BACTERIOLOGICAL AGENTS OF DIARRHOEA IN CHILDREN AGED 0-5 YEARS, IN MINNA, NIGER STATE, NIGERIA.

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M.TECH/SSSE/2007/1670

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FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA,

NIGER STATE.

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DECEMBER, 2010.

DECLARATION

I OLOYEDE, Olusayo Oyeronke (Mrs.) do declare that this research thesis "Bacteriological Agents of Diarrhoea in Children Aged 0-5 years, In Minna, Niger State Nigeria" was carried out by me.

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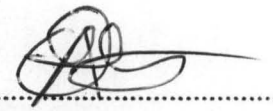

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Signature

CERTIFICATION

This research thesis "Bacteriological Agents of Diarrhoea in Children Aged 0-5 years, In Minna, Niger State Nigeria." was written by OLOYEDE, Olusayo Oyeronke and has been read, examined and found to meet the regulations governing the award of the degree of Master of Technology, Federal University of Technology, Minna and is approved for its contribution to knowledge and literary presentation.

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My profound gratitude is also extended to my husband, children, my in-laws and my entire family members for their love and understanding and above all I return all the glory to almighty God.

DEDICATION

This thesis is dedicated to my caring husband Engr. Stephen Sunday K and my loving children Israel Goodluck and Ifeoluwa Gloria for their understanding and support during the course of the programme.

ABSTRACT

One hundred and seventy six stool samples from children with diarrhoea attending the General Hospital Minna, Nigeria were analysed for the presence of different types of bacteria using standard bacteriological methods. Isolates were subjected to antimicrobial susceptibility using the disc diffusion method. The bacteria identified were as follows: *Escherichia coli* 84 (47.734%), *Shigella* species 34(19.32%), *Salmonella* species 29 (16.6532%). Others were *Citrobacter* species 8 (4.55%), *Enterobacter* species 11 (6.65%), *Vibro cholerae* 4 (2.23%). Ten (5.68%) samples yielded no bacteria growth. The highest percentage prevalence was recorded for the 6-12 months age group in homes where parents occupy multiple tenant apartments, and where wells and streams served as source of drinking water. No isolate was recovered from the 0-5months age group or from exclusively breast fed children, and children whose parents boiled their water before drinking. The highest number of isolates, (51%), belonged to those in the 0157 sero-group of *E.coli* This study has shown that *Escherichia coli* accounts for most cases of infantile diarrhoea in Minna, Nigeria. All isolates were resistant to chloramphenicol (30ug) and streptomycin (30ug) and were highly sensitive to amoxicillin and ciprofloxacin. 75% of *Salmonella species* belonged to *Salmonella* paratyphi A; 19%were positive for *Salmonella* paratyphi B and 8% for *Salmonella* typhi. 66% of the examined samples were positive for *Shigella dysenteriae* while 30% were positive for *Shigella flexneri.* In addition, findings indicate that the incidence of diarrhoea in children can be traced primarily to mode of child feeding, poor personal hygiene and type of apartment occupied by parents, and source of drinking water.

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CHAPTER ONE

1.0 INTRODUCTION

Diarrhoea is the passing of loose and watery stools (Kandakai-Oluyemi *et al.*, 2009) and occurs at some point in the life of nearly every child. Diarrhoea is not a disease, but is a symptom of a number of illnesses (Kandakai-Oluyemi *et al.*, 2009). Diarrhoea is defined by the World Health Organization as having 3 or more loose or liquid stools per day, or as having more stools than is normal for that person (WHO, 2009)

Acute diarrhoea is a common problem that usually lasts 1 or 2 days and resolves on its own without special treatment (Chen *et al.*, 2010). Prolonged diarrhoea persisting for more than 2 days may be a sign of a more serious problem and poses the risk of dehydration (Katribe *et al.*, 2008). Chronic diarrhoea may be a feature of a chronic disease.

Dehydration alters the child's natural balance of water and electrolytes (sodium, potassium, chloride) and can be serious if not treated promptly. Dehydration is particularly dangerous in children and older people (Katribe *et al.*, 2008), and it must be treated promptly to avoid serious health problems.

People of all ages can get diarrhoea and the average adult has a bout of acute diarrhoea about four times a year. Each child will have had seven to 15 episodes of diarrhoea by the age of 5 years (Cravioto *et al.*, 1998). The normal consistency and frequency of bowel movements varies with a child's age and diet.

Frequency — It is normal for young infants to pass up to three to ten stools per day, although this varies depending upon the child's diet (breastmilk versus formula). Breastfed children

usually have more frequent stools (Wilson, 2005). Older infants, toddlers, and children normally have one to two bowel movements per day (Viswanatha *et al.*, 2009).

Consistency: The consistency and color of a child's stool normally changes with age, which highlights the importance of knowing what is normal for your child. Young infants' stools may be yellow, green, or brown, and may appear to contain seeds or small curds (Viswanatha *et al.*, 2009). Developing stools that are runny, watery, or contain mucus is a significant change that should be monitored. The presence of blood in stool is never normal.

1.1 CAUSES OF DIARRHOEA

Acute diarrhoea is usually due to bacterial, viral, or parasitic infection. Chronic diarrhoea is usually related to functional disorders such as irritable bowel syndrome or inflammatory bowel disease.

The most common cause of acute diarrhoea is a viral infection. Other causes include bacterial infections, side effects of antibiotics, and infections not related to the gastrointestinal system (Kayser *et al.*, 2005).

1.11 Viral infection: Viral infection is the leading cause of diarrhoea in children, and is seen most commonly in the winter months (Rupnik *et al.* , 2009). Symptoms of viral infection can include watery diarrhoea, vomiting, fever (temperature greater than 100.4°F or 38°C), headache, abdominal cramps, lack of appetite, and muscle aches(Patel *et al*, 2009). Diarrhoea due to viral infection usually begins 12 hours to four days after exposure, and resolves within three to seven days. No specific treatment is available for viral causes of diarrhoea (Longstreth *et al.*, 2006). Children with diarrhoea from viral infections are best treated with supportive measures (oral rehydration solution, limited diet, rest). Many viruses including rotavirus, Norwalk virus,

cytomegalovirus, herpes simplex virus, and hepatitis virus causes diarrhoea (Viswanatha *et al.*, 2009).

1.12 Bacterial infection: Bacterial infection is sometimes hard to distinguish from viral infection. Persistent high fever (greater than 40°C or 104°F) and diarrhoea that is bloody or contains mucus are somewhat more common with bacterial infection (Viswanatha *et al.*, 2009). Most children with bacterial infection do not require antibiotics and will improve with time and supportive measures. Several types of bacteria consumed through contaminated food or water can cause diarrhoea. Common microorganisms that cause diarrhoea include *Campylobacter, Salmonella, Shigella, and Escherichia coli.* (Viswanatha *et al.*, 2009).

1.13 Parasitic infection: Generally, infection with a parasite is uncommon, but may be seen in children who have recently ingested contaminated water or who have traveled or lived in developing countries. Diarrhoea from parasitic infections may last longer than two weeks. Parasites can enter the body through food or water and settle in the digestive system. Parasites that cause diarrhoea include *Giardia lamblia, Entamoeba histolytica*, and *Cryptosporidium* (Viswanatha *et al*, 2009).

1.14 Antibiotic-associated diarrhoea: Prolong usage of a number of antibiotics can cause diarrhoea in both children and adults. The diarrhoea is usually mild, and typically does not cause dehydration or weight loss (Gonzales *et al.*, 2009). The diarrhoea usually resolves one to two days after antibiotics are withdrawn. Antibiotics, blood pressure medications, cancer drugs, and antacids containing magnesium can all cause diarrhoea.

1.15 Other causes of diarrhoea

Food intolerances: Some people are unable to digest food components such as artificial sweeteners and lactose (the sugar found in milk).

Intestinal diseases: Inflammatory bowel disease, colitis, Crohn's disease, and celiac disease often lead to diarrhoea.

Functional bowel disorders. Diarrhoea can be a symptom of irritable bowel syndrome.

Some people develop diarrhoea after stomach surgery or removal of the gallbladder (Alam *et al.*, 2003). The reason may be a change in how quickly food moves through the digestive system after stomach surgery or an increase in bile in the colon after gallbladder surgery.

People who visit foreign countries are at risk for traveler's, diarrhoea which is caused by eating food or drinking water contaminated with bacteria, viruses, or parasites. Traveler's diarrhoea can be a problem for people visiting developing countries. Visitors to the United States, Canada, most European countries, Japan, Australia, and New Zealand do not face much risk for traveler's diarrhoea.

In many cases, the cause of diarrhoea cannot be found. As long as diarrhoea goes away on its own, an extensive search for the cause is not usually necessary.

1.2 The symptoms of diarrhoea

Diarrhoea may be accompanied by cramping, abdominal pain, bloating, nausea, or an urgent need to use the toilet. Depending on the cause, a person may have fever or bloody stools and dehydration (Kiser *et al.*, 2008).

1.21 Dehydration

Signs of dehydration include

- thirst
- less frequent urination
- dry skin
- fatigue
- light-headedness
- dark-colored urine

1.3 Diarrhoea in children

Children can have acute and chronic forms of diarrhoea. Causes include bacteria, viruses, parasites, medications, functional bowel disorders, and food sensitivities (Viswanatha *et al.*, 2009). Infection with rotavirus is the most common cause of acute childhood diarrhoea. Rotavirus diarrhoea usually resolves in 3 to 9 days. .

Signs of dehydration in children include

- dry mouth and tongue
- no tears when crying
- no wet diapers for 3 hours or more
- sunken abdomen, eyes, or cheeks
- high fever
- listlessness or irritability
- skin that does not flatten when pinched and released

1.4 Preventing Dehydration

The fluid and electrolytes lost during diarrhoea need to be replaced promptly because the body cannot function without them. Electrolytes are the salts and minerals that affect the amount of water in the body, muscle activity, and other important functions (WHO, 2009).

Although water is extremely important in preventing dehydration, it does not contain electrolytes. Broth and soups that contain sodium, and fruit juices, soft fruits, or vegetables that contain potassium, help restore electrolyte levels.

1.5 Diagnosis of Diarrhoea

Diagnostic tests to find the cause of diarrhea may include the following:

- **Medical history and physical examination.** The doctor should ask about the eating habits and medication use and examine the patient for signs of illness.
- **Stool culture.** A sample of stool is analyzed in a laboratory to check for bacteria, parasites, or other signs of disease and infection.
- **Blood tests.** Blood tests can be helpful in ruling out certain diseases.
- **Fasting tests.** To find out if a food intolerance or allergy is causing the diarrhea, can tell the patient to avoid lactose, carbohydrates, wheat, or other foods to see whether the diarrhoea responds to a change in diet.
- **Sigmoidoscopy.** For this test, the doctor uses a sigmoidoscope to look at the inside of the rectum and lower part of the colon.
- **Colonoscopy.** This test is similar to a sigmoidoscopy, but it allows the doctor to view the entire colon.

1.6 JUSTIFICATION

- Bacteria are among the major causes of diarrhoea in children.
- Diarrhoea account for high morbidity and mortality rates in children below five years of age in developing and underdeveloped countries.
- Inadequate water supply and poor sanitation are some of the contributory factors of diarrhoea and these remain problems in developing and underdeveloped Countries.
- Characterization of diarrhoea agents will provide information on the epidemiology of diarrhoea. The antibiotic susceptibility will provide useful information for the management of diarrhoea especially in Minna.

1.6 The main aims and objectives are:

- To identify and characterize the species of bacteria that causes diarrhoea in children in Minna.
- To relate diarrhoea to the type of resident occupied by their parents.
- To relate diarrhoea to sources of drinking water.
- To determine incidence of diarrhoea in infants who are breast fed and those fed with other baby formula foods.
- To determine the antibiotic susceptibility of the bacteria isolated.

CHAPTER TWO

2.0 LITERATURE REVIEW

Diarrhoea (from the Greek, διἀρροια meaning "a flowing through" (WHO, 2008)), is the condition of having three or more loose or liquid bowel movements per day (Odukoya, 1998). It is a common cause of death in developing countries and the second most common cause of infant deaths worldwide. The loss of fluids through diarrhoea can cause dehydration and electrolyte imbalances. In 2009 diarrhoea was estimated to have caused 1.1 million deaths in people aged 5 years and over and 1.5 million deaths in children under the age of 5years (Odukoya, 1998).

Diarrhoeal disease continues to be a health problem worldwide, especially in developing countries. In these regions, it accounts for approximately 2.5 million deaths per year in children under 5 years of age. Furthermore, acute diarrhoea considerably contributes to morbidity and increases health care costs in children from industrialized countries (Mahmood *et al.*, 1987). Bloody diarrhoea (BD) represents approximately 20-30% of all cases, causing important inflammatory intestinal illness and, under some circumstances, producing severe complications, such as sepsis, hemorrhagic colitis and hemolytic uremic syndrome (HUS) (Odukoya, 1998). The bacterial pathogens associated with BD include species of *Shigella*, *Campylobacter*, *Salmonella*, *Escherichia coli* pathotypes, especially Shiga toxin-producing *Escherichia coli* (STEC) and enteroinvasive *E. coli* (EIEC), as well as *Yersinia enterocolitica*.

During the period 1950 to 1970s it was estimated that 4.6 million children died annually from diarrhoea in developing world (Robin *et al.*, 1980). Mortality due to diarrhoea declined to approximately 3.3 million annually in the 1980s (Robin *et al.*, 1980).

Diarrhoea has been reported to account for 1.6–2.5 million deaths annually (Odukoya, 1998). Despite the decline in mortality, diarrhoea still remains one of the principal causes of morbidity in the developing world, with each child experiencing an average of three episodes of diarrhoea per year (WHO, 2008). In these countries, diarrhoeal diseases are the second most common illness of children after acute respiratory illness (Cravioto *et al.*, 1998). The causes of diarrhoea include a wide range of viruses, bacteria, and parasites (Longstreth *et al.*, 2006). Among the bacterial causes diarrhoeagenic *Escherichia coli* (DEC) is the most important etiologic agent of childhood diarrhoea and represents a major public health problem in developing countries (Kandakai-Oluyemi *et al.,* 2009). DEC strains can be divided into six main categories on the basis of distinct molecular, clinical and pathological features (Kandakai-Oluyemi *et al.,* 2009): enteroaggregative *E. coli* (EAEC), enterohemorrhagic (Shiga-toxin producing *E. coli* (EHEC/STEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and diffusely adherent *E.coli* (DAEC). The epidemiological significance of each DEC category in childhood diarrhoea varies with geographical area.

Diarrhoea has been recognized as a prominent killer of infants and young children in most developing countries (Odukoya, 1998). Reports indicated that over five million deaths per year occur in children under the age of five year as a result of diarrhoea in these countries (Kebede *et al.*, 1990). The association between bacteria and diarrhoea has been reported in many countries (Robin *et al.*, 1980). Studies have also shown that bacteria are important causative agent in sporadic infantile diarrhoea in many developing countries (WHO, 2009). In previous studies, it has been demonstrated that the incidence of community acquired bacteria infection is highest in the first two years following childbirth (Cravioto *et al.*, 1998), and that the infection is more severe in younger children (Cravioto *et al.*, 1998). Infants are more likely to develop diarrhoea

during the first episode of colonization than during subsequent exposures. Illiteracy in parents (especially mothers), faulty weaning practices, source of drinking water, the environment, and the use of bottle feeding are possible factors that could be behind the high prevalence of this infection in most developing countries(Kandakai-Oluyemi *et al.,* 2009). In Nigeria, cases of gastroenteritis due to bacteria infection have been reported; however, common serotypes associated with the infection have not yet been well established among children from different parts of the country. Hence, this study was undertaken to investigate the prevalence of bacteria and its sero-groups in children presenting with diarrhoea at the General Hospital Minna, Niger state.

Worldwide, 1.5 billion cases of diarrhoea occur each year, accountable for 1.5-2.5 million deaths yearly (Odukoya, 1980), more than 20% of all mortalities (Kebede *et al.*, 1990). Diarrhoea is still a leading cause of illness and death among children under 5 years of age in developing countries. In Iran it has been estimated that diarrhoea is responsible for 18 million cases of illness (Mohammad, 1987), 12 million medical visits (NCCLS, 2000). Acute diarrhoea is the fifth leading cause of death due to infectious diseases and responsible for 16.2% of infectious diseases burden (Bauer *et al.*, 1988).

Acute diarrhoea has been recognized as a prominent killer of infants and young children in most developing countries (Odukoya, 1998). Reports in the literature indicate that over five million deaths per year occur in children under the age of five year as a result of diarrhoea in these countries.

2.1 Evolution

According to two researchers, (Williams and Nesse, 1996), diarrhoea may function as an evolved expulsion defense mechanism. As a result, if it is stopped, there might be a delay in recovery

(WHO, 2008). They cite in support of this argument research published in 1973 which found that treating *Shigella* with the anti-diarrhoea drug (Co-phenotrope, Lomotil) caused people to stay feverish twice as long as those not so treated. The researchers indeed themselves observed that: "Lomotil may be contraindicated in shigellosis. Diarrhoea may represent a defense mechanism" (WHO, 2008).

2.2 CLASSIFICATION OF DIARRHOEA

Diarrhoea is defined as the abrupt onset of abnormally high fluid content in the stool: more than the normal value of approximately 10 ml/kg/d in the infant and young child, and more than 200 g/d in the teenager and adult. This situation typically implies an increased frequency of bowel movements, which can range from 4-5 to more than 20 times per day (Longstreth *et al.*, 2006). The augmented water content in the stools is due to an imbalance in the physiology of the small and large intestinal processes involved in the absorption of ions, organic substrates, and thus water. A common disorder in its acute form, diarrhoea has many causes and may be mild to severe.

Childhood diarrhoea is usually caused by infection; however, numerous disorders may cause this condition, including a malabsorption syndrome and various enteropathies. Acute-onset diarrhoea is usually self-limited; however, an acute infection can have a protracted course (Spandorfer *et al.*, 2005). By far, the most common complication of acute diarrhoea is dehydration.

Although the term "acute gastroenteritis" is commonly used synonymously with "acute diarrhoea," the former term is a misnomer. The term gastroenteritis implies inflammation of both the stomach and the small intestine, whereas, in reality, gastric involvement is rarely if ever seen in acute diarrhoea (including diarrhoea with an infectious origin); enteritis is also not

consistently present. Examples of infectious diarrhoea syndromes that do not cause enteritis include *Vibrio cholerae*– induced diarrhoea and *Shigella* -induced diarrhoea. Thus, the term acute diarrhoea is preferable to acute gastroenteritis.

Diarrhoeal episodes are classically distinguished into acute and chronic (or persistent) based on their duration. Acute diarrhoea is thus defined as an episode that has an acute onset and lasts no longer than 14 days; chronic or persistent diarrhoea is defined as an episode that lasts longer than 14 days. The distinction, supported by the World Health Organization (WHO, 2008), 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.

2.3 TYPES OF DIARRHOEA

2.3.1 Secretory Diarrhoea

Secretory diarrhoea means that there is an increase in the active secretion, or there is an inhibition of absorption. There is little to no structural damage. The most common cause of this type of diarrhoea is a cholera toxin that stimulates the secretion of anions, especially chloride ions. Therefore, to maintain a charge balance in the lumen, sodium is carried with it, along with water. In this type of diarrhoea intestinal fluid secretion is isotonic with plasma even during fasting (Longstreth *et al.*, 2006).

2.3.2 Osmotic Diarrhoea

Osmotic diarrhoea occurs when too much water is drawn into the bowels. This can be the result of maldigestion (e.g., pancreatic disease or celiac disease), in which the nutrients are left in the

lumen to pull in water. Osmotic diarrhoea can also be caused by osmotic laxatives (which work to alleviate constipation by drawing water into the bowels). In healthy individuals, too much magnesium or vitamin C or undigested lactose can produce osmotic diarrhoea and distention of the bowel (Viswanatha *et al.*, 2009). A person who has lactose intolerance can have difficulty absorbing lactose after an extraordinarily high intake of dairy products. In persons who have fructose malabsorption, excess fructose intake can also cause diarrhoea. High-fructose foods that also have high glucose content are more absorbable and less likely to cause diarrhoea. Sugar alcohols such as sorbitol (often found in sugar-free foods) are difficult for the body to absorb and, in large amounts, may lead to osmotic diarrhoea (Kandakai-Oluyemi *et al.*, 2009).

2.3.3 Exudative Diarrhoea

Exudative diarrhoea occurs with the presence of blood and pus in the stool. This occurs with inflammatory bowel diseases, such as Crohn's disease or ulcerative colitis, and other severe infections such as *E. coli* or other forms of food poisoning (Longstreth *et al.*, 2006).

2.3.4 Motility-related Diarrhoea

Motility-related diarrhoea is caused by the rapid movement (hypermotility) of food through the intestines. If the food moves too quickly through the GI tract, there is not enough time for sufficient nutrients and water to be absorbed. This can be due to a vagotomy or diabetic neuropathy, or a complication of menstruation (Viswanatha *et al.*, 2009). Hyperthyroidism can produce hypermotility and lead to pseudodiarrhoea and occasionally real diarrhoea. Diarrhoea can be treated with antimotility agents (such as loperamide). Hypermotility can be observed in

people who have had portions of their bowel removed, allowing less total time for absorption of nutrients.

2.3.5 Inflammatory Diarrhoea

Inflammatory diarrhoea occurs when there is damage to the mucosal lining or brush border, which leads to a passive loss of protein-rich fluids, and a decreased ability to absorb these lost fluids. Features of all three of the other types of diarrhoea can be found in this type of diarrhoea. It can be caused by bacterial infections, viral infections, parasitic infections, or autoimmune problems such as inflammatory bowel diseases. It can also be caused by tuberculosis, colon cancer, and enteritis (Longstreth *et al.*, 2006).

2.3.6 Dysentery Diarrhoea

Generally, if there is blood visible in the stools, it is not diarrhoea, but dysentery. The blood is trace of an invasion of bowel tissue. Dysentery is a symptom of, among others, *Shigella*, *Entamoeba histolytica*, and *Salmonella* infections.

Diarrhoea is most commonly due to viral gastroenteritis with rotavirus accounting for 40% of cases in children under five. In travelers however bacterial infections predominate (Mahmood *et al.*, 1987).

It can also be the part of the presentations of a number of medical conditions such as Crohn's disease or mushroom poisoning.

2.3.7 Infectious diarrhoea

There are many causes of diarrhoea, which include viruses, bacteria and parasites. Norovirus is the most common cause of viral diarrhoea in adults, but rotavirus is the most common cause in children under five years old (Cheesbrough, 2004).

The bacterium campylobacter is a common cause of bacterial diarrhoea, but infections by *salmonellae, shigellae* and some strains of *Escherichia coli (E.coli)* are frequent (NCCLS, 2000). In the elderly, particularly those who have been treated with antibiotics for unrelated infections, a toxin produced by *Clostridium difficile* often causes severe diarrhoea (Olanipekun, 1996).

Parasites do not often cause diarrhoea except for the protozoan *Giardia*, which can cause chronic infections if these are not diagnosed and treated with drugs such as metronidazole (Martinez *et al.*, 1999).

Other infectious agents such as parasites and bacterial toxins also occur (Mahmood *et al.*, 1987).

In sanitary living conditions where there is ample food and a supply of clean water, an otherwise healthy person usually recovers from viral infections in a few days. However, for ill or malnourished individuals, diarrhoea can lead to severe dehydration and can become life-threatening (Requa *et al.*, 1990).

2.4 MALABSORPTION

Malabsorption is the inability to absorb food.

Causes of malabsorption include:

- enzyme deficiencies or mucosal abnormality, as in food allergy and food intolerance, (e.g. celiac disease, lactose intolerance (intolerance to milk sugar, common in non-Europeans), fructose malabsorption) or deficiency of certain enzyme which causes food intolerance.
- pernicious anemia (impaired bowel function due to the inability to absorb vitamin B_{12}),
- loss of pancreatic secretions (may be due to cystic fibrosis or pancreatitis),
- structural defects, such as short bowel syndrome (surgically removed bowel) and radiation fibrosis (usually following cancer treatment and other drugs, including agents used in chemotherapy),
- certain drugs (like orlistat, which inhibits the absorption of fat).

2.4.1 Inflammatory bowel disease

The two overlapping types here are of unknown origin:

- Ulcerative colitis is marked by chronic bloody diarrhoea and inflammation and mostly affects the distal colon near the rectum.
- Crohn's disease typically affects fairly well demarcated segments of bowel in the colon and often affects the end of the small bowel.

2.4.2 Irritable bowel syndrome

Another possible cause of diarrhoea is irritable bowel syndrome (IBS) which usually presents with abdominal discomfort relieved by defecation and unusual stool (diarrhoea or constipation) for at least 3 days a week over the previous 3 months (WHO, 2000).

2.4.3 Other types include

Diarrhoea can be caused by chronic ethanol ingestion (Kayser *et al.*, 2005).

Ischemic bowel disease. This usually affects older people and can be due to blocked arteries.

Hormone-secreting tumors: some hormones (e.g., serotonin) can cause diarrhoea if excreted in excess (usually from a tumor).

Chronic mild diarrhoea in infants and toddlers may occur with no obvious cause and with no other ill effects; this condition is called toddler's diarrhoea.

2.5 Treatment of diarrhoea.

In most cases of diarrhoea, replacing lost fluid to prevent dehydration is the only treatment necessary. Medicines that stop diarrhoea may be helpful, but they are not recommended for people whose diarrhoea is caused by a bacterial infection or parasite. If you stop the diarrhoea before having purged the bacteria or parasite, you will trap the organism in the intestines and prolong the problem. Rather, doctors usually prescribe antibiotics as a first-line treatment. Viral infections are either treated with medication or left to run their course, depending on the severity and type of virus.

2.6 Management

In many cases of diarrhoea, replacing lost fluid and salts is the only treatment needed. This is usually by mouth – oral rehydration therapy – or, in severe cases, intravenously (WHO, 2009). Research does not support the limiting of milk to children as doing so has no effect on duration of diarrhoea. Gastroenteritis is usually an acute and self-limited disease that does not require pharmacological therapy (King *et al.*, 2003).The objective of treatment is to replace lost fluids and electrolytes. Oral rehydration is the preferred method of replacing these losses in children with mild to moderate dehydration (WHO, 2004).

2.6.1 REHYDRATION

The primary treatment of gastroenteritis in both children and adults is rehydration, i.e., replenishment of water and electrolytes lost in the stools. This is preferably achieved by giving the person oral rehydration therapy (ORT) although intravenous delivery may be required if a decreased level of consciousness or an ileus is present (King *et al.*, 2003). Complex-carbohydrate-based Oral Rehydration Salts (ORS) such as those made from wheat or rice have been found to be superior to simple sugar-based ORS (Kandakai-Oluyemi *et al.*, 2009).

Sugary drinks such as soft drinks and fruit juice are not recommended for gastroenteritis in children below 5 years of age as they may make the diarrhoea worse (Loureiro *et al.*, 1998). Plain water may be used if specific ORS are unavailable or not palatable (WHO, 2000).

2.6.2 Medications

Antiemetics

Antiemetic drugs may be helpful for vomiting in children. Ondansetron has some utility with a single dose associated with less need for intravenous fluids, fewer hospitalizations, and

decreased vomiting (Greenberg and Estes, 2009). Metoclopramide also might be helpful (Mitchell, 2009). However there was an increased number of child who returned and were subsequently admitted in those treated with ondansetron. The intravenous preparation of ondansetron may be given orally.

Antibiotics

Antibiotics are not usually used for gastroenteritis, although they are sometimes used if symptoms are severe (such as dysentry) or a susceptible bacterial cause is isolated or suspected. If antibiotics are decided on, a fluoroquinolone or macrolide is often used (NCCLS, 2000).

While antibiotics are beneficial in certain type of acute diarrhoea they are usually not used except in specific situations (Kandakai-Oluyemi *et al.,* 2009).There are concerns that antibiotic may increase the risk of hemolytic uremic syndrome in people infected with *Escherichia coli* O157:H7 (Gomes *et al.*, 1991). In resource poor countries treatment with antibiotics may be beneficial. However, some bacteria are developing antibiotic resistance, particularly *Shigella.*

Pseudomembranous colitis, usually caused by antibiotics use, is managed by discontinuing the causative agent and treating with either metronidazole or vancomycin (NCCLS, 2000).

Anti motility agents and Bismuth compounds

Anti motility agents like loperamide are effective at reducing the duration of diarrhoea. While bismuth compounds (Pepto-Bismol) decrease the number of bowel movements in those with travelers' diarrhoea it does not decrease the length of illness (Alam *et al.*, 2003). These agents should only be used if bloody diarrhoea is not present.

Codeine Phosphate

Codeine Phosphate is used in the treatment of diarrhoea to slow down Peristalsis and the passage of fecal material through the bowels - this means that more time is given for water to be reabsorbed back into the body, which gives a firmer stool, and also means that feces is passed less frequently.

2.6.3 Alternative therapies

The probiotic *lactobacillus* can help prevent antibiotic associated diarrhoea in adults but possibly not in children. For those who suffer from lactose intolerance, taking digestive enzymes containing lactase when consuming dairy products is recommended (Alam *et al.*, 2003).

2.7 Epidemiology

Worldwide in 2004 approximately 2.5 billion cases of diarrhoea occurred, which results in 1.5 million deaths among children under the age of five. Greater than half of these were in Africa and South Asia. This is down from a death rate of 5 million per year two decades ago (Rupnik *et al.*, 2009). Diarrhoea remains the second leading cause of death (16%) after pneumonia (17%) in this age group (Rupnik *et al.*, 2009).

2.8 Nosocomial Diarrhoea

Diarrhoea commonly occurs in the hospital, where patients (often with coexisting conditions) are receiving drugs and feedings and there is exposure to *Clostridium difficile* spores. Although *C. difficile* accounts for a minority of antibiotic-associated and hospital-associated diarrhoea, it should be considered in patients with clinically significant diarrhoea (passage of three or more

unformed stools per day), toxic dilatation of the colon or otherwise unexplained leukocytosis, or both. Patients with this infection often pass watery diarrhoea stools but may also pass grossly bloody stools. *C. difficile* diarrhoea is increasing in frequency (Wilson, 2005) and is associated with an increasing mortality rate (Cohen, 2000). Although *C. difficile* diarrhoea has been viewed as a nosocomial condition, it is increasingly being seen in the outpatient setting. Risk factors for *C. difficile* diarrhoea in the inpatient or outpatient setting include advanced age and coexisting conditions, alteration of intestinal flora by antimicrobial agents, and probably host genetics. The indigenous human intestinal microbiota is important to colonization resistance and recovery from antibiotic-associated and *C. difficile* diarrhoea (Greenberg *et al.*, 2009). *C. difficile* diarrhoea was recently reviewed in the *Journal*. Diarrhoea is one of the main causes of morbidity and mortality in children younger than 5 years of age, in developing countries, where the average number of episodes of diarrhoea per child per year within this age group is 3.2 (Greenberg and Estes, 2009). Twenty-one percent of childhood mortality in children younger than 5 years old in these countries is associated with diarrhoea, resulting in 2.5 million deaths per year (Kale *et al.*, 2010). In sub-Saharan Africa, mortality caused by acute diarrhoea varies from 1.9% of all deaths in The Gambia to 37% in Nigeria, with most of the deaths occurring during the first year of life. Even though morbidity caused by diarrhoea is still high, mortality has been decreasing worldwide, mainly because of improved management (Greenberg and Estes, 2009).

In Mozambique, diarrhoea has been recently reported to be the third cause of death (10%) among children from 0 to 14 years old in Maputo City. In the Manhiça district, diarrhoea is the fourth cause of death among children between 12 and 59 months of age (Schiller, 2007).

2.9 Pathophysiology

Diarrhoea is the reversal of the normal net absorptive status of water and electrolyte absorption to secretion. Such a derangement can be the result of either an osmotic force that acts in the lumen to drive water into the gut or the result of an active secretory state induced in the enterocytes. In the former case, diarrhoea is osmolar in nature, as is observed after the ingestion of nonabsorbable sugars such as lactulose or lactose in lactose malabsorbers. Instead, in the typical active secretory state, enhanced anion secretion (mostly by the crypt cell compartment) is best exemplified by enterotoxin-induced diarrhoea.

In osmotic diarrhoea, stool output is proportional to the intake of the unabsorbable substrate and is usually not massive; diarrhoeal stools promptly regress with discontinuation of the offending nutrient, and the stool ion gap is high, (WHO, 2009) In fact, the fecal osmolality in this circumstance is accounted for not only by the electrolytes but also by the unabsorbed nutrient(s) and their degradation products. The ion gap is obtained by subtracting the concentration of the electrolytes from total osmolality (assumed to be 290 ml/kg), according to the formula: ion gap = $290 - [(Na + K) \times 2]$.

In secretory diarrhoea, the epithelial cells' ion transport processes are turned into a state of active secretion. The most common cause of acute-onset secretory diarrhoea is a bacterial infection of the gut. Several mechanisms may be at work. After colonization, enteric pathogens may adhere to or invade the epithelium; they may produce enterotoxins (exotoxins that elicit secretion by increasing an intracellular second messenger) or cytotoxins. They may also trigger release of cytokines attracting inflammatory cells, which, in turn, contribute to the activated secretion by inducing the release of agents such as prostaglandins or platelet-activating factor. Features of

secretory diarrhoea include a high purging rate, a lack of response to fasting, and a normal stool ion gap, indicating that nutrient absorption is intact (Greenberg and Estes, 2009).

2.10 PREVALENCE

United States

In the United States, one estimate assumes a cumulative incidence of 1 hospitalization for diarrhoea per 23-27 children by age 5 years, with more than 50,000 hospitalizations in 2000. By these estimates, rotavirus is associated with 4-5% of all childhood hospitalizations, and 1 in 67 to 1 in 85 children are hospitalized due to rotavirus by age 5 years. Furthermore, acute diarrhoea is responsible for 20% of physician referrals in children younger than 2 years and for 10% in children younger than 3 years (Rupnik *et al.*, 2009).

2.10.1 International

In developing countries, an average of 3 episodes per child per year in children younger than 5 years is reported; however, some areas report 6-8 episodes per year per child. In these settings, malnutrition is an important additional risk factor for diarrhoea, and recurrent episodes of diarrhoea lead to growth faltering (Viswanatha *et al.*, 2009). Childhood mortality associated with diarrhoea has constantly but slowly declined during the past 2 decades, mostly because of the widespread use of oral rehydration solutions; however, it appears to have plateaued over the past few years.

Because the single most common cause of infectious diarrhoea worldwide is rotavirus, and because a vaccine has been in use for over 3 years now, a reduction in the overall frequency of diarrhoea episodes is hoped for in the near future.

2.11 Mortality/Morbidity

Mortality from acute diarrhoea is overall globally declining but remains high. Most estimates have diarrhoea as the second cause of childhood mortality, with 18% of the 10.6 million yearly deaths in children younger than age 5 years (Iwamoto *et al.*, 2010). Despite a progressive reduction in global diarrhoeal disease mortality over the past 2 decades, diarrhoea morbidity in published reports from 1990-2000 slightly increased worldwide compared with previous reports.

Furthermore, in countries where the toll of diarrhoea is highest, poverty also adds an enormous additional burden, and long-term consequences of the vicious cycle of enteric infections, diarrhoea, and malnutrition are devastating (Greenberg *et al.*, 2009).

2.12 Sex

Most cases of infectious diarrhoea are not sex specific. Females have a higher incidence of *Campylobacter* species infections and hemolytic uremic syndrome (HUS) (Parissi-Criulli *et al.*, 2000).

2.13 Age

Viral diarrhoea is most common in young children. Rotavirus and adenovirus are particularly prevalent in children younger than 2 years. Astrovirus and norovirus usually infect children younger than 5 years. *Yersinia enterocolitis* typically affects children younger than 1 year, and the *Aeromonas* organism is a significant cause of diarrhoea in young children.

Very young children are particularly susceptible to secondary dehydration and secondary nutrient malabsorption. Age and nutritional status appear to be the most important host factors in

determining the severity and the duration of diarrhoea. In fact, the younger the child, the higher the risk for severe, life-threatening dehydration as a result of the high body-water turnover and limited renal compensatory capacity of very young children. Whether younger age also means a risk of running a prolonged course is an unsettled issue. In developing countries, persistent post enteritis diarrhoea has a strong inverse correlation with age.

2.14 DIAGNOSIS

Gastroenteritis is diagnosed based on symptoms, a complete medical history and a physical examination. An accurate medical history may provide valuable information on the existence or inexistence of similar symptoms in other members of the patient's family or friends. The duration, frequency, and description of the patient's bowel movements and if he experiences vomiting are also relevant (Greenberg and Estes., 2009).

No specific diagnostic tests are required in most patients with simple gastroenteritis. If symptoms including fever, bloody stool and diarrhoea persist for two weeks or more, examination of stool for *Clostridium difficile* may be advisable along with cultures for bacteria including *Salmonella, Shigella, Campylobacter* and Enterotoxic *Escherichia coli* (kale-Pradhan *et al.*, 2010). Microscopy for parasites, ova and cysts may also be helpful (Mitchell, 2009).

A complete medical history may be helpful in diagnosing gastroenteritis. A complete and accurate medical history of the patient includes information on travel history, exposure to poisons or other irritants, diet change, food preparation habits or storage and medications. Patients who travel may be exposed to *E. Coli* infections or parasite infections contacted from beverages or food. Swimming in contaminated water or drinking from suspicious fresh water

such as mountain streams or wells may indicate infection from Giardia - an organism found in water that causes diarrhoea.

Food poisoning must be considered in cases when the patient was exposed to undercooked or improperly stored food. Depending on the type of bacteria that is causing the condition, the reactions appear in 2 to 72 hours (Greenberg and Estes , 2009).Detecting the specific infectious agent is required in order to establish a proper diagnosis and an effective treatment plan.

Conditions such as appendicitis, gallbladder disease, pancreatitis or diverticulitis may cause similar symptoms but a physical examination will reveal a specific tenderness in the abdomen which is not present in gastroenteritis (Pawlowski *et al.*, 2009).

Diagnosing gastroenteritis is mainly an exclusion procedure. Therefore in rare cases when the symptoms are not enough to diagnose gastroenteritis, several tests may be performed in order to rule out other gastrointestinal disorders. These include rectal examinations, complete blood count, electrolytes and kidney function tests. However, when the symptoms are conclusive, no tests apart from the stool tests are required to correctly diagnose gastroenteritis especially if the patient has traveled to at-risk areas.

2.15 PREVENTION

A rotavirus vaccine has been given to children between 2000 and 2009 which decreased the number of cases of diarrhoea due to rotavirus in the United States (WHO, 2000).

Gastroenteritis may be prevented through immunization (loureiro *et al.*,1998). The U.S. Food and Drug Administration approved in 2006 a rotavirus vaccine called Rotateq that may be given to infants aged 6 to 32 weeks to prevent getting infected with viral gastroenteritis rotavirus

(Requa *et al.*, 1990). The vaccines may however have side effects that are similar to the mild flu symptoms.

Different types of vaccinations are available for *Salmonella typhi* and *Vibrio cholereae* and which may be administered to people who intend traveling in at-risk areas. However, the vaccines that are currently available are not effective on gastroenteritis caused by other viruses than rotaviruses.

Prevention may also be done by means of observing proper hygiene through hand wash especially for those who are prone to this type of infection. One is advised to thoroughly wash their hands before eating, after using the toilet or changing diapers and not to eat or drink something that might be contaminated. Viral gastroenteritis is a highly infectious disease and thus avoiding crowded spaces such as markets, theaters or shopping centers may also help in preventing infection for those who have weak resistance.

2.16 CLINICAL PRESENTATION OF DIARRHOEA

Acute diarrhoea in developed countries is almost invariably a benign, self-limiting condition, subsiding within a few days. The clinical presentation and course of illness depends on the etiology of the diarrhoea and on the host. For example, rotavirus is more commonly associated with vomiting, dehydration, and a greater number of work days lost than non rotavirus gastroenteritis (Kiser *et al.*, 2008).

A prospective study conducted in the United States in 604 children aged 3-36 months in community settings found that the highest incidence of acute diarrhoea (Chen *et al.*, 2010) was in January and August, with an overall incidence of 2.21 episodes per person per year (Odukoya

et al., 1998). Close to 90% of episodes were acute (i.e, lasting more than 14 days, with a median duration of 2 days and a median of 6 stools per day).

Individual stooling pattern widely varies; for example, breastfed children may normally have 5-6 stools per day.

Flatulence associated with foul-smelling stools that float suggests fat malabsorption, which can be observed with infection with *Giardia lamblia.*

Knowledge of the characteristics of consistency, color, volume, and frequency can be helpful in determining whether the source is from the small or large bowel. Outlines of these characteristics will demonstrate that an index of suspicion can be easily generated for a specific set of organisms (Longstreth *et al.*, 2006).

The most common clinical manifestation of diarrhoea in children and adult includes

Excessive passage of diarrhoea, stools containing blood or black stools with pus, a sign of dehydration and increase in body temperature. Signs of dehydration in adults include, thirst, less frequent urination, dry skin, fatigue, light-headedness and dark-coloured urine.

Signs of dehydration in children include dry mouth and tongue, no tears when crying, no wet diapers for 3 hours or more, sunken abdomen, eyes, or cheeks, high fever, listlessness or irritability, skin that does not flatten when pinched and released (Kiser *et al.*, 2008).

Associated systemic symptoms include the following:

Some enteric infections commonly have systemic symptoms, whereas others less commonly are associated with systemic features.

The frequency of some of these symptoms with particular organisms, outlines incubation periods and usual duration of symptoms of common organisms are shown below. Certain organisms (eg, *C difficile, Giardia, Entamoeba* species) may be associated with a protracted course (WHO, 2009).

Table 2.1 Organisms and Frequency of Symptoms

Organism	**Incubation days**	**Duration days**	**Vomiting**	**Fever**	**Abdominal Pain**
Rotavirus	1-7	4-8	Yes	Low	No
Adenovirus	8-10	5-12	Delayed	Low	No
Norovirus	1-2	2	Yes	No	No
Astrovirus	1-2	4-8	+/-	+/-	No
Calicivirus	1-4	4-8	Yes	+/-	No
***Aeromonas* species**	None	0-2 wk	+/-	+/-	No
***Campylobacter* species**	2-4	5-7	No	Yes	Yes
C difficile	Variable	Variable	No	Few	Few
C perfringens	Minimal	1	Mild	No	Yes
Enterohemorrhagic *E coli*	1-8	3-6	No	+/-	Yes

Organism	Incubation days	Duration days	Vomiting	Fever	Abdominal Pain
Enterotoxigenic *E coli*	1-3	3-5	Yes	Low	Yes
***Salmonella* species**	0-3	2-7	Yes	Yes	Yes
***Shigella* species**	0-2	2-5	No	High	Yes
***Vibrio* species**	0-1	5-7	Yes	No	Yes
Y. enterocolitica	None	1-46	Yes	Yes	Yes
***Giardia* species**	2 wk	1+ wk	No	No	Yes
***Cryptosporidium* species**	5-21 d	Months	No	Low	Yes
***Entamoeba* species**	5-7 d	1-2+ wk	No	Yes	No

(Mitchell, 2009).

Daycare considerations are as follows:

Certain organisms are spread quickly in daycare. These organisms include rotavirus; astrovirus; calicivirus; C*ampylobacter, Shigella, Giardia,* and *Cryptosporidium* species.

Increase in daycare usage has raised the incidence of rotavirus and *Cryptosporidium* species.Ingestion of raw or contaminated food is a common cause of diarrhoea.

Organisms that cause food poisoning include the following:

Campylobacter, *Salmonella* species,*C perfringens, Aeromonas,* Enterohemorrhagic *E coli, Y enterocolitica,* Astrovirus, *Plesiomonas,* and *Vibrio* species, Calicivirus and *Plesiomonas.*

Water exposure can contribute to diarrhoea. Water is a major reservoir for many organisms that cause diarrhea. Swimming pools have been associated with outbreaks of infection with *Shigella* species; *Aeromonas* organisms are associated with exposure to the marine environment.

Giardia, Cryptosporidium, and *Entamoeba* are resistant to water chlorination; therefore, exposure to contaminated water should raise index of suspicion for these parasites. A history of camping suggests exposure to water sources contaminated with *Giardia.*

Travel history may indicate a cause for diarrhoea. Enterotoxigenic *E coli* are the leading cause of traveler's diarrhoea. Rotavirus, *Shigella, Salmonella,* and *Campylobacter* organisms are prevalent worldwide and need to be considered regardless of specific travel history. Risk of contracting diarrhoea while traveling is by far, highest for persons traveling to Africa. Travel to Central and South America and Eastern European countries is also associated with a relatively high risk of contracting diarrhoea.

Other organisms that are prevalent in some parts of the world include the following: Nonspecific foreign travel history - Enterotoxigenic *E coli, Aeromonas, Giardia, Plesiomonas, Salmonella,* and *Shigella* species. Others include, *C perfringens, Entamoeba* species, *Vibrio cholerae, Yersinia* species, *and Vibrio parahaemolyticus.*

Animal exposure can contribute to diarrhoea.

Exposure to young dogs or cats is associated with *Campylobacter* organisms. Exposure to turtles is associated with *Salmonella* organisms.

2.17 PHYSICAL EFFECTS OF DIARRHOEA

The following may be observed:

- Dehydration
    - Dehydration is the principal cause of morbidity and mortality.
    - Assess every patient with diarrhoea for signs, and symptoms.
    - Lethargy, depressed consciousness, sunken anterior fontanel, dry mucous membranes, sunken eyes, lack of tears, poor skin turgor, and delayed capillary refill are obvious and important signs of dehydration (Martinez *et al.*, 1999).
- Failure to thrive and malnutrition
    - Reduced muscle and fat mass or peripheral edema may be clues to the presence of carbohydrate, fat, and/or protein malabsorption.
    - *Giardia* organisms can cause intermittent diarrhoea and fat malabsorption (Requa *et al.*, 1990).
- Abdominal pain
    - Nonspecific nonfocal abdominal pain and cramping are common with some organisms.
    - Pain usually does not increase with palpation.

- With focal abdominal pain worsened by palpation, rebound tenderness, or guarding, is alert for possible complications or for another noninfectious diagnosis (Greenberg *et al.*, 2009).

- Borborygmi: Significant increases in peristaltic activity can cause an audible and/or palpable increase in bowel activity.
- Perianal erythema
  - Frequent stools can cause perianal skin breakdown, particularly in young children.
  - Secondary carbohydrate malabsorption often results in acidic stools.
  - Secondary bile acid malabsorption can result in a severe diaper dermatitis that is often characterized as a "burn" (Loureiro, 1998).

CHAPTER THREE

3. 0 MATERIALS AND METHODS

3.1.0 STUDY BASE

The study was based at General Hospital, Minna, and Maternal and Children Hospital old airport road Minna.

3.1.1Study Population

The study population included infants and young children between 0 – 5years attending the Paediatric unit and Out Patient Department (OPD) of the General Hospital in Minna, Nigeria, and Maternal and Children Hospital, Minna.

A total of 176 diarrhoea stool samples were collected. Informed consent was obtained from patients' mothers, hospital authorities, laboratory technician and

Clinicians involved in the management of the patients examined.

3.1.2 Sample Collection

Stool samples were collected from the patients in clear, transparent, wide mouthed bottles. Information were also obtained from each patient regarding age, sex, feeding patterns, types of apartment occupied by the parents, source of their drinking water and maternal education.

The sample size of the stool samples collected for the research work was determined by the formula below.

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

Description

n = required sample size

t = Confidence level at 95% (standard value of 1.96)

p = Prevalence rate of the disease (9%)

m = Margin of error at 5% (Standard value of 0.05)

The samples were transported to the Microbiology laboratory Federal University of Technology, Minna immediately after collection in a cold flask containing ice block.

3.2 Processing of Specimens

The specimens were processed according to the guidelines provided by Cheesbrough (2004) for the laboratory diagnosis of enteric pathogens. These include: macroscopy, microscopy, Gram's stain, motility testing, culture and biochemical tests.

3.3 MACROSCOPIC EXAMINATION

The stool samples were examined macroscopically and the appearances, consistency and colour were recorded.

3.4 CULTURING THE SPECIMEN

A loopful of liquid stool or fecal suspension was inoculated by streak method on the following media:

1 .MacConkey agar (MAC)

2. Desoxycholate citrate agar (DCA)

3. Sorbitol MacConkey Agar

The plates were incubated at 37°C for 24hrs. The isolates were purified and stored on nutrient agar slant for further characterization.

3.5 CHARACTERIZATION OF THE ISOLATES.

The isolates were characterised based on the following biochemical tests.

3.5.1 Citrate utilisation test

The pure isolates were inoculated on Simmons citrate agar and incubated at 37°C for 24hrs. A change in colour from blue to green signified positive result.

3.5.2 Indole test

Each isolate was inoculated in 3ml of sterile tryptone water and incubated at 37°C for 48hrs. About 0.5ml of Kovac's reagent was then added after incubation and shaken gently. Production of red surface layer within 10mins signified positive result.

3.5.3 Hydrogen sulphide production and Gas production test

The isolates were individually inoculated into triple sugar iron agar and inoculated at 37°C for 24hrs. The results were read for hydrogen sulphide production, gas production and metabolism of the sugars.

3.5.4 Motility test

A semi solid nutrient agar medium was inoculated by stabbing method with the isolate and was incubated at 37°C for 24hrs. Growth is confined to the line of inoculation for non motile organisms and diffused hazily for motile organisms.

3.5.5 Urease test

The test organism was inoculated on a sterile urea agar medium and incubated at 37°C for 24hrs. Production of pink coloration indicated positive result.

3.5.6 Carbohydrate fermentation test

The isolates were inoculated into test tubes containing peptone water and specific sugar with phenol red as an indicator. The sugars tested were Glucose, Lactose, Manitol, and Sucrose. A change of colour from straw yellow to pink indicated fermentation of the sugar.

3.5.7 Voges-poskauer test

The isolate was inoculated into sterile peptone water and incubated at 37°C for 24hrs. About 2ml of growth suspension was measured into a sterile test tube and 1ml of 10% ethanol and 2ml of 40% potassium hydroxide were added and the tube was left for1hhr. Formation of pink ring at the top of the test tube indicated a positive result.

3.5.8 LYSINE DECARBOXLASE TEST

The test isolate was inoculated into a medium of peptone water and lysine (amino acid) with bromocresol purple as an indicator. The medium was sealed with paraffin oil and incubated at 37°C for 72hrs. A colour change to purple indicated positive result.

3.6 SEROLOGICAL IDENTIFICATION OF THE ISOLATES

A drop of normal saline was placed on a clean grease-free slide. A colony of the test organism was picked from nutrient agar and was emulsified to a smooth emulsion in the drops of saline. A drop of *Salmonella paratyphi* groupA polyvalent antiserum was added to the emulsion and mixed with a sterile wire loop. The suspension was mixed further by titling to and fro for 1minute while viewing against black background. Distinct clumping indicates positive agglutination test.

3.7 Antimicrobial Susceptibility Testing

A colony of test organism was inoculated in to nutrient broth and was incubated at 37°C for 2hrs. A sterile swab stick was dipped into the nutrient broth. The swab was pressed against the test tube to reduce excess broth. The swab stick was then used to inoculate on a sterile nutrient agar. Multiple antibiotic susceptibility disc was carefully picked with sterile forceps and placed on the inoculated plate. The plate was incubated at 37°C for 24hrs. The antibiotics used were Septrin (30ug), sparfloxacin (25ug), amoxacillin (25ug), augmentin (30ug), gentamycin (25ug), pefloxacin (25ug), tarivid (30ug), streptomycin (10ug), and chloramphenicol(30ug).

CHAPTER FOUR

4.0 RESULTS

A total of 176 diarrhoeal stool samples were examined for the presence of different enteric bacteria in children between the ages of 0 – 5 years. Of the total number of 253 isolates examined, 139 (54.94%) were identified as *Escherichia coli,* 49(19.37%) *Shigella* species, 42 (16.60%) *Salmonella* species, and 8 (3.16%) *Citrobacter* species. Others were *Enterobacter* species11 (4.34%), and *Vibro cholereae* 4 (1.58%).38 (15%) samples did not yield any growth. Figure 1 shows the frequency of occurrence of bacteriogical agents in examined samples. The highest number of isolates was observed for *E. coli*, 139(54.94%), There was no bacterial growth in 38 (15%) of the samples which suggested that the cause of the diarrhoea might be due to other causes other than bacteria.

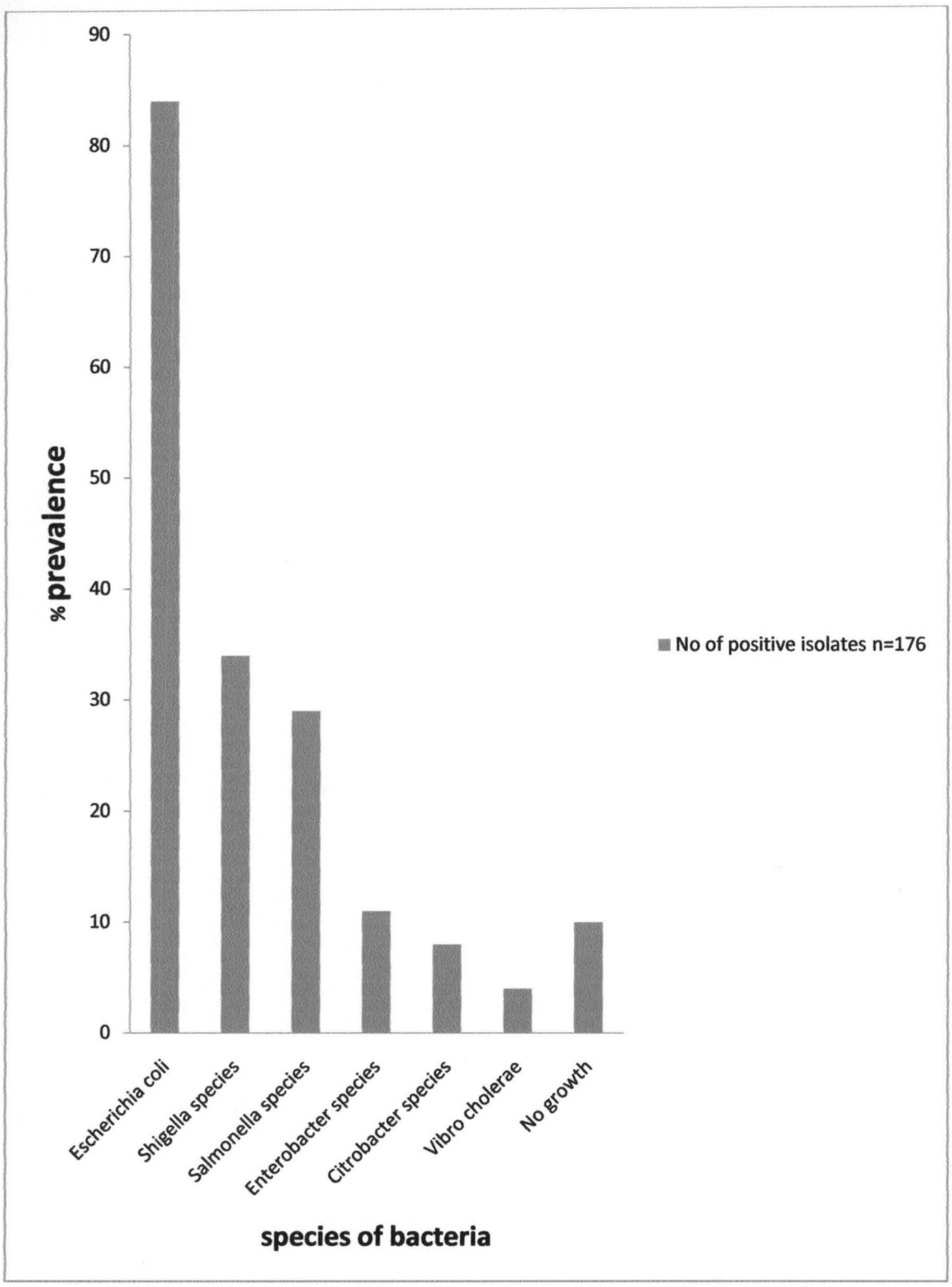

Fig 4.1: Prevalence of enteropathogenic bacteria in children.

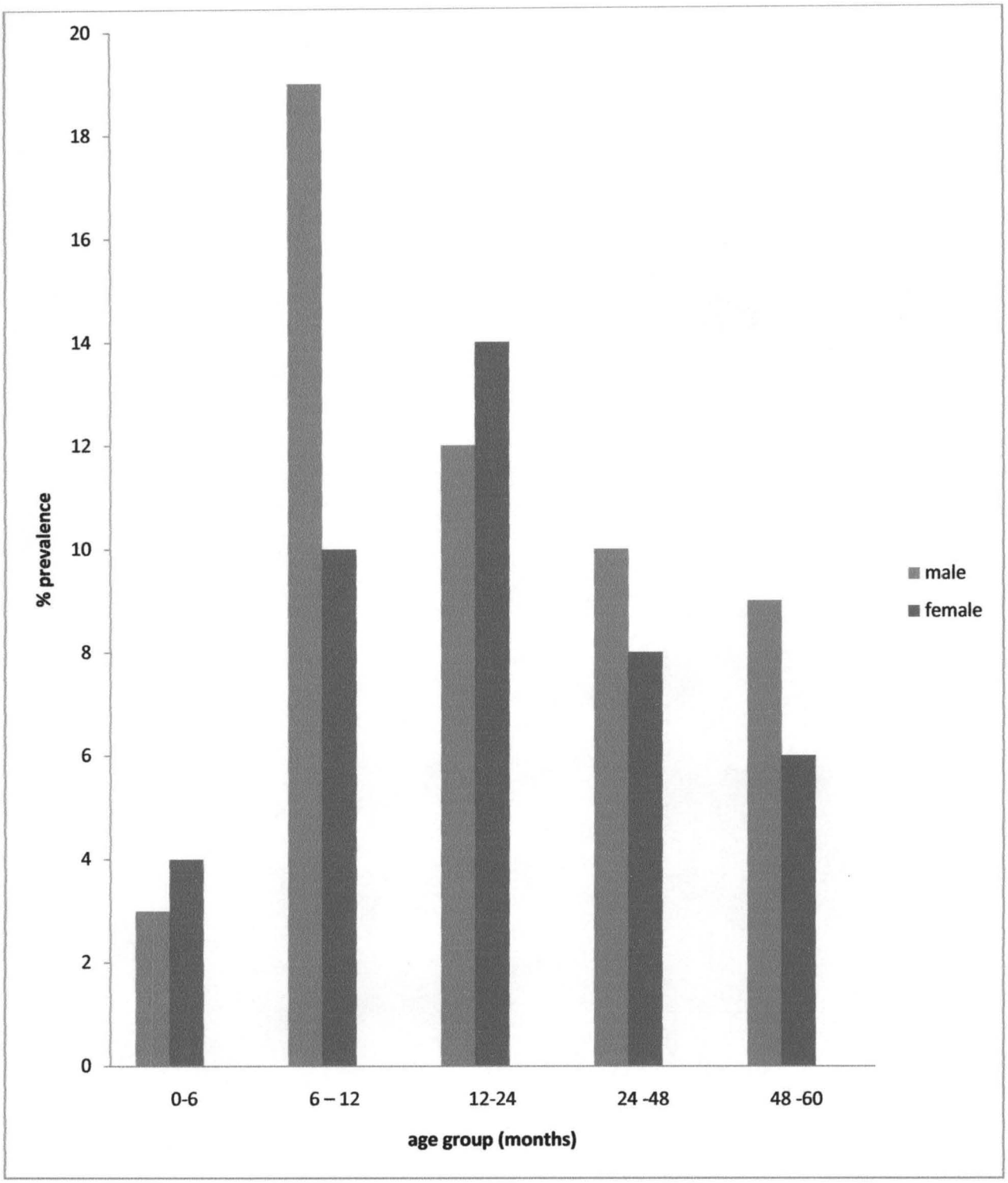

Fig 4.2: Prevalence of E.*coli* in relation to age and sex of the children.

The age group 6 –12 month had the highest percentage prevalence 59 (88%) for E.*coli* and was followed by the 12 – 24 month age group which had 34 (80.04%) isolates. The age group 24-48 months had the prevalence of 75% while those aged 0-6 months had the lowest number of *E.coli* isolates. The difference between these groups was found to be statistically significant (P>0.05).

The age group 6 –12 months had the highest percentage prevalence 21 (31.34%) for *Shigella* species, and was followed by the 24 – 48 month age group which gave 6 (25.80%) isolates. The lowest number of isolates was recorded for the 48 – 60 month age group 4 (19.04%). No isolate was recovered from 0- 6 months age group.

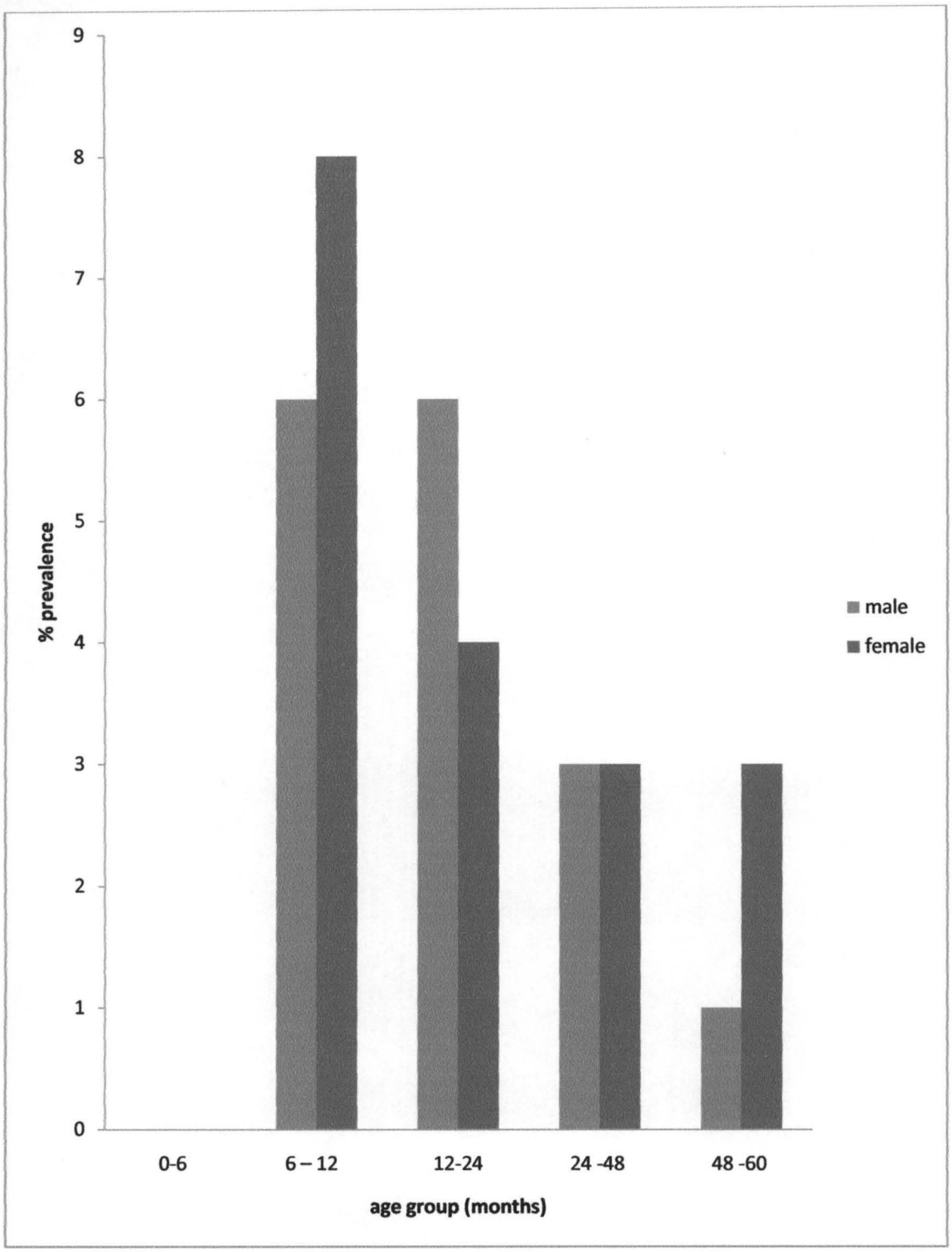

Fig 4.3: Prevalence of *Shigella* species in relation to age and sex of the children.

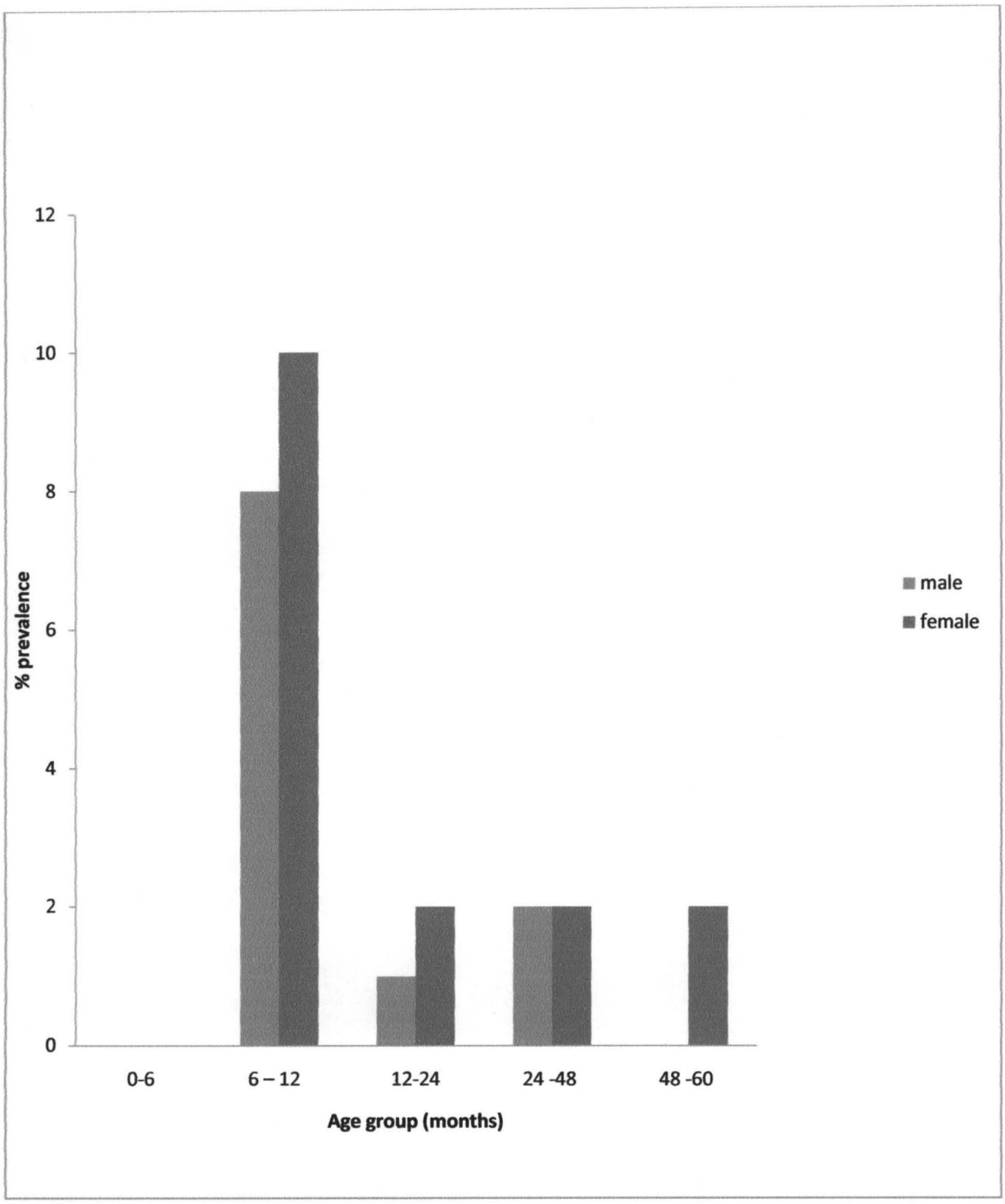

Fig 4.4: Prevalence of *Salmonella* species in relation to age and sex of the children.

The age group12-24 months had highest percentage prevalence 18 (42.86%) for *Salmonella* species. The 6 – 12 month age group had prevalence of 18 (26.87%). No isolate was recovered from 0- 6 months age group.

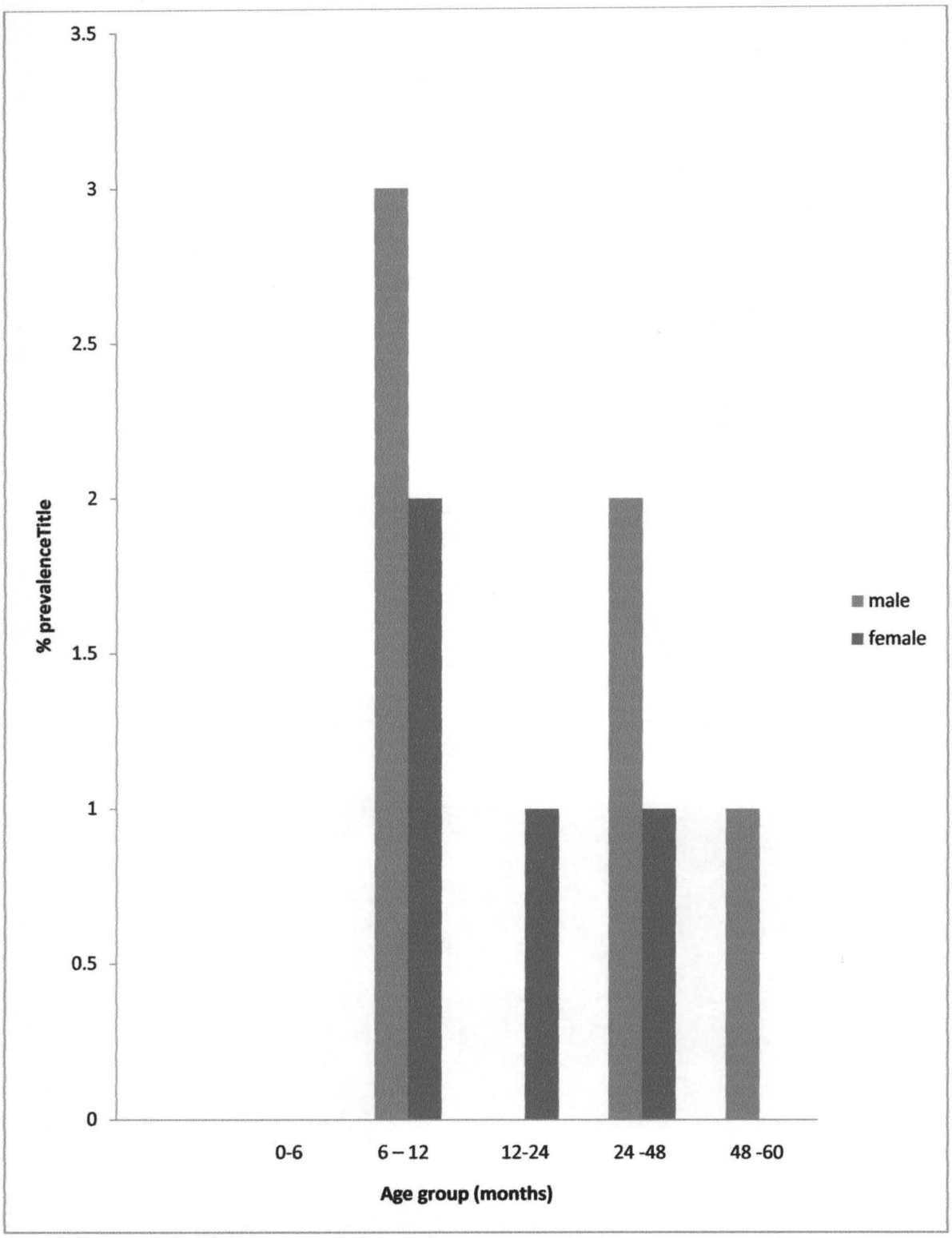

Fig 4.5: Prevalence of *Enterobacter species* in relation to age and sex of the children.

The age group 24-48 months had highest percentage prevalence 3 (12.50%) for *Enterobacter species*. The 6 – 12 month age group which produced 5 (7.46%) isolates, and no isolate was recovered from 0- 6 months age group.

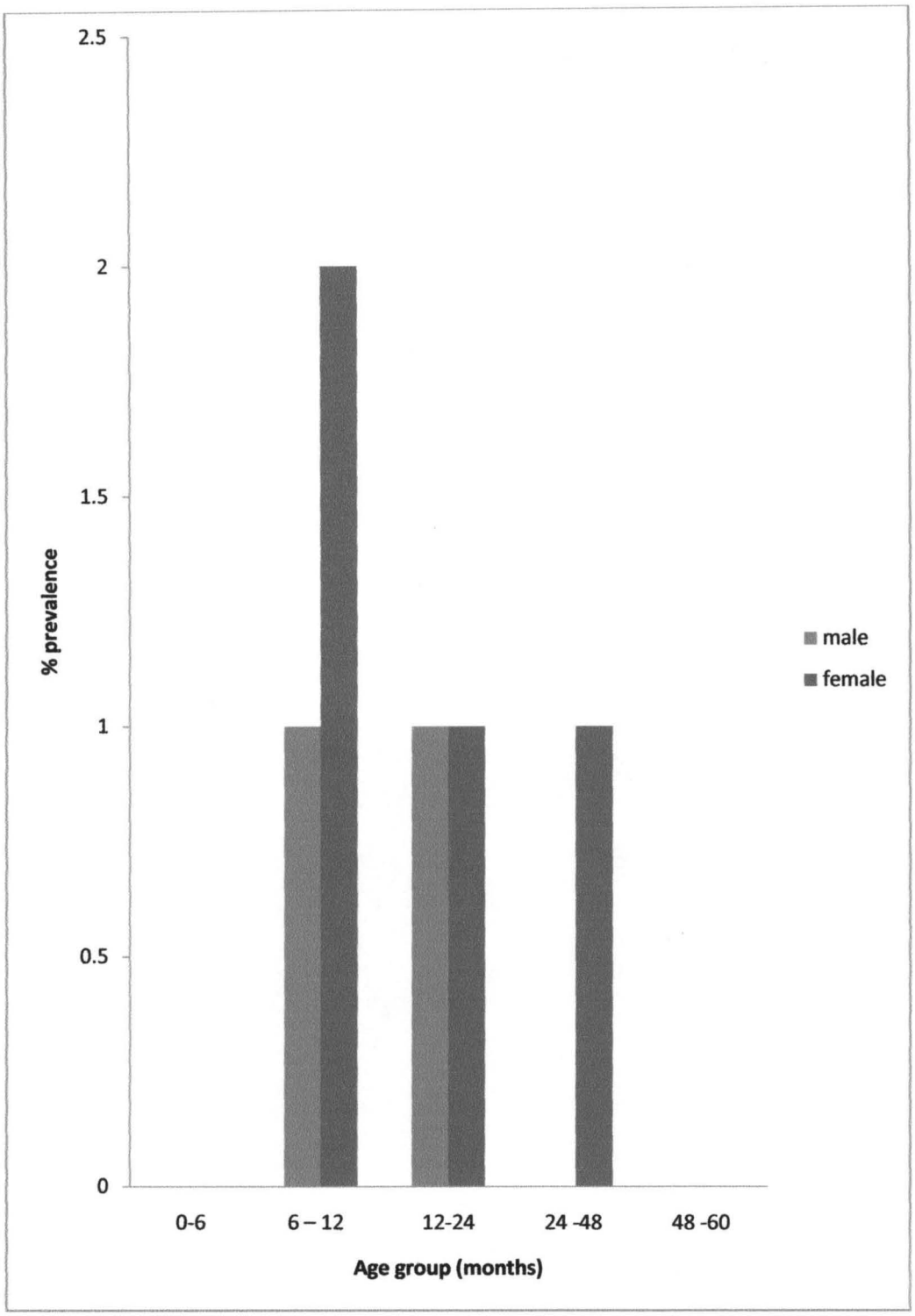

Fig 4.6: Prevalence of *Citrobacter* species in relation to age and sex of the children.

The age group 6-12 months had the highest prevalence percentage 5 (11.90%) for *Citrobacter* species with the female having slightly higher percentage than the male. The 12 – 24 months age group which produced 1 (4.16%) isolates. No isolate was recovered from 0- 6 and 48 -60 months age group.

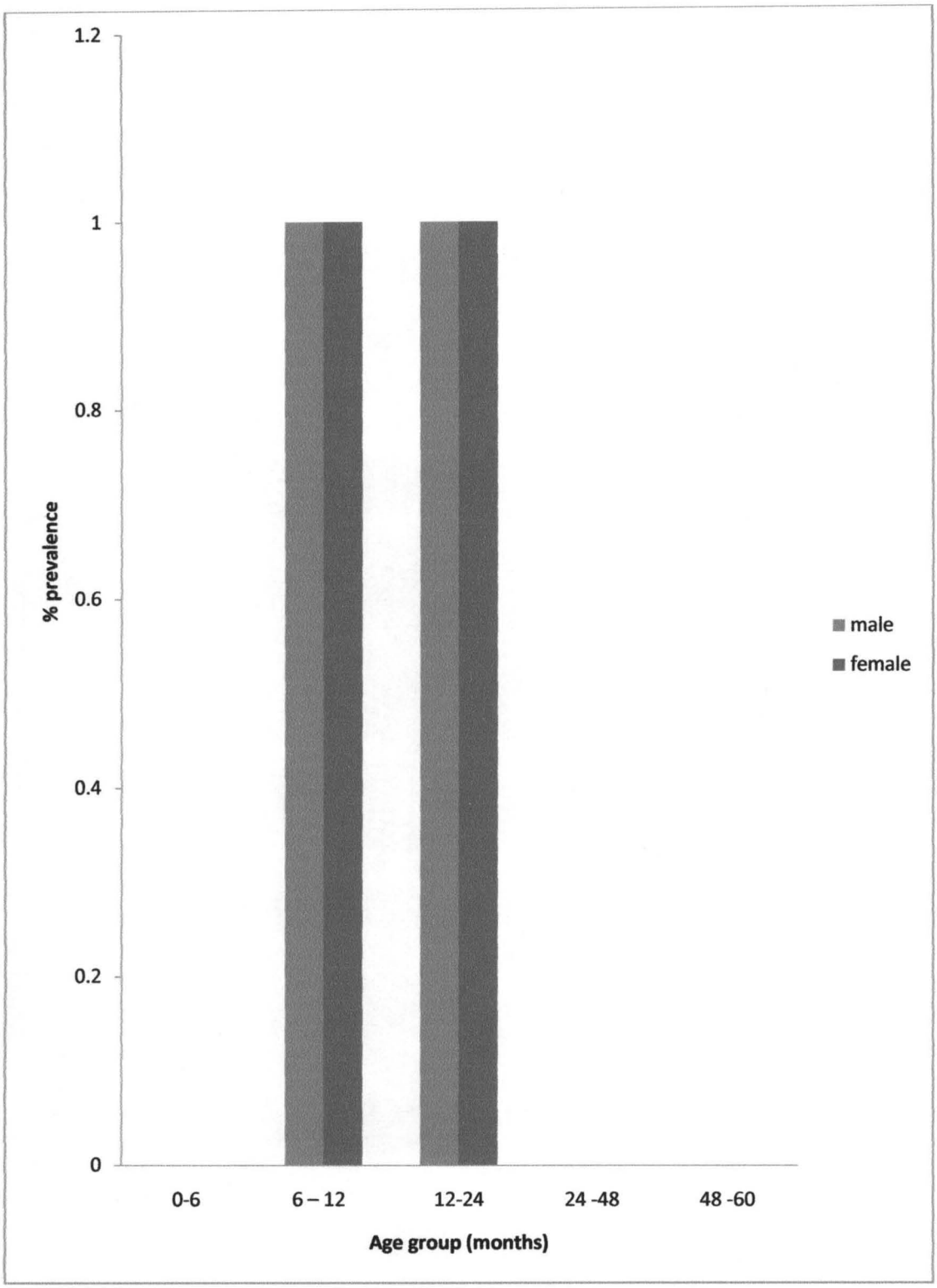

Fig 4.7: Prevalence of *Vibro cholerae* in relation to age and sex of the children.

The age group that has highest percentage 1 (4.76%) of positive isolates of *Vibro cholerae* species was 48 -60 month age group, and was followed by the 6 – 12 month age group which produced 2 (2.99%) isolates, and followed by age 12-24 months which produces 1(2.38%) of the isolates. No isolate was recovered from 0- 6 and 24 -48 months age group.

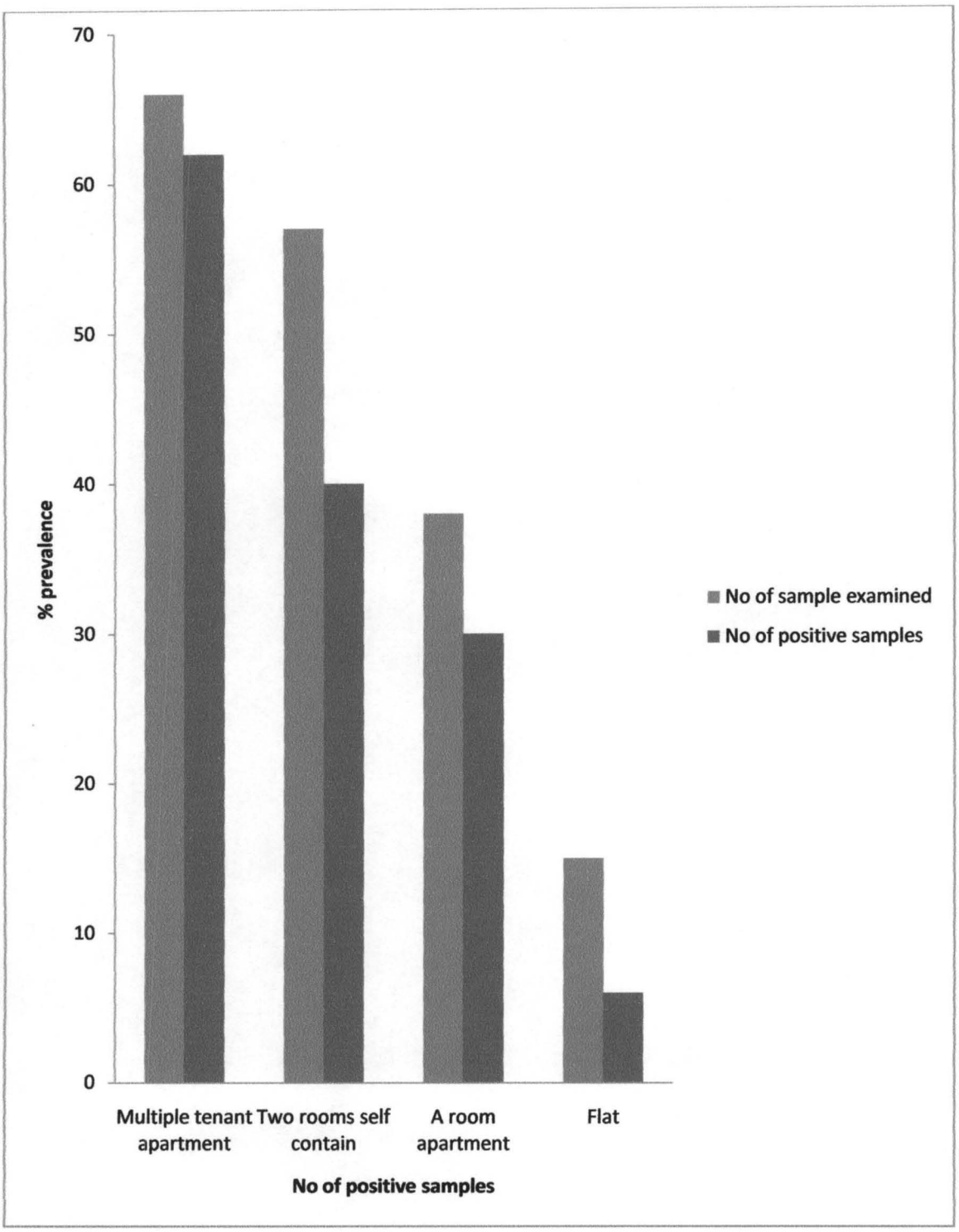

Fig 4.8: Prevalence of diarrhoea in relation to types of residence of the parents.

Children of parents living in multiple tenant apartments had the highest percentage prevalence 66 (93.94%), and were followed by those whose parents live in a room apartment with 30 (78.94%) percentage prevalence rates. The lowest prevalence rates were recovered from children whose parents lived in flat apartment.

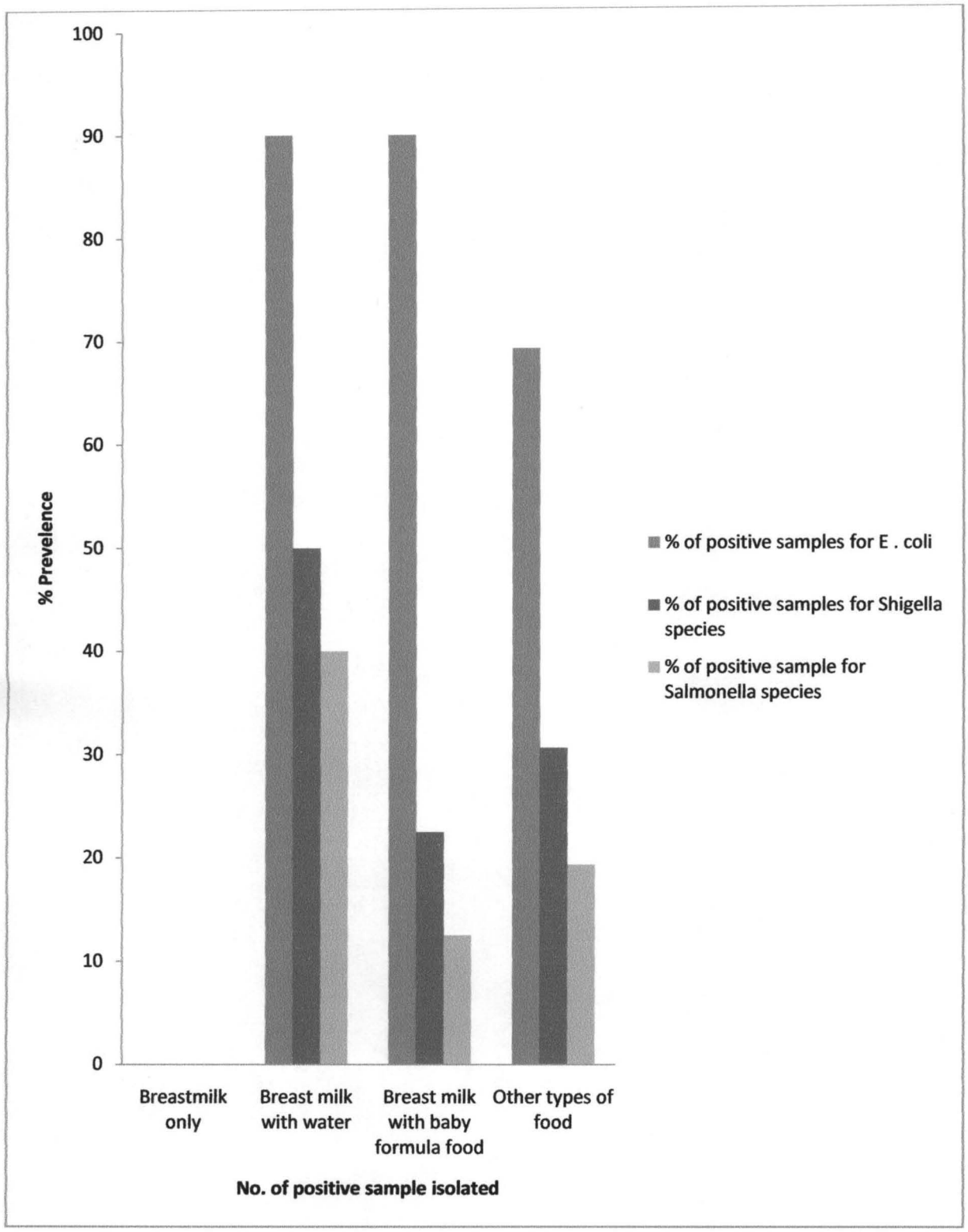

Fig 4.9: Feeding pattern of infants in relation to frequency of diarrhoea.

Children who had mixed feeding (either with water or baby formula food) yielded the highest number, with a total of ninety (90.00% positive for *E.coli*) isolates, 10 (50%) and 18 (22.5%) for *Shigella* species, and *Salmonella* species respectively while those who were not breast-fed had (69.35%, 30.65%, 19.35% positive) for *E.coli*, *Shigella*, and *Salmonella* respectively. No isolates were recovered from exclusively breast fed children from 0-6 months of age. The difference was found to be statistically significant ($P<0.05$).

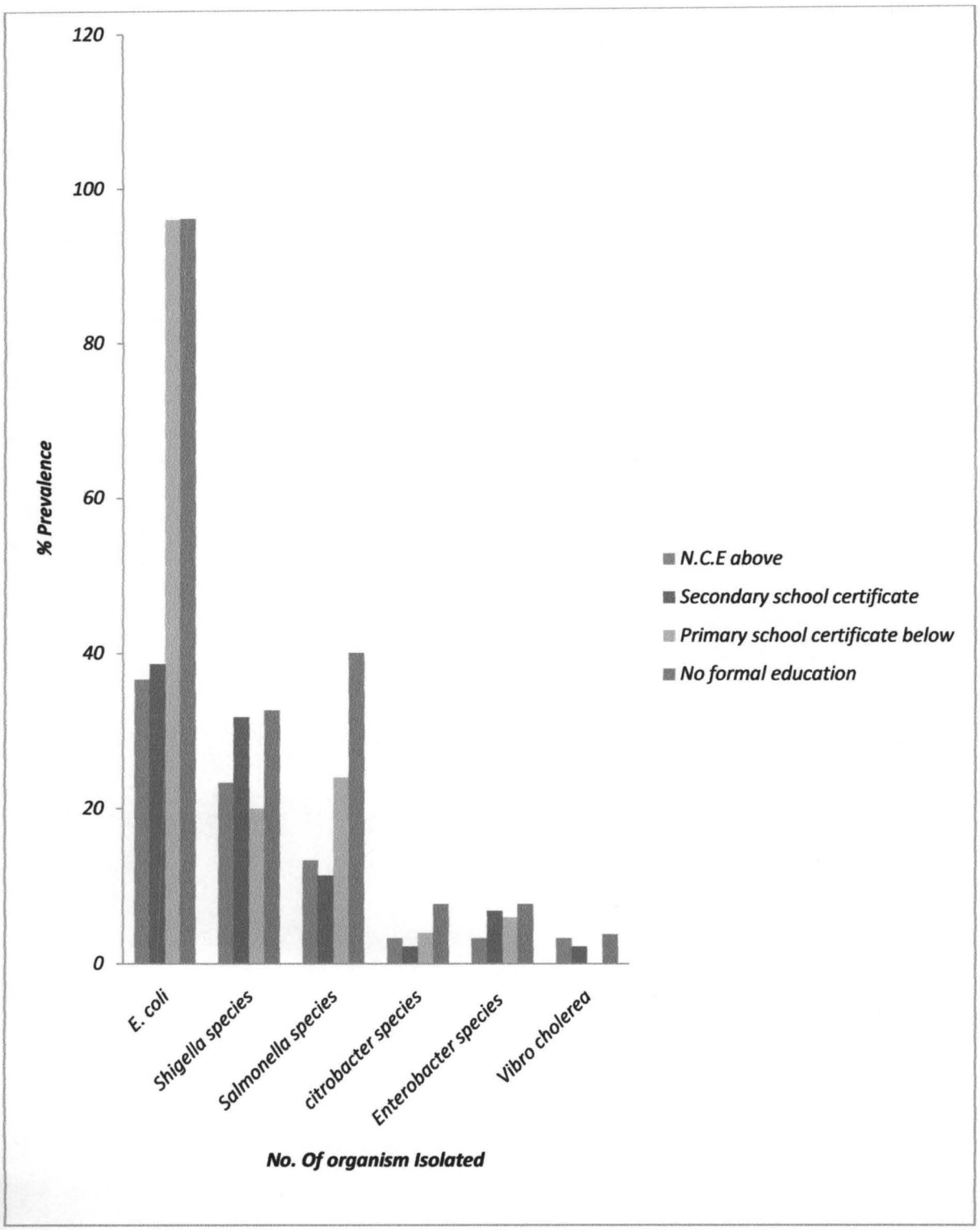

Fig 4.10: occurrence of diarrhoea in relation to mother's literacy level

The relationship between maternal education level and number of bacteria isolated was presented in fig 10. Prevalence rate for infants whose mother had no formal education was highest for the enteropathogenic bacteria. This was followed by infants whose mothers had only received primary school education tested positive. The lowest percentage prevalence was from children whose mother had post NCE qualifications The results of this analysis were not statistically significant.

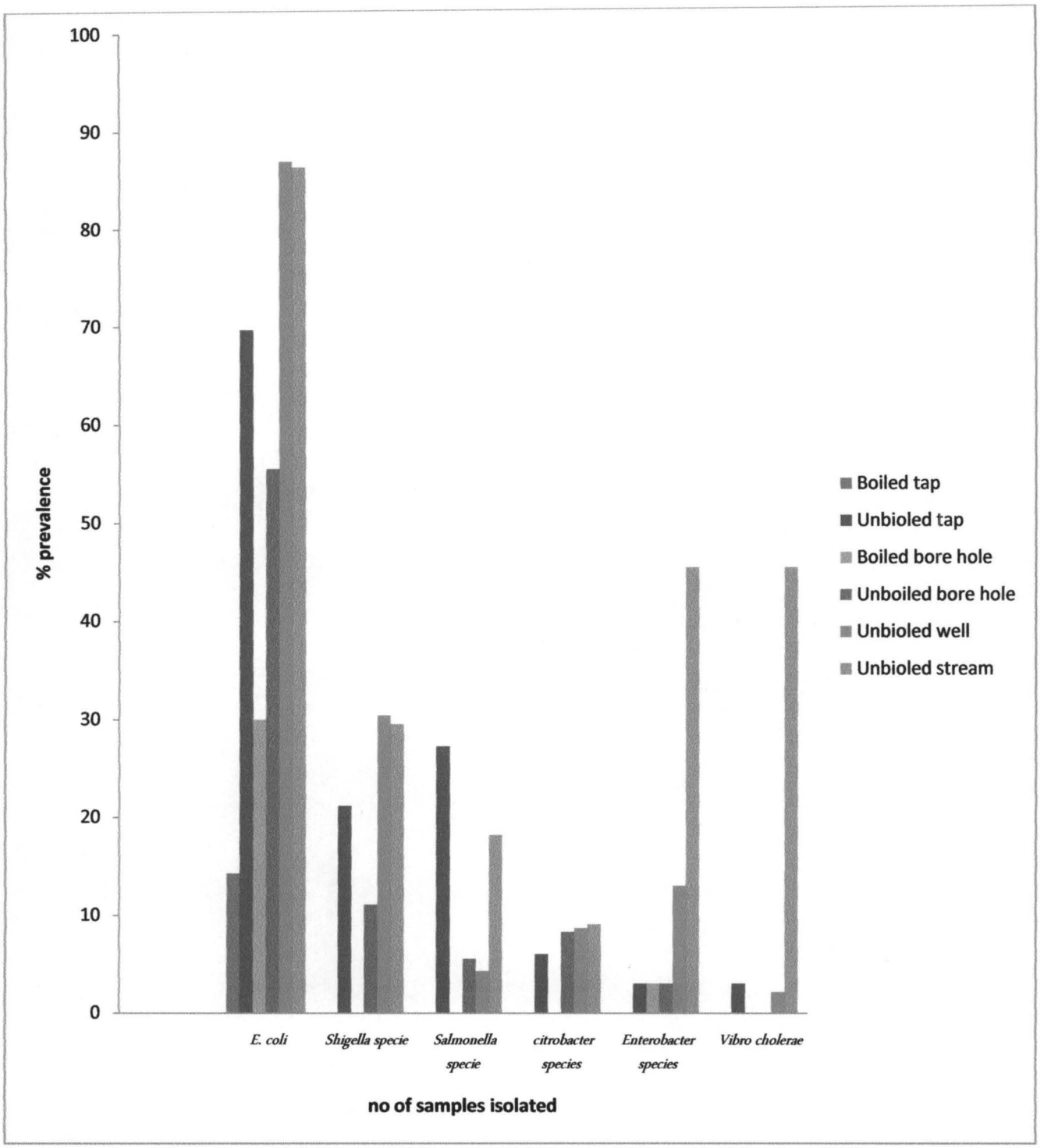

Fig 4.11: Occurrence of diarrhoea in relation to source of drinking water.

The highest prevalence rate of isolates of each of the isolated bacteria were from children whose parents used unboiled bore hole water and unboiled stream water. Children whose parents prefer to boil their water before drinking had the lowest prevalence rate.

BIOCHEMICAL RESULTS

About 51% of the isolates were *E. coli* 0157. 66% of the examined samples yeilded *Shigella dysenteriae* while 30% for *Shigella flexneri.*

SEROTYPING RESULTS

72% of *Salmonella* species isolates were positive for *Salmonella* paratyphi A

19%were positive for *Salmonella* paratyphi B and 8% for *Salmonella* typhi.

SUSCEPTIBILITY PROFILE

All isolates of E.*coli were* moderately sensitive to septrin, sprafloxacin amoxicillin, augmentin, gentamycin, pefloxacin, and tarivid. About 89% of the isolates were resistant to chloramphenicol and streptomycin and 91% were highly sensitive to amoxicillin and ciprofloxacin.

All isolates of *Salmonella species* were resistant to chloramphenicol streptomycin, and ciprofloxacin. 70% were highly sensitive to septrin, sprafloxacin, augmentin, gentamycin, and pefloxacin. 86% of *Salmonella* isolates are moderately sensitive to amoxicillin and tarivid.

All *Shigella species* isolates were moderately sensitive to sprafloxacin, chloramphenicol, gentamycin, pefloxacin, and tarivid, 56% were highly sensitive to amoxicillin and ciprofloxacin, 45% were resistant to septrin and streptomycin.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION OF RESULTS

A total of 176 diarrhoeal stool samples were examined for the presence of enteric bacteria in children between the ages of 0 – 5 years. Out of the total number of 253 isolates, 139 were E. *coli*, 49 were *Shigella* species, 42 were *Salmonella* species, 8 were *Citrobacter* species, 11 were *Enterobacter* species, 4 were *Vibro Cholera* species, and there were no growth in 38 (15%) of the samples (fig4.1).

The prevalence rate of E.*coli* was 2.5 times higher than that of *Shigella* which are the major bacteriological causes of diarrhoea in children below five years of age. This is higher than the 26% prevalence rate document by Olanipekun (1996) for children with diarrhoea attending the Jos University Teaching Hospital in Jos, Nigeria and 15% by kandakai- Oluyemi for children attending the Abuja national hospital but similar to report by Rotimi *et al.(1994)* for children attending Obafemi Awolowo University Teaching Hospital in Ile-ife and several other international authors who reported that the prevalence rate of E.*coli* is 2.5 higher than that of *Shigella* which are the major bacteriological causes of diarrhea in children below two years (King *et al,*. 2003, Longstreth *et* al., 2006).

Figures 4.2-4.5 show distribution of each bacteria species in relation to age of the children examined. From figures 4.2-4.5 the highest isolation rates were found in children between the ages of 6 – 12 months followed by age group12-24 months. This finding is similar to reports published by Olanipekun (1996) and Rotimi *et al., (1994)* and several authors (King *et al,*. 2003, Longstreth *et* al., 2006) who observed that the highest incidence of gastroenteritis in children was found within the age range of 7 – 12 months. During this period, children are weaned and start moving around putting few contaminated toys and unguided things into their mouths. Figure 5

shows the distribution of *Enterobacter* species in relation to the age of the children examined. The highest isolation rates were found in children between the ages of 24 -48 months followed by age group 0-6 months.

Figure 4.6 shows the distribution of *Citrobacter* species in relation to the age of the children examined. The highest isolation rates were found in children between the ages of 12 -24 months followed by age group 24-48 months. Figure 6 shows distribution of *Vibro cholereae* in relation to the age of the children examined. The highest isolation rates were found in children between the ages of 48- 60 months followed by age group 6- 12 months. Most cases of diarrhoea are not sex specific. Females had a higher occurrence in some cases while in some males had higher percentage prevalence.

No bacteria were isolated from 0 -6 months age group. One of the reason for this is because the percentage of mothers that breast fed their children exclusively is higher than those that breast fed with either water or baby formula food reported

to contain high levels of immunoglobulin A (IgA) antibodies against bacteria (Cravioto *et al.*, 1998).

Figure 4.8 shows the prevalence of diarrhoea in relation to types of residence of the parents. The children whose parents live in multiple tenant apartment had the highest percentage 66 (93.94%) of isolates, and was followed by the parents living in one room apartment which recorded 30 (78.94%) isolates. The lowest isolates were recovered from children of parents occupying flat apartments possibly due to the enclosed nature of the apartment. The high percentage of positive cases was found in children whose parents occupied multiple tenant apartments because in such environment, there is possibility of having a lot of children and this might promote transmission of diarrhoea. Poor hygiene during food preparation may also contribute to increased gastroenteritis around the environment. The parents prepare their food in an open place and the

children share whatever they have among themselves without washing their hand or sanitizing their environments.

Figure 4.9 shows the relationship between feeding patterns and the prevalence diarrhoea. Children who had mixed feeding had the highest prevalence rate. No isolates were recovered from exclusively breast fed children, and the difference was found to be statistically significant ($P<0.05$).

Breast milk (colostrums) from mothers living in endemic areas has been reported

By Ogunsola and Adenuga (2009) to contain high levels of immunoglobulin A (IgA) antibodies. Bacteria were not isolated from infants younger than 6 months old because of the feeding pattern adopted by their parents (exclusive breast feeding. Data obtained in this study indicate that all children below six months old were exclusively breast fed, whereas those between 7 – 12 months had their breast feeding interrupted with mixed feeding, if they had not stopped completely. These findings therefore corroborate findings from previous studies by Ogunsola and Adenuga (2009) regarding the protective role of breast milk against bacterial gastroenteritis. Faulty weaning practices and poor hygiene during food preparation may also contribute to increased gastroenteritis around the age of 7 – 12 months. The low isolation rate of *E.coli* in children older than 12 months may be associated with the development of immunity or the loss of receptors for some specific adhesion molecules.

The relationship between maternal education level and occurrence of diarrhoea is presented in figure 4.10. Infants whose mother either had no formal education or primary school certificate had higher prevalence rate. Illiteracy of mothers has been reported to be a predisposing factor that contributes to infants and young children acquiring the infection.

Figure 4.11 illustrates the relationship of sources of drinking water to the occurrence of diarrhoea. There was higher prevalence rate among children whose parents used either unboil

well water or stream water. Few of the reasons for high prevalence rate include poor hygienic standard of handling drinking water; poor sanitation and the drinking of untreated water by the children. Several authors have reported that stream and well water contains several bacteriological and parasitic agents that cause diarrhoea in developing countries Nigeria inclusive (Cohen, 2000). Children whose parents use boiled water had the lowest prevalence rate. The highest number of E.*coli* isolated belonged to the 0157 sero-group. 0157 sero-group has been recognized by the World Health Organization (WHO) to be one of the major causes of diarrhoea. However, due to the unavailability of the monovalent typing sera, further specific typing was not carried out to show which serotype occurs more frequently in infantile diarrhoea within the study's geographic area.

The antimicrobial susceptibility profile shows that high rates of resistance were recorded for streptomycin,septrin, chloramphenicol . Several reports have indicated that these drugs are also less effective against other bacterial agents isolated in Nigeria, largely because they are inexpensive and can be obtained easily without a doctor's prescription (Chatkaeomorakot *et al.*, 1987).

Resistance is probably due to indiscriminate antibiotic usage (drug abuse) which could result in plasmid mediated antibiotic resistance found to be common in *Escherichia coli*. All isolates were highly sensitive to ciprofloxacin, amoxicillin, and pefloxacin. These antimicrobial agents are expensive, cannot be easily purchased without prescription. These are few of the reasons why these drugs are highly effective in treating these infections. Fortunately, diarrhoea is usually self-limited and rehydration is the most effective treatment.

The use of antibiotics in general is of minor importance and has been criticized on the grounds of drug toxicity and the risk of increased wide-spread antimicrobial resistance.

5.2 CONCLUSION

This study has shown that *Escherichia coli* is the commonest cause of infantile diarrhoea in Minna, Nigeria, and that the species isolated were moderately sensitive to septrin, sprafloxacin amoxicillin, augmentin, gentamycin, pefloxacin, and tarivid. All isolates were resistant to chloramphenicol and streptomycin but were highly sensitive to amoxicillin and ciprofloxacin. Therefore, amoxillin and ciprofloxacin should be used in the treatment of E.*coli* infections in the study area.

In addition, the findings indicate that children feeding pattern, poor personal hygiene, type of apartment occupied by the parents, and source of drinking water contribute to the incidence of diarrhoea in children.

5.3 RECOMMENDATIONS

Government should give rehydration salts to women without charging for money and be trained on proper preparation. There should be effective legislation by the government to control the indiscriminate purchase of antibiotics to prevent its abuse in Nigeria. Mothers should be encouraged to practice good personal hygiene and to breast-feed infants for at least 12 months because of the protective role that breast milk plays against bacterial gastroenteritis. Environmental sanitation, good hygiene, treatment of drinking water and washing of hand before and after eating and defecations should be preached and encouraged between women especially those that live in highly populated area.

REFERENCES:

Alam, N.H., Ashraf, H. (2003). "Treatment of infectious diarrhoea in children". *Journal of Paediatric Drugs* 5 (3): 151–65. PMID 12608880.

Bauer, A., Kirby, W.M.M., Sharris, J.C., Truck M. (1988). Antibiotic susceptibility testing by standardized single disk method. *American Journal of Clinical Pathology* ; 45: 493–496.

Bruyn, G. (2008). "Diarrhoea in adults (acute)". *Clinical Evidence journal*. PMID 19450323.

Chatkaeomorakot, A., Echeverria, P., Taylor, D.N. (1987). Hela cell – adherent E. coli in children with diarrhoea in Thailand. *Journal of Infectious Diseases;* 160: 243–247.

Cheesbrough, M. (2004). District Laboratory practice in Tropical countries Part 2. Cambridge University Press, Cambridge: 178–180.

Chen, C.Y., Chen, W.C., Chin, S.C., Lai, Y.H., Tung, K.C., Chiou, C.S., Hsu, Y.M., Chang, C.C. (2010). Prevalence and antimicrobial susceptibility of salmonellae isolates from reptiles in Taiwan. 44-50

Cohen, M.B. (2000). Etiology and mechanisms of acute infectious diarrhoea in infants in the United States. *Journal of Paediatric Drugs*; 118:S34.

Cravioto, A., Reyes, R.and Ortega, R. (1998). Prospective study of diarrhoeal disease in a cohort of rural Mexican children: Incidence and isolated pathogens during the first two years of life. *Journal of Epidemiology Infectious disease*, 101: 123-134.

Cravioto, A., Villafan, H. and Ruiz, J. (1991). Introduction of localized adhesion of EPEC to HEp-2 cells by immunoglobin and oligosaccharide fractions of human colostrums and breast milk. *Journal of Infectious disease;* 163: 1247–1255.

Dans, L. and Martínez, E. (2006). "Amoebic dysentery". Clinical Evidence (15): 1007–13.

De la Torre, E., Tello, M., Mateu, E. M., Torre E. (2005). Use of Biochemical Kinetic Data To Determine Strain Relatedness among *Salmonella enterica* subsp. enterica Isolates. *Journal of Clinical Microbiology* 43: 5419-5424

Dryden, M.S., Gabb, R.J., Wright, S.K. (1996). "Empirical treatment of severe acute community-acquired gastroenteritis with ciprofloxacin". *Clinical Infectious Disease Journal* 22 (6): 1019–25.

DuPont, H.L., Ericsson, C.D. and Farthing, M.J. (2009). "Expert review of the evidence base for self-therapy of travelers' diarrhoea". *Journal of Travel Medicine* 16 (3): 161–71.

DuPont, H.L.and Hornick, R.B. (1973). "Adverse effect of lomotil therapy in shigellosis". JAMA 226 (13): 1525–8.

Elmahde, E.H. (1987). Epidemiology of diarrhoeal diseases in Kartoum Metropolitan area. *Journal of Tropical Paediatric Disease*; 33: 315–317.

Fadeel, M. A., Crump, J. A., Mahoney, f. J., Nakhla, I. A., Mansour, A. M., Reyad, B., El melegi, D., Sultan, Y., Mintz, E. D., Bibb, W. F. (2004). Rapid diagnosis of typhoid fever by enzyme-linked immunosorbent assay detection of salmonella serotype typhi antigens in urine. *American Journal of Tropical Medical Hygiene* 70: 323-328

Foley, S. L.and Lynne, A. M. (2008). Food animal-associated Salmonella challenges: Pathogenicity and antimicrobial resistance. *Journal of Animal Science* 86: E173-E187

Gomes, T.A.T., Rassi, V., MacDonald, K.L. (1991). Enteropathogenic *E. coli* associated with acute diarrhoea in urban infants in Sao PauloBrazil. *Journal of Infectious disease;* 164: 331–337.

Gonzales, M.L., Dans, L.F. and Martinez, E.G. (2009). "Antiamoebic drugs for treating amoebic colitis". *Cochrane Database of Systematic Reviews(2):*

Greenberg, H.B. and Estes, M.K. (2009). "Rotaviruses: from pathogenesis to vaccination". Gastroenterology 136 (6): 1939–51.

Guard-Bouldin, J., Morales, C. A., Frye, J. G., Gast, R. K., Musgrove, M. (2007). Detection of *Salmonella enterica* Subpopulations by Phenotype Microarray Antibiotic Resistance Patterns. *Applied Environmental Microbiology.* 73: 7753-7756

Iwamoto, M., Ayers, T., Mahon, B. E., Swerdlow, D. L. (2010). Epidemiology of Seafood-Associated Infections in the United States. *Clinical Microbiology Journal Review* 23: 399-411

Johnson, J. R., Clabots, C., Azar, M., Boxrud, D. J., Besser, J. M., Thurn, J. R. (2001). Molecular Analysis of a Hospital Cafeteria-Associated Salmonellosis Outbreak Using Modified Repetitive Element PCR Fingerprinting. *Journal of Clinical Microbiology* 39: 3452-3460

Kandakai-Olukemi, Y.T., Mawak, J.D., Ochai I.J., Olukemi, M.A. (2009). Isolation of Enteropathogenic Escherichia coli from Children with Diarrhoea Attending the National Hospital in Abuja, Nigeria. *Shiraz E-Medical Journal; 10(3):* 99-106

Kandakai-Olukemi, Y.T., Mawak, J.D., Ochai, I.J., Olukemi, M.A. (2007). Isolation of Aeromonas species from children with and without diarrhoea in Jos, Nigeria. *Africal Journal of Experimental Microbiology*; 8(1): 54-62.

Kaniuk, N. A., Vinogradov, E., Whitfield, C. (2004). Investigation of the Structural Requirements in the Lipopolysaccharide Core Acceptor for Ligation of O Antigens in the Genus *Salmonella*: *Journal of Biological Chemistry;* 279: 36470-36480

Katribe, E., Bogomolnaya, L. M., Wingert, H., Andrews-Polymenis, H. (2008). Subspecies IIIa and IIIb *Salmonellae* Are Defective for Colonization of Murine Models of Salmonellosis Compared to *Salmonella* enterica subsp. I Serovar Typhimurium. *Journal of Bacteriology.* 191: 2843-2850

Kayser, F.H., Bienz, K.A., Eckert, J., Zinkernafel, R.M. (2005). Medical Microbiology. Thieme veriag, Stuttgart. ; 194.

Kale-Pradhan, P.B., Jassal, H.K., Wilhelm, S.M. (2010). "Role of Lactobacillus in the prevention of antibiotic-associated diarrhoea: a meta-analysis". Pharmacotherapy 30 (2): 119–26.

Kasper, D.L., Braunwald, E., Fauci, A.S., Hauser, S.L., Longo, D.L., Jameson, J.L. (2005). Harrison's Principles of Internal Medicine. New York: McGraw-Hill, ISBN 0-07-139140-1.

Kebede, D., Ketsela, T., Asfaw, W. (1990). Patterns of breast feeding in Western Ethiopia and their relationship to acute diarrhoea in infants. *Journal of Paediatric;* 36: 180-183.

King, C.K., Glass, R., Bresee, J.S., Duggan, C. (2003). "Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy". *MMWR Recommendation* Rep 52 (RR-16): 1–16. PMID 14627948.

King, C.K., Glass, R., Bresee, J.S., Duggan,. (2003). Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy. *MMWR Recomm* Rep; 52:1.

Kiser, J.D., Paulson, C.P., Brown, C. (2008). "Clinical inquiries. What's the most effective treatment for giardiasis?". *The Journal of Family Practice* 57 (4): 270–2.

Lau, S. K. P., Woo, P. C. Y., Chan, C. Y. F., Woo, W.L., Woo, G. K. S., Yuen, K.Y. (2005). Typhoid Fever Associated with Acute Appendicitis Caused by an H1-j Strain of Salmonella enterica Serotype Typhi. *J. Clin. Microbiol.* 43: 1470-1472

Longstreth, G.F., Thompson, W.G., Chey, W.D., Houghton, L.A., Mearin, F., Spiller, R.C. (2006). "Functional bowel disorders". Gastroenterology 130 (5): 1480–91.

Loureiro, I., Frankel, G., Adu-Bobie J(1998). Human colostrum contains IgA antibodies reactive to enteropathonogic Escherichia colivirulence associated proteins: *Journal of Paediatrics Gastroenterology and Nutrition.*

Mahmood, D.A .and Feachern, R.G. (1987). Clinical and epidemiological characteristics of Rota virus and EPEC – associated hospital infantile diarrhoea in Basrah, Iraq. *Journal of Tropical Paediatrics* ; 33: 319-322.

Martinez, M.B., Tadder, C.R.and Ruiz-Tagle, A. (1999). Antibody response of children with enteropathogenic *Escherichia coli* infection to the bundle-forming pilus and locus of enterocyte effacement encoded virulence determinants. *Journal of Infectious disease*; 179: 269–274.

McQuiston, J. R., Parrenas, R., Ortiz-Rivera, M., Gheesling, L., Brenner, F., Fields, P. I. (2004). Sequencing and Comparative Analysis of Flagellin Genes fliC, fljB, and flpA from Salmonella. *Journal of Clinical Microbiology;* 42: 1923-1932

Medterms dictionary. "Definition of Diarrhoea". Medterms.com. http://www.medterms.com/script/main/art.asp?articlekey=2985.

Mitchell, D.K. (2009). "Astrovirus gastroenteritis". *The Pediatric Infectious Disease Journal* 21 (11): 1067–9.

Nataro, J.P. and Kaper, J.B. (1998). Diarrheogenic Escherichia coli. *Clinical Microbiology Review Journal;* 11: 142–201.

National Community for Clinical Laboratory Standards (2000). Performance standard for antimicrobial susceptibility testing eleventh informational supplement, Wayne Pennsylvania National Community for Clinical Laboratory Standards, NCCLS Documents M100- S11 .

Navaneethan, U. and Giannella, R.A. (2008). "Mechanisms of infectious diarrhea". Nature Clinical Practice. Gastroenterology & Hepatology 5 (11): 637–47.

Octavia, S. and Lan, R. (2006). Frequent recombination and low level of clonality within Salmonella enterica subspecies I. *Microbiology* 152: 1099-1108

Odukoya, D.K. and Olasupo, N.A. (1998). Drug resistance and plasmid profile of diarrhoeagenic bacteria isolated in Nigeria. *Journal of Hospital Medicine;* 7(1): 29-31.

Ogunsola F.T. and Adenuga A. (2009). Significance of of breast feeding in Nigeria. *Journal of Tropical Paediatric*; 36: 176–178.

Olanipekun, O.O. (1996). Prevalence of Enteropathogenic *Escherichia coli* in children with diarrhoea attending Jos University Teaching Hospital (MSc Thesis). Jos: University of Jos.

Onile, B.A. (1997). Rational use of antibiotic/ antimicrobial agents. *Nigerian Medical Practitioner*; 33: 14.

Parissi-Crivelli, A., Parissi-Crivelli, J. and Giron, J. (2000). Recognition of enteropathogenic *Escherichia coli* virulence determinants by human colostrum and serum antibodies. *Journal of Clinical Microbiology*; 38: 2696–2700.

Patel, M.M., Hall, A.J., Vinjé, J., Parashar, U.D. (2009). "Noroviruses: a comprehensive review". *Journal of Clinical Virology;* 44 (1): 1–8.

Pawlowski, S.W., Warren, C.A., Guerrant, R. (2009). "Diagnosis and treatment of acute or persistent diarrhoea". Gastroenterology 136 (6): 1874–86.

Requa, A.H., Bravo, V.L.P., Lead, M.G., Lobe, M.E. L. (1990). Epidemiology survey of the enteropathogenic *Escherichia coli* isolated from children with diarrhoea. *Journal of Tropical Paediatric*; 36: 176–178.

Robins – Browne, R., Still, C.S. and Miliotis, M.D., (1980). Summer diarrhoea in African infants and children. Archive of Diseases in Children; 55: 923-928.

Rotimi, V.O., Onyemefa, P.I., Banjo, T.O., Ogunsola, F.T., Adenuga, A. (1994). Significance of antibiotic resistance amongst clinical bacterial isolates in agos. *West African Journal of Medicine;* 13 (12): 81-86.

Rupnik, M., Wilcox, M.H. and Gerding, D.N. (2009). "Clostridium difficile infection: new developments in epidemiology and pathogenesis". *Nature Reviews Microbiology* 7 (7): 526–36.

Ruuska, T. and Vesikari, T. (1990). "Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes". *Journal of Infectious Disease* 22 (3): 259–67.

Schiller, L.R. (2007). "Management of diarrhea in clinical practice: strategies for primary care physicians". *Review Gastroenterology* 3: 27–38.

Spandorfer, P.R., Alessandrini, E.A., Joffe, M.D. (2005). Oral versus intravenous rehydration of moderately dehydrated children: a randomized, controlled trial. *Pediatrics* ;115:295.

Standards Stuttgart (2005). Performance standard for study of diarrhoeal disease a NCCLS Documents ; 194.

Sukhnanand, S., Alcaine, S., Warnick, L. D., Su, W.L., Hof, J., Craver, M. P. J., McDonough, P., Boor K. J., Wiedmann, M. (2005). DNA Sequence-Based Subtyping and Evolutionary Analysis of Selected *Salmonella* enterica Serotypes. *Journal of Clinical Microbiology.* 43: 3688-3698

Trabulsi, L.R., Keller, R. and Gomes, T.A.T. (2002). Typical and atypical enteropathogenic *Escherichia coli. Emergence Infectious Disease*; 8(5): 508–513.

Uhnoo, I., Svensson, L. and Wadell, G. (1990). "Enteric adenoviruses". Baillière's *Clinical Gastroenterology* 4 (3): 627–42.

Viswanatha, V.K. Hodges, K. and Hecht, G. (2009). "Enteric infection meets intestinal function: how bacterial pathogens cause diarrhoea". *Nature Reviews. Microbiology* 7 (2): 110–9.

White, A. P. and Surette, M. G. (2006). Comparative Genetics of the rdar Morphotype in Salmonella. *Journal of Bacteriology.* 188: 8395-8406

Whitfield, C., Kaniuk, N., Frirdich, E. (2003). Molecular insights into the assembly and diversity of the outer core oligosaccharide in lipopolysaccharides from Escherichia coli and Salmonella. *Innate Immunity* 9: 244-249

Williams, George Nesse, Randolph M. (1996). Why we get sick: the new science of Darwinian medicine. New York: Vintage Books. pp. 36–38. Wilson, M.E. (2005). "Diarrhea in nontravelers: risk and etiology". *Clinical Infectious Disease Journal. 41 Suppl* 8: S541–6..

Wong, C.S., Jelacic, S., Habeeb, R.L., Watkins, S.L., Tarr, P.I. (2000). "The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections". *Journal of Medicine.* 342 (26): 1930–6.

Woo, P. C. Y., Fung, A. M. Y., Wong, S. S. Y., Tsoi, H.W., Yuen K.Y. (2001). Isolation and Characterization of a *Salmonella* enterica Serotype Typhi Variant and Its Clinical and Public Health Implications. *Journal Clinical Microbiology;* 39: 1190-1194

World Health Organization (2004). "Mortality and Burden of Disease Estimates for http://www.who.int/entity/healthinfo/global_burden_disease/gbddeathdalycountry estimates.

WHO (2009). Diarrhoeal Diseases . World Health Organization. http://www.who.int/vaccine_research/diseases/diarrhoeal/en/i.

World Health Organization (2009). "Diarrhoea" http://www.who.int/topics/diarrhoea/en/.

World Health Organization. http://whqlibdoc.who.int/publications/2009/15.

World Health Organization Scientific Working Group (1990). *Escherichia coli* Diarrhoea.

Bulletin of the World Health organization; 58: 23–36.

APPENDIX A

Preparation of culture media.

The culture media used was prepared from dehydrated powder supplied by Oxford medical services.

MacConkey agar

52g of the dehydrated powder was dissolved in 1000ml of distilled water. This was sterilized by autoclaving at 121°C for 15 minutes. The sterile medium was dispensed aseptically in sterile petri dishes after cooling to 50°C.

Deoxycholate citrate agar (DCA)

53g of the dehydrated powder was dissolved in 1000ml of distilled water. This was sterilized by boiling and shaking. The sterile medium was dispensed aseptically in sterile petri dishes after cooling to 50°C.

Sorbitol MacConkey agar

52g of the dehydrated powder was dissolved in 1000ml of distilled water. This was sterilized by autoclaving at 121°C for 15 minutes. The sterile medium was dispensed aseptically in sterile petri dishes after cooling to 50°C.

Nutrient agar

28g of the dehydrated powder was dissolved in 1000ml of distilled water. This was sterilized by autoclaving at 121°C for 15 minutes. The sterile medium was dispensed aseptically in sterile petri dishes and slant bottles after cooling to 50°C.

Simmon's Citrate agar

28g of the dehydrated powder was dissolved in 1000ml of distilled water. This was sterilized by autoclaving at 121°C for 15 minutes. The sterile medium was dispensed aseptically in sterile slant bottles after cooling to 50°C.

Urea agar

28g of the dehydrated powder was dissolved in 1000ml of distilled water. This was sterilized by autoclaving at 121°C for 15 minutes. The sterile medium was dispensed aseptically in sterile slant bottles after cooling to 50°C and addition of cold sterile urea solution.