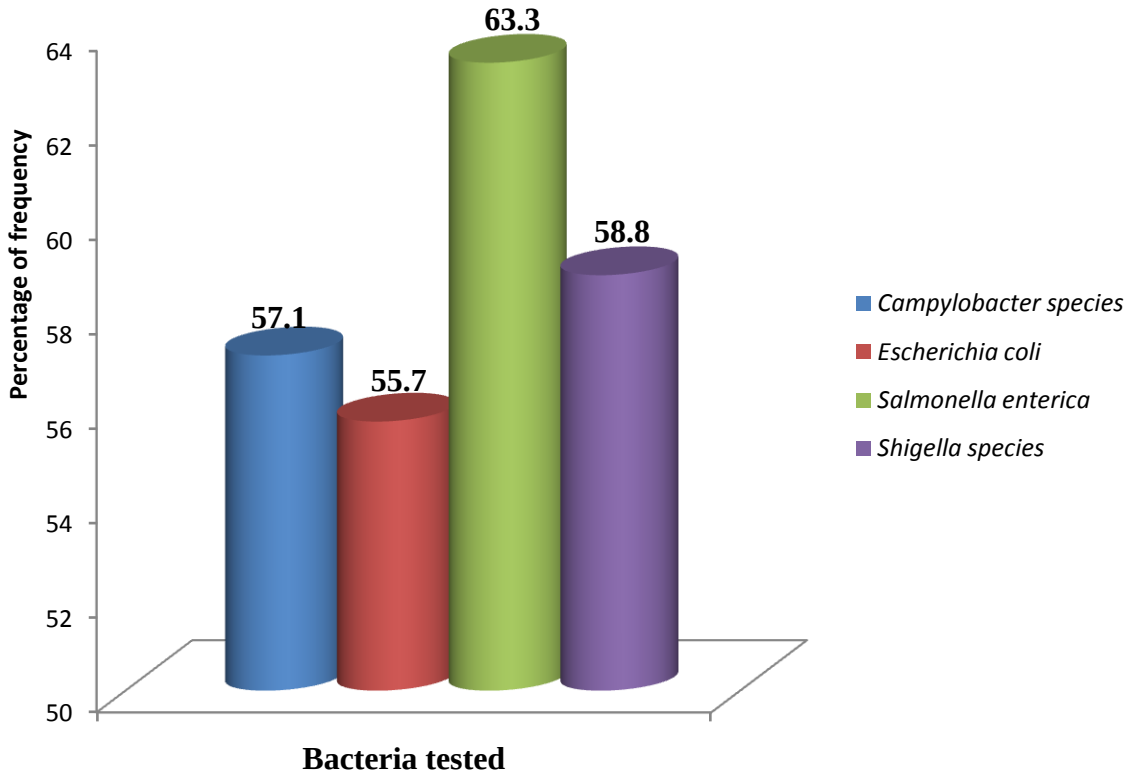


Figure 4.6: Percentage of frequency MAR index value ≥0.2 (%)

Table 4.8: Assessment of risk factors associated with diarrhoea in children from Sokoto state.

Factors	Number examined	Number infected	Percentage
Toilet types;			
Open field	208	135	66.5
Pit latrine	175	66	32.5
Water system	37	2	1.0 (χ^2=30.116, *P*=0.0001*)
Main source of drinking water;			
Pipe-borne	22	2	1.0
Private well	36	5	2.5
Public well	194	99	8.8
Pond	62	39	19.2
Borehole water	106	58	28.5 (χ^2=19.706, *P*=0.0006*)

χ^2 = chi square

* = significant at P ≤ 0.05

The assessment of the main source of drinking water as reported indicated that of the total population 194 (46.2%) that used public well as the only source of water for both drinking and domestic uses 99 (48.8%) were infected. Records, also showed that 106 (25.2%) were those that uses borehole water and 58 (28.5%) among them were infected, 62 (14.8%) used water from the ponds of which 19.2% were infected, whereas 36 (8.6%) were reported as those that uses water from their private well at the houses with 2.5% infection. None was reported to use water from river or streams for drinking. The frequency of those that treat their water before drinking either by boiling filtering or other means was found to be very low and just reported as 2%. The variation observed was considered to be statistically significant (χ^2=19.706, *P*=0.0006).

4.15: Enumeration of coliform organism from water samples.

Table 4.9 and 4.10 shows the mean total and faecal coliform counts from the well, borehole, package/satchet and river water used in the state and according to senatorial zones. Means values of total coliform count varied from 118 to 807 Cfu/100 ml while mean values of faecal coliform counts ranged from 43 to 331 Cfu/100 ml for all the sampled sites. The total and faecal coliform counts exceeding acceptable limits are indicative of pollution of the primary water source in the area.

4.16: Relationship between faecal coliform density and bacterial diarrhoeal disease occurrence

The Pearson correlation coefficient analysis conducted on various mean concentrations of faecal coliform density of water samples from the state to determine the relationship between bacterial diarrhoeal disease occurrence and quality of water source was represented in Table 4.11. The result of the analysis shows a statistical significant different (r = 0.9526571, P=0.013).

Table 4.9: Mean Values of Total Coliform Count from Different Water Sources According To Senatorial Zones in Sokoto State.

Sources of Drinking Water	No. of sample	Senatorial Zones East (Cfu/100 ml)	North (Cfu/100 ml)	South (Cfu/100 ml)	Accepted level (WHO, 2003, SON, 2007)
Well	*n*=90	$5.14\times10^2 \pm 1.93\times10^2$	$7.27\times10^2 \pm 2.24\times10^2$	$6.17\times10^2 \pm 2.16\times10^2$	≤ 10 Cfu/100 ml

Borehole	*n*=56	$1.38\times10^{2}\pm5.58\times10^{1}$	$1.98\times10^{2}\pm5.47\times10^{1}$	$1.18\times10^{2}\pm4.82\times10^{1}$	≤ 10 Cfu/100 ml
Satchet	*n*=60	$3.42\times10^{2}\pm1.06\times10^{2}$	$4.37\times10^{2}\pm1.44\times10^{2}$	$3.32\times10^{2}\pm1.12\times10^{2}$	≤ 2 Cfu/100 ml
River	*n*=6	$6.08\times10^{2}\pm8.84\times10^{1}$	$8.07\times10^{2}\pm1.94\times10^{2}$	$7.07\times10^{2}\pm5.33\times10^{2}$	≤ 10 Cfu/100 ml

Values are represented as means ± SD (Standard deviation)

Cfu = Colony forming unit

Table 4.10: Means Values of Faecal Coliform Count from Different Water Sources According to Senatorial Zones in Sokoto State.

Sources of Drinking Water	No. of sample	Senatorial Zones East (Cfu/100 ml)	North (Cfu/100 ml)	South (Cfu/100 ml)	Accepted level (WHO, 2003, SON, 2007)
Well	*n*=90	$1.03\times10^{2}\pm4.30\times10^{1}$	$1.79\times10^{2}\pm3.19\times10^{1}$	$1.49\times10^{2}\pm5.06\times10^{1}$	≤ 2 Cfu/100 ml
Borehole	*n*=56	$1.12\times10^{1}\pm6.68\times10^{0}$	$2.07\times10^{1}\pm5.30\times10^{0}$	$1.62\times10^{1}\pm5.68\times10^{0}$	≤ 2 Cfu/100 ml
Satchet	*n*=60	$9.08\times10^{1}\pm5.5\times10^{1}$	$11.09\times10^{1}\pm5.13\times10^{1}$	$4.3\times10^{1}\pm2.06\times10^{1}$	0 Cfu/100 ml
River	*n*=6	$2.53\times10^{2}\pm6.36\times10^{1}$	$2.82\times10^{2}\pm 7.34\times10^{1}$	$3.31\times10^{2}\pm 4.57\times10^{1}$	≤ 2 Cfu/100 ml

Values are represented as means ± S.D (Standard deviation)

Cfu = colony forming unit

Table 4.11: Pearson Correlation Coefficient Analysis Between Fecal Coliform Density And Bacterial Diarrhoeal Cases Among Children In Sokoto

Senatorial zones	Mean density (mean feacal coliform count)	Infection rate	
ESZ	140	43.6	
NSZ	194.8	55.0	
SSZ	171.3	46.4	
State wide	169.2	33.3	(r =0.9526571, P=0.013*)

ESZ = East senatorial zone

NSZ = North senatorial zone

SSZ = South senatorial zone

γ = Correlation Coefficient

*= P ≤0.05

4.17 Prevalence of enteropathogenic bacteria detected by their target genes and product size of PCR.

The occurrence of enteric bacteria in the stool samples examined with the PCR using the Seeplex Diarrhoea-B1 and B2 ACE was represented in Table 4.12. Out of 420 stool samples collected from diarrhoeic children a total of 177 (42.1%) bacterial pathogens were identified. *Campylobacter* spp. (*Campylobacter jejuni*, with target gene *hip* and *coli* with target gene *asp*) were identified in 34 (8.1%) specimen. A total of 87 specimens (20.7%) were identified with *Escherichia coli. Escherichia coli* were identified as verocytotoxin *E. coli, VTEC* (*VT1, VT2)*, 48 (11.4%), *E. coli* H7 (*fliC*), 2 (0.5%) and *E. coli* O157, 37 (8.8%). *Salmonella enterica, sop*B genes were identified in 35 (8.3%) specimen. *Shigella* spp. (*Shigella flexnerii and Shigella dysenteriae, vif* and *ipa*H genes) were identified in 22 (4.8%) stool specimen.

4.18 PCR results

The Agarose gel electrophoresis results of enteric bacterial DNA detected using Seeplex Diarrhoea-B2 ACE. Lane M shows bands for 100 base pair molecular weight ladder is presented in Plate I. Lanes 56, 57, 58, 61, 62 and 63 shows positive amplification band of 291 bp corresponding to the expected band size for VTEC species tested. Lane numbers 59 also shows positive band, at 476 bp corresponding to *Escherichia* O157 tested. Lane numbers 60 shows no positive bands, lane -ve is negative control.

Plate II shows PCR results of enteric bacterial DNA detected using Seeplex Diarrhoea-B2 ACE.Lane M shows bands for 100 base pair molecular weight ladder. Lanes 64, 65, 66, 67, 69, 70 and 71 shows positive amplification band of 476 bp corresponding to the expected band size for *E. coli* O157 species tested. Lane 65 and 67 also shows positive amplification band of 291 bp corresponding to the expected band size for VTEC species tested. Lane numbers 68 shows no positive bands, lane -ve is negative control.

Table 4.12: Prevalence of enteropathogenic bacteria detected by their target genes and product size of PCR.

Bacteria	Target gene	Product size(*bp*)	Number detected	Percentage
Campylobacter spp.		227	34	8.1
Campylobacter jejuni	*hip*			
Campylobacter coli	*asp*			
Escherichia coli				

Verocytotoxin *E. coli* (VTEC)	*VT1,VT2*	291	48	11.4
Escherichia H7	*fliC*	370	2	0.5
Escherichia O157	*rfb*	476	37	8.8
Shigella spp. *(flexnerii and dysenteriae)*	*vif, ipa*H	330	22	4.8
Salmonella enterica	*sop*B	395	35	8.3
Total			177	42.1

hip- hippuricase gene

asp- aspartokinase gene

VT-verocytotoxin gene

fliC- chromosomal flagella

rfb- somatic antigen expression gene

*ipa*H- invasion plasmid antigen H

*sop*B- superoxide dismutase gene

hly- hemolysin gene

ivn- inversion gene

bp- base pair

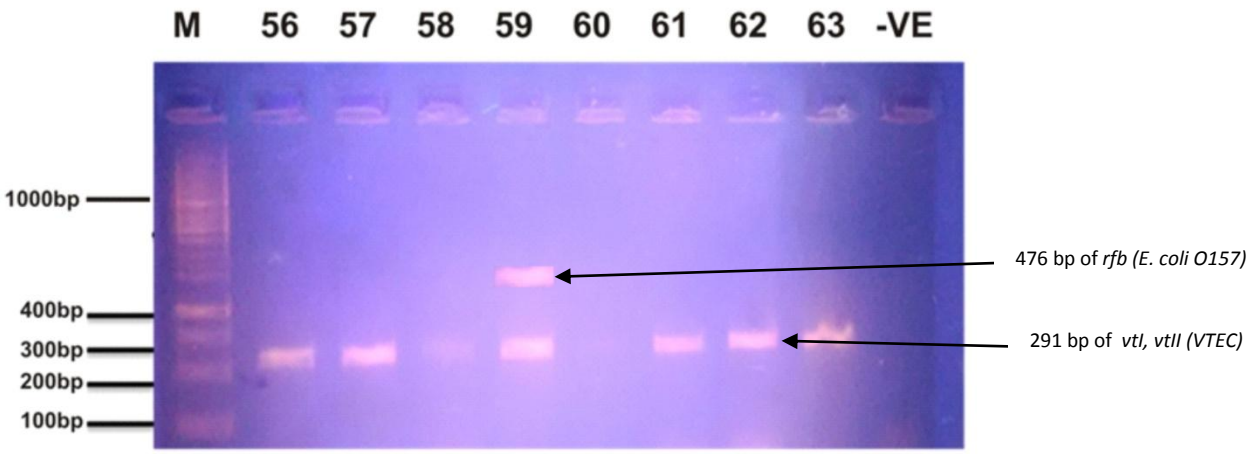


Plate I: PCR results of enteric bacterial DNA (*E. coli* 0157 and VTEC) detected following Agarose gel electrophoresis using Seeplex Diarrhoea-B2 ACE.

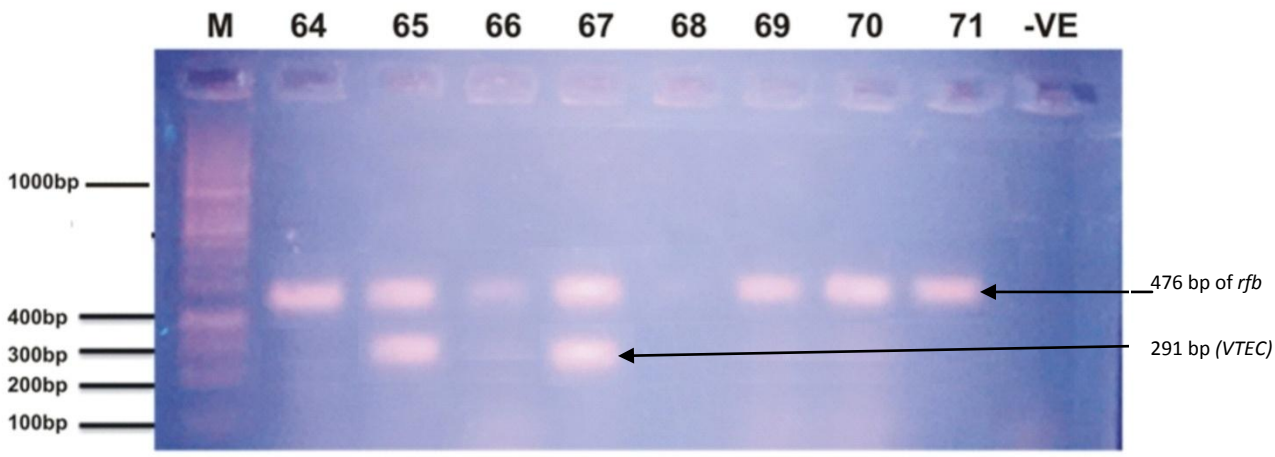


Plate II: PCR results of enteric bacterial DNA (*E. coli* 0157) detected following Agarose gel electrophoresis using Seeplex Diarrhoea-B2 ACE.

Plate III is the PCR results of enteric bacterial DNA detected using Seeplex Diarrhoea-B1 ACE. Lane M shows bands for 100 base pair molecular weight ladder. Lanes 13, 21 and 22 shows positive amplification band of 395 bp corresponding to the expected band size for *Salmonella enterica* tested. Lane numbers 19, 20, and 21 shows positive bands of 227 corresponding to the expected band size for *Campylobacter* species tested. Lane numbers 14, 15, 16, 17 and 18 shows no positive bands.

PCR results of enteric bacterial DNA detected using Seeplex Diarrhoea-B1 ACE is shown in Plate IV. Lane M shows bands for 100 base pair molecular weight ladder. Lanes 95 and 96 shows positive amplification band of 395 bp corresponding to the expected band size for *Shigella* species tested. Lane numbers 94, 97, 98 and 99 shows no positive bands, lane -ve is negative control.

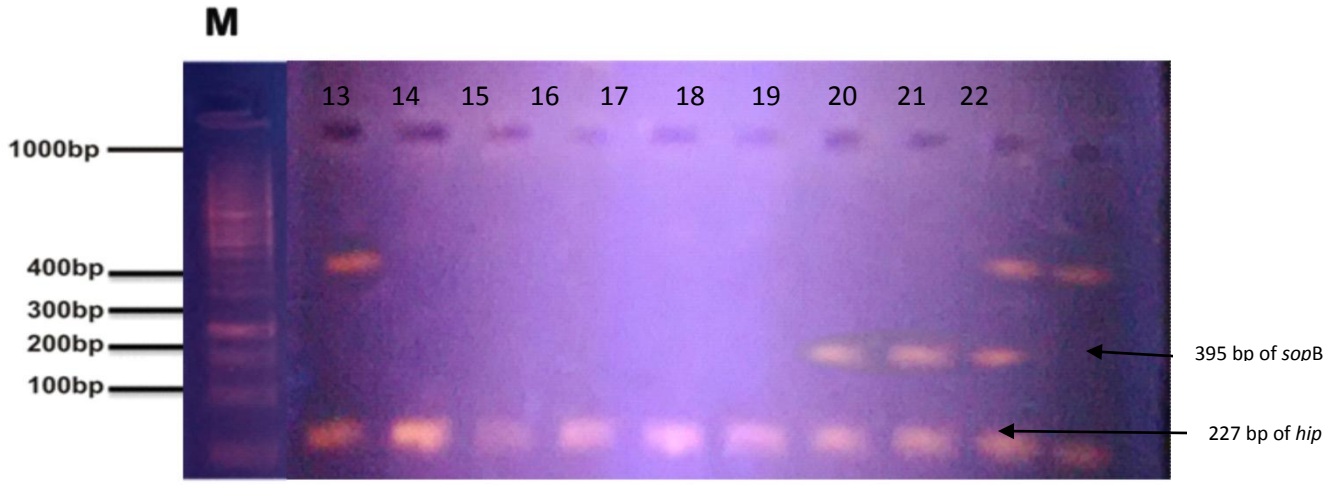


Plate III: PCR results of enteric bacterial DNA (*Salmonella enterica* and *Campylobacter jejuni*) detected following Agarose gel electrophoresis using Seeplex Diarrhoea-B1 ACE.

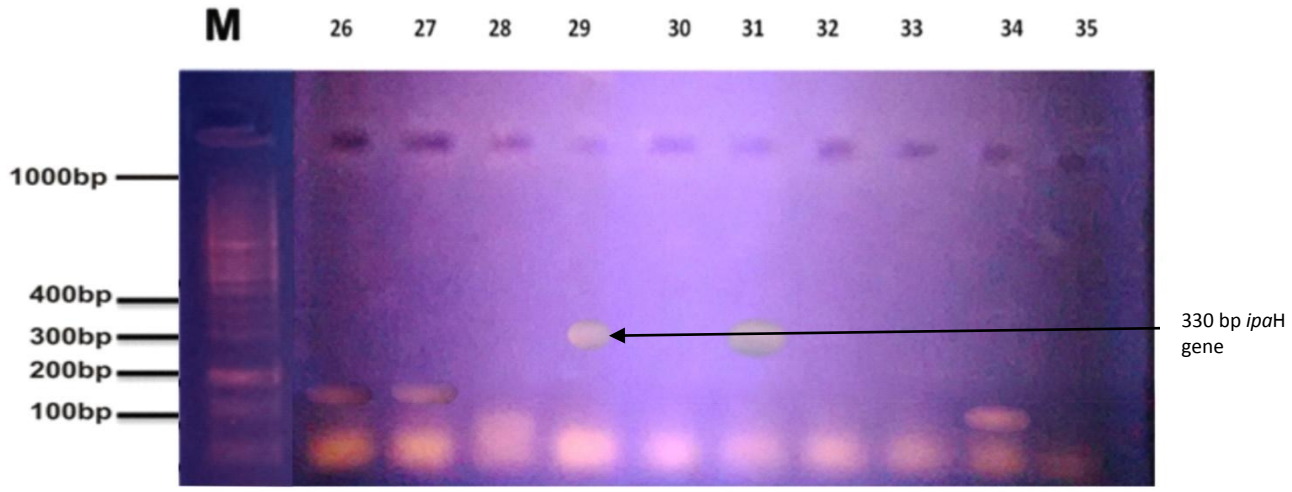


Plate IV: PCR results of enteric bacterial DNA (*Shigella* species) detected following Agarose gel electrophoresis using Seeplex Diarrhoea-B1 ACE.

CHAPTER FIVE

5.0 DISCUSSION

This study determined the prevalence (33.3%) and the association of the bacterial strains namely *Campylobacter jejuni, Salmonella, Shigella* and *Escherichia coli* in faecal samples of diarrhoeic children zero to five years old as predominant bacterial entropathogen causing diarrhoea in the study area. Observation from the present study was that infection with bacterial pathogens was very common. Among enteric pathogens, *viral organisms* were not studied in the present study. However, a previous study within the region found that these pathogens are extremely rare in this population were it found evidence of enteric rotaviruses infection in up to 9% of children with acute diarrhea (Aminu *et al*., 2007 and Aminu *et al*., 2008). The yield from examination of stools by microscopic techniques for ova, cyst or adult parasite was very low. This low yield had been reported also by studies in Nigeria (Oyerinde *et al*., 1977).

The overall prevalence of enteric pathogens by conventional culture method in this study (33.3%) is comparable with other previous studies conducted in Abuja 29.4% (Ifeanyi *et al*., 2010) and Kano 27.9% (Abdullahi *et al*., 2010). However, the prevalence of the bacterial pathogens is found to be lower when compared with previous study conducted also in Lagos, Nigeria (59.1%) (Ogusanya *et al*., 1994) and this could be due to the densely populated nature of the area with limited community hygiene. From the results obtained from this study *Escherichia coli* were found in 79 (18.8%), *Salmonella* 30 (7.1%), *Shigella* species 17 (4.1%) and *Campylobacter* species in 14 (3.3%) of the 420 diarrhoiec stool sample of the children under five years old with diarrhoea in the study areas by conventional culture.

This is in line with several other related studies according to the WHO report of 2009 and those conducted in Nigeria and most part of Africa. Comparable bacterial rates of isolates were reported from studies conducted in Kogi State, Nigeria (*Escherichia coli* 46.9%, *Salmonella* species 28.1%, *Shigella* species 21.9%, and *Staphylococcus aureus* 3.1%) (Okolo *et al*., 2013), Kano (*Escherichia coli* 21%, *Salmonella* species 33.6 %, and *Shigella* species 30%) (Abdullahi *et al*., 2010). From Ethiopia records shows that frequencies of isolated bacteria among under five children with diarrhoea were *Campylobacter* species, 20 (12.7%), *Shigella* species, 11 (7.0%), and *Salmonella* species, 4 (2.5%) (Mulatu *et al*., 2014).

The percentage prevalence of *Campylobacter* species from the culture method as revealed in this study is in agreement with global reports of increasing number of cases of campylobateriosis often exceeding those of salmonellosis and shigellosis (Coker and Dosunmu, 1984). From Ile-Ife, Nigeria 19% were reported (Aboderin *et al*., 2002), from Thailand 18.8% (Tailor *et al*., 1993) and Bangladish 38.8% (Haq and Rahman 1991). *Campylobacter jejuni*/*coli* are well established causative agents of diarrhoea in Nigeria. Gastroenteritis due to *C. jejuni* was first reported in the Northern part of the country in 1981 (Low *et al*., 1981) and the South-Western part in Ile-Ife in 1983 (Olusanya *et al*., 1983). In 1984, the most comprehensive report on *C. jejuni* as an agent of diarrhoea particularly in children was given by Coker and Dosunmu. Since then, the isolation rate of *Campylobacter* spp. from cases of diarrhoea in the country has increased and has led to a series of careful biological studies on the local strains (Aboderin, *et al*., 2002).

The prevalence of *Escherichia coli* from culture in this study was 18.8%. This prevalence was however not markedly different from the study conducted in Kano (Abdullahi *et al*., 2010), Calabar (Oko *et al*., 2014), and India (Ajjampur *et al*., 2008), in which the prevalences were 18.18%, 15% and 15.8% respectively. But, it is lower than the rate reported in studies carried out at Zaria (Sani *et al*., 2015), Abuja (Ifeanyi *et al*., 2010) and Anyigba (Okolo *et al*., 2013) where the prevalence rates were 45%, 62.8% and 46.9% respectively. *Escherichia coli* was the most frequently identified potential pathogen in this study and many studies have also documented *Escherichia coli* as common bacterial pathogens in childhood diarrhoea (Okeke *et al*., 2000, Black *et al*., 2003 and WHO 2010). Diarrhoegenic *Escherichia coli* (DEC) remains an important aetiological agent of infantile diarrhoea in Nigeria and from Sokoto as indicated by this study. A high prevalence of DEC among Nigerian children with diarrhoea was previously described (Nweze, 2009) where the majority of isolates in that study were classified as atypical, and most of them (35/43 isolates) did not belong to EPEC serogroups as previously defined (WHO, 2009). In Nigeria, the epidemiology of DEC and the virulence of the various *E. coli* pathotypes have not been well studied because most of the currently published data were from the southwestern axis and the southeast leaving the north unstudied. This study has thereby provided the current situation of DEC in Sokoto in Northwest of Nigeria.

In this study *Samonella* spp. was the second dominant bacterial aetiology in under-five children with diarrhoea from Sokoto state. The overall prevalence of *Salmonella enterica* (7.1%.) in this study by culture was lower than the findings of other similar studies conducted in Kano (13.5%) (Abdullahi 2010) and Kogi (28.1%) (Okolo *et al*., 2014). But it is comparable with studies carried out in other countries

such as Mozambique 7.5 % (Ina´ Cio *et al*., 2007), Tanzania 6.4% (Gascon and Schellenberg, 2000) and Botswana 6.3% (Urio *et al*., 2001). The prevalence of *Salmonella* from this study had further reveled concern on the importance of this pathogen in the epidemiology of bacterial childhood diarrhoea in developing countries and Nigeria in particular.

The prevalence in our study of *Shigella* species (4%) is similar to those found in other African countries, where prevalence ranged from 5% (the lowest) in Northwestern Ethiopia (Aseffa *et al*.,1997), to 14% in Tanzania (Temu *et al*.,2006) and 16% (the highest) in Kenya (Brooks *et al*., 2006) . But, the percentage prevalence reported in this study was much lower than the rate reported in studies carried out in Iran (20.1%) (Herwana *et al*., 2010) and Indonesia (34.5%) (Mashouf *et al*., 2006), respectively. The various variations may be due to the nature of different water supply at various communities. Coupled with the fact that *Shigella*, accounts for less disease prevalence in this study, but it is also worthy to note that it was reported to be highly infectious (WHO, 2010) and its detection in any stool samples is important to prevent further disease from spreading through person to person.

The occurrence of diarrhoeal cases in the three senatorial zones of Sokoto state as recorded was found to be significantly higher in less than three years old children, this is in consistence with several other study on diarrhoeic children both from develop and developing countries (Yongsi 2008, Abdullahi 2010, Okolo *et al*., 2013). The high incidence of diarrhoea in age group zero to eleven mouths (88/420) though essentially under their mothers care may be exposure to predisposing factors that enhance the risk of diarrhoea in children which include failure to breast feed exclusively for the first four to six months of life as recommended by WHO (Abdullahi 2010). It was also reported that most diarrhoea episodes occur during the first 1-2 years of life due to combined effects of declining levels of maternal acquired antibodies, lack of active immunity in the infant, the introduction of food that may be contaminated with faecal pathogens and direct contact with human or animal faeces when the infant start to grow (Patwari *et al*., 1993). In this study, the diarrhoeal cases recruited were the males (51.4%) and females (48.6%) and by statistical analysis indications shows that there was no statistical significant difference between the sexes and diarrhoeic condition ($P \geq 0.05$). Previous study from Vietnam (Nguyen *et al*., 2005) also reported similar diarrhoeic distribution among gender with no significant difference between male (52.2%) and female (46.8%).

Antibiotic resistance has emerged as a major challenge in the treatment of enteric bacterial pathogens as multi-drug-resistant bacteria are being increasingly reported. High rates of resistance to commonly used, inexpensive, oral antimicrobial agents such as ampicillin, tetracyclines, and trimethoprim-sulfamethaxosole were observed in isolates of enteric pathogens such as adherent *E. coli*, ETEC, EPEC, and *Shigella* spp. in a survey in Somalia (Casalino *et al*., 1988). Similarly, high overall rates of resistance, often to multiple drugs, were seen in isolates from children with acute diarrhea in Nigeria (Lamikanra *et al*.,1989), unfortunately, detailed resistance patterns of each pathogen were not reported. In this study *Campylobacter* spp. isolates revealed reasonably high rate of resistance to a number of commonly used antibiotics in Nigeria such as Amoxycillin-clavulanic acid (80%), Erythromycin (60.9%), Ampicillin (63.6%), trimethoprim-sulfamethaxosole (71.5%) and Tetracycline (50.5%). High levels of resistance to commonly used antibiotics have also been observed in *Campylobacter* spp. in

Hawassa, Southern Ethiopia (Malatu *et al*., 2014). There appears to be considerable regional variation in the sensitivity patterns of C. *jejuni* to erythromycin, with resistance rates of 48.2% and 0% in Nigeria (Coker, *et al*., 1989) and Djibouti (Mikhail, *et al*., 1990), respectively. Earlier data from Nigeria showed a much lower rate of erythromycin resistance of 18% (Coker *et al*., 1989). The increased prevalence of strains exhibiting resistance to commonly available, less costly oral antibiotics represents a growing obstacle to the simple, cost-effective therapy of invasive childhood diarrhoeas in Sub-Saharan Africa. The alarming increase in rates of resistance of many diarrheal pathogens may relate to the frequent use and abuse of antibiotics.

The overall resistance of *Escherichia coli* was found to be high for Cloxacillin, Erythromycin and Streptomycin. But, relatively low rate resistance was observed against Cefuroxime and Cotrimoxazole. The antibiotic resistance of *E. coli* to cloxacillin (50%), erythromycin (50%), Streptomycin (47%), and Gentamicin (43%) recorded in this study are similar to those obtained by Okeke *et al*., (2000) and Okoli *et al*., (2002). Recent data from Nigeria, Gabon and Tanzania also suggest similar resistance among causative organisms of diarrhoel infections, such as enterotoxigenic, enteropathogenic, and enteroaggregative *Escherichia coli*, as high and appears to be rising. Also notable drug-resistant enteropathogenic *E*. *coli* outbreaks and sporadic cases have been reported from several African countries, including Kenya and Tanzania (Senerwa *et al*., 1989 and Vila *et al*., 1999).

All the *Salmonella* isolates in this study were susceptible to ofloxacin (100%), ciprofloxacin (97%), chloramphenicol (93.3%), and ceftriaxone (83.3%). But lower rate was observed against Cotrimoxazole (16.7%) and erythromycin (33.3%). The development of multiple resistance of *Salmonella* species to commonly used antibiotics was reported by other investigators in different times. In Harar, eastern Ethiopia, a high rate of resistance of *Salmonella* to amoxicillin (100%), ampicillin (100%) and tetracycline (71.4%) was reported by Ayalu *et al*. (2011). A study in Jos, Nigeria, reported all *Salmonella* isolates as susceptible to ciprofloxacin and ceftriaxone (MIC≤ 1 µg/ml). However, varying degrees of susceptibilities were also recorded for the other antimicrobial agents: 55% were susceptible to Amoxicillin-clavulanic acid, 37% to Chloramphenicol, 25.8% to Amoxicillin and 14.6% to cotrimoxazole (Solomon *et al*., 2014).

In the current study, all *Shigella* isolates showed susceptibility to Ciprofloxacin (100%), Ofloxacin (100%) and Ceftriaxone (94.2%). This is similar to reports from other parts of the country (Okeke *et al*., 2000, Abdu *et al*., 2014 and Oko *et al*., 2014). In a study carried out to determine the prevalence of antimicrobial resistance among different serogroups of Shigellae isolated from children up to sixty months of age with acute diarrhoea in Ile-Ife, Nigeria records shows that more than 50% exhibited resistance to Trimethoprim (80.4%), Nalidixic acid (64.7%), Cephalothin (64.7%), Chloramphenicol (59.8%), Sulphonamide (56.9%), and Cefotaxime (52.0%). However, only five (4.9%) of the isolates were found to be resistant to gentamicin, tobramycin and ciprofloxacin (Abdu *et al*., 2014). Susceptibility to Ciprofloxacin, Nalidixic acid, and Cotrimoxazole was also reported in Hawassa, south Ethiopia (Mulatu *et al*., 2014). The development of high resistance among *Shigella* species against commonly used antibiotics has also been reported in Nigeria (Meremikwu *et al*., 1997, Iwalokun *et al*., 2001, Egah *et al*.,

2003 and Enabulele *et al*., 2006) and from other low-income countries such as Tanzania (Navia *et al*., 2005) and Ethiopia (Belay *et al*., 2000 and Mulatu *et al*., 2014).

The multiple antibiotic resistant (MAR) index analysis showed that all the isolates had great percentage of bacteria recovered had MAR index value >0.2%. The percentage frequency of MAR index value greater than 0.2% were recorded as 57.1% (8), 55.7% (44), 63.3% (19) and 58.8% (10) for *Campylobacter* species, *Escherichia coli*, *Salmonella* and *Shigella* species isolates respectively. The high frequency of multiple antibiotic resistant isolates observed in this study most probably reflect the ease of access and the extensive use of these antibiotics in this environment and probably across the entire country. The reason behind this may be due to excessive use of antibiotics in treatment of infectious bacterial diseases as well as due to environmental cross contamination through other risk factors such as contact with reservoirs like domestic animals and their faeces.

Studies have shown that lack of latrine, inadequate disposal of the faeces and household refuse, visible faeces on latrines floor and sharing latrines were strongly associated with increasing incidence of diarrhoeal diseases (Jinadu *et al*., 1991, Oni *et al*., 1991 and Ekanem *et al*., 1994). The fecal-oral transmission route for diarrhoeal diseases is enhanced by the contamination or pollution of drinking water sources like the unprotected wells, rivers, ponds by faecal materials indiscrimately disposed into the environment. The unhygienic practice of defecating in the bush may be a major source of ground water contamination. These fecal matters pollute the unprotected water sources after being transported down slopes by run-off or overland flow. The run-off is largely experienced in the wet seasons within the tropics; hence high level of pollution should occur in the wet season in such regions (Adefunke *et al*., 1998). When the association between the various potential risk factors, personal hygiene practices and the incidence of diarrhoea were considered, it was observed that; there was strong association ($P<0.05$). The risk factors so far identified in this study, however, are just traits of a great context dwindling in the State: poverty, lack of safe water, improper sewage disposal and poor hygienic practices (NDHS, 2008). The bacteriological analysis to determine whether faecal contamination of domestic used water is present was justified by this study. The result signifies gross faecal contaminations of drinking water sources and were found to be at high risk and unacceptable for consumption or grossly polluted according to World health organization guidelines for drinking-water quality (WHO, 2003). In this region, most of the rural households rely mainly on treated packaged (sachet), well and borehole water sources for their daily water needs and these water sources are usually faecally contaminated and devoid of adequate treatment. A similar previous study carried out by Obi *et al.* (2002) in Nigeria also reported that several water sources harboured diarrhoeagenic pathogens and unsafe for consumption, microbiologically unacceptable and likely to be potential sources of transmission of water-borne diseases to humans. Hence the general public is exposed to high risk of pathogenic bacteria which are important agents of water borne diseases including diarrhoea. There was high bacterial contamination of the water samples exceeding those recommended by WHO (1996 and 2003). Similar poor microbiological quality of drinking water samples associated with diarrhoea had also been reported in Nigeria and many other countries (Flora and Michael, 1996, Olabisi *et al*., 2008 and Folarin *et al*., 2013).

The molecular diagnostic assay using the Seeplex ACE bacterial diarrhoea detection kit directly on stool specimens to screen for several common bacterial enteropathogens has been validated in this study. The PCR based analysis of stool specimen detected (8.1%) *hip* and *asp* genes of *Campylobacter jejuni and Campylobacter coli* respectively, (20.7%) *vt1*, *vt2*, *fliC*, and *rfb* genes of *E*. *coli*, (2.6%) *vif, ipa*H genes of *Salmonella* and (8.3%) *sop*B gene of *Shigella.* This indicated that the PCR assay detected an additional 8.8% of infections for *Campylobacter*, *E*. *coli, Salmonella*, and *Shigella*, in the clinical specimens beyond what was ascertained by conventional culture means. The results obtained, which revealed PCR-positive but culture-negative results may be the results of a non-culturable state of some organisms in selective media, delicate nature of some bacteria and damaged bacterial cells during sampling processes (Pawlowski *et al.,* 2009).

The striking feature of this study is the low sensitivity of culture (41.2%), in detecting *Campylobacter* spp. from stool specimen, in contrast to molecular tests (95.1%). This was also similar to other related study that reported a significantly increased in detection rate with a PCR sensitivtity of 96.6% (Min-chul *et al*., 2010) and 78.6% (Richard *et al*., 2010). *Escherichia coli* 0157 and verocytotoxigenic *Escherichia coli* (VTEC) which share clinical and epidemiological features were the most prevalent group, accounting for 11.4 and 8.8% cases of diarrhoea detected by PCR. This is a relatively high prevalence bearing in mind that the study was community-based where mild cases tend to dominate, and was similar to other finding (12.2%) by Nguyen *et al*, (2006). *Escherichia* bacteria are transmitted primarily by person-to-person spread, but part of the reason for the high prevalence could also be due to exposure to wastewater contaminated with human faeces.

The diagnostic sensitivity and specificity of *Salmonella* spp. by culture when compared to PCR were similar to the study by Gentry-Weeks *et al*. (2002) that uses PCR evaluation to determine whether it is as sensitive and specific as conventional culture method in detecting *Salmonella* spp. from feces. The detection revealed a sensitivity of 93.3 and 80%, respectively, and a specificity of 85.6 and 98.6%, respectively, when compared with bacterial culture. The conventional culture method used in identification of *Shigella* spp. detects only a small fraction of the actual shigellosis cases (4.1%) compared to PCR (4.8%). The common reasons for the low sensitivity (81%) of traditional culture methods than PCR (99%) probably may include the low number of causative organisms, competition from other commensal organisms, as well as inappropriate changes in ambient temperature and pH during specimen transport and thus hindering the growth on media (Taylor and Schelhart, 1975). Taken together with the data presented in this study, it support the view that PCR based testing is more sensitive than conventional culture for the detection of enteric bacterial pathogens in stool samples obtained from diarrhoeic children (Amar *et al.,*2004).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The prevalence of bacterial diarrhoea in children in Sokoto state was determined; *Campylobacte*r, *E. coli, Shigella* and *Salmonella enterica* were isolated at different rates and played a dominant role in causing diarrhoea in children from Sokoto State. The prevalence of enteric bacterial pathogens in diarrhoeal cases remains a significant threat to the health of communities in the State.

The overall antibiotic resistance levels against some commonly prescribed drugs were determined and were found to be higher than those reported from other regions in Nigeria, possibly due to the higher levels of exposure and usage of those antimicrobials in the study area.

Risk factors associated with incidence of bacterial diarrhoea in children from the study area were determined and were found to include: lack of safe water, improper sewage disposal system and poor hygienic practices.

Enteropathogenic bacteria were detected directly from the stool sample by PCR and pathogen detection was greater using PCR compared to routine culture methods. The findings highlight the value of using a combination of traditional and molecular techniques in the diagnosis of diarrheal disease in this population.

6.2 Recommendation

1. Future improvement programmes to control diarrhoea should be carried out and aimed at educating the communities on improvement on apposite waste disposal and sanitation, raise awareness among the residents about good personal hygiene practices, drinking clean water, exclusive breast feeding for six months (which helps in providing passive immunity in the newborn for at least 6 months) in order to minimize the infection rate by many enteropathogens and on ORT as the main stay for treatment of diarrhoea. Caregivers and parents need to be made aware of the signs and symptoms of dehydration and when to seek medical treatment.

2. Periodic surveys of antibiograms of bacterial agents of diarrhoea is recommended in order to keep trends in antibiotic resistance and to provide updated guidelines for the management of childhood diarrhoea.

3. The study also recommends that all hospital and primary health care laboratories in the state should be provided with adequate laboratory facilities and expertise which will improve on the quality of pathogens detection. This will make the clinical diagnosis better and help the clinicians in treatment of children with diarrhoea.

4. The PCR technique can be chosen as an alternative to the culture technique, and can further be used for identifying asymptomatic carriers, serving as potential reservoirs of enteric pathogens silently transmitting the disease within communities.

6.3 Limitationss

The limited budget prevented us from conducting the real-time PCR that will determine quantitatively the enteric bacterial pathogens directly from the stool sample. Nevertheless, using PCR in the enteric laboratory for the detection of pathogens is cost effective and further disadvantage is lack of isolate and hence the inability to perform simple antibiotic sensitivity testing and in some cases further identification or detailed typing for epidemiological purposes.

Samples were collected from children attending clinics, however, we should also have gone to subjects' houses to carry out observation in sanitation, hygiene and water related practices. This was impossible in context of limited time and manpower. Moreover, some questions may have been so sensitive that some interviewees did not feel confident to give an honest answer.

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