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*RESEARCH ARTICLE*

# *E. COLI* AND *VIBRIOCHOLERAE* PREVALENCE IN INFANTS 0 - 5 YEARS OLD WITH DIARRAHOEA VISITING SOME HOSPITALS

**Iyevhobu K. O.[1,2], Airefetalor A .I.[1,2], Abinokhauno, S. O.[3], Ken-Iyevhobu B. A.[4] and Oseni D. I.[4]**

1. Department of Public Health, National Open University of Nigeria, Uromi Community Study Centre, Uromi, Edo State, Nigeria.
2. Lassa Fever Enable Study CEPI/ISTH Irrua, Edo State, Nigeria.
3. Quality Assurance Department, R-Jolad Hospital, Lagos State, Nigeria
4. St. Kenny Research Consult, Ekpoma, Edo State, Nigeria.

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***Abstract***

Diarrhoea can be defined as the occurrence of three or more loose, liquid or watery stools or at least one bloody loose stool in a 24h period. Cholera is worldwide problem, especially in developing countries. It has been very rare in industrialized nations for the hundred years, however, the diseases is common today in other part of the world, including the Indian sub-continent and sub-Sahara Africa. This study is to ascertain the prevalence of *Escherichiacoli* and *Vibriocholera* in infants 0 – 5 years with diarrhoea attending some private hospitals in Ekpoma. The study population consisted of children between the ages of 0-5 years, presenting with diarrhoea, whose parents gave consent and the exclusion criteria were children under the ages of 0-5 years, whose parents did not give their consent and those above the age range in study. Fifty (50) stool samples were collected from children under 5 years of age visiting some selected hospital in Ekpoma due to acute diarrhoea. Stool samples were collected using sterile stool containers and transferred to the microbiology laboratory of St Kenny Research Consult, Ekpoma on ice packs for laboratory analysis. Stool samples collected from all of the children enrolled in the study were tested for *E. coli* and *Vibrio cholera* during the period of the study. There were altogether 50 stool samples processed for *Vibrio cholerae*and *Escherichia coli*. Out of 50 diarrhoeal patients (children) studied, 22 (44%) were male and 28 (56%) were female. Majority of the children with diarrhea were between 13 – 24 months 16 (32%), followed by 25 - 36 months 12 (24%), 0 – 12 months 10 (20%), 37 – 48 months 8 (16%) and 49 – 60 months 4 (8%). Among the 4, children within the age of 0 – 12 months had a prevalence of 1 (5.6%), those within the age range of 13 – 24 months had a prevalence of 2 (20%) while those within the age range of 25 – 36 months had a prevalence of 1 (16.7%). While for Vibrio, out of the fifty (50) stool samples examined, three (3) were positive to *Vibrio cholerae*. Among the 3, one (1) child from within the age of 13 – 24 months, 25 – 36 months and 37 – 48 months. None was found among children within the age range of 0 – 12 months and 49 – 60 months. This study suggests that *E. coli*are an important diarrhoea pathogen in subjects in Ekpoma. The implication of this is that our health inspectors in the local government areas of the state must be vigilant and ensure that campaigns are mounted to educate our citizens on ways of improving on the unsanitary environment. The presence of antibiotic

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

**Corresponding Author:-***Iyevhobu K.O.**

## Introduction:-

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

The World Health Organisation defined diarrhoea as the voiding of more than two unformed watery stools in any 24 h period, or any voiding of watery stools if accompanied by fever, abdominal pain and / or vomiting (Gondwe et al., 2006). Diarrhoea accounts for more deaths in childhood than any other disease in the developing world. It has been pointed out that in developing countries; a child of less than seven years of age still has a 50% chance of dying from diarrhoeal diseases (Gondwe *et al.,* 2006). Infantile diarrhoea remains one of the leading causes of childhood morbidity and mortality in developing countries, with children in the developing world having an average of 5-6 episodes a year. There are three major forms of diarrhoea namely: acute watery diarrhoea, acute bloody diarrhoea and persistent diarrhoea. Diarrhoeal disease forms one of the two major killer diseases in children under five years of age in the developing world. *E. coli*is one of the major bacterial causes of diarrhoeal diseases (Abdullahi *et al.,* 2010). The bacterium *E. coli*is one of the best and most thoroughly studied free-living organisms. It is also a remarkably diverse species because some *E. coli*strains live as harmless commensals in animal intestines. External contact and subsequent ingestion of bacteria from faecal contamination can cause detrimental health effects (Money *et al.,* 2009).

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

*Escherichia coli* conferred beta-lactam antibiotics resistance used for treatment of bacterial infection and relates the term extended spectrum beta lactamase (ESBLs) producing bacteria which are beta-lactamases capable of conferring bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and are inhibited by B-lactamase inhibitors such as clavulanic acid. This was first described in Germany in 1983 and France in 1985 among Klebsiella spp (Pathak et al., 2013). ESBLs exist in every region of the world and in most genera of enterobacteria (Tenover et al., 2003).

Diarrhoea is one of the leading causes of death among children under five globally. More than one in ten child deaths – about 800,000 each year – are due to diarrhoea. Today, only 44% of children with diarrhoea in low-income countries receive the recommended treatment, and limited data in literature suggests that there has been little progress since 2000 (UNICEF/WHO, 2013). Acute diarrhoeal disease is a major public health problem throughout the world, with over two million deaths occurring each year, and affecting mostly children under 5 years of age in developing countries (Iyevhobuet al., 2021). This disease is especially common in developing countries with poor hygiene and sanitation, and with limited access to safe drinking water. Underlying conditions such as malnutrition, which increase the risk of contracting diarrhoea, are also common in these countries. Bacteria such as *E.*

*coli*,*Shigella*and *Vibrio cholerae*are all known to cause diarrhoeal diseases and are identifiable through electron microscopy and culturing of stool samples (Bonkongou *et al.*, 2010, 2013). This study is to ascertain the prevalence of *Escherichia coli* and *Vibriocholera* in infants 0 – 5 years with diarrhoea attending some private hospitals in Ekpoma.

## Materials and Methods:-

### Study Area and Population

The study was carried out in Ekpoma town located in Esan West Local Government Area of Edo State, Nigeria. Ekpoma lies within longitude $6.13^0$E and latitude $6.73^0$N with a population of about 61,870 people (Population of Cities, 2007) and incidentally, the headquarters of Esan West Local Government Area. Ekpoma is situated at elevation 364 meters above sea level and apparently the fourth biggest city in Edo State (World atlas, 2015).

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

### Collection of Samples and Processing

Fifty (50) stool samples were collected from children under 5 years of age visiting some selected hospital in Ekpoma due to acute diarrhoea. Stool samples were collected using sterile stool containers and transferred to the microbiology laboratory of St Kenny Research Consult, Ekpoma on ice packs for laboratory analysis.

### Identification of Test Organisms

All isolates for this study were identified by their colonial appearance on the media, gram staining reaction and biochemical tests.

### Isolation and Characterization of *Escherichia coli*:

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

Each stool sample was streaked onto MacConkey medium using a sterile inoculating wire loop. Plates were incubated overnight at $37^0$C. Lactose fermenting colonies appearing on the plates were Gram-stained, and those showing as gram negative bacilli were tested for indole production. Indole positive colonies were further plated out on Sorbitol MacConkey agar and incubated for 18 – 2hrs at $37^0$C. All non-Sorbitol fermenting colonies were further subjected to biochemical tests using ducitol, β-glucuronidase, potassium cyanide and cellubiose.

### Isolation and preliminary identification of Vibrio species:

Aliquots of the samples were inoculated into alkaline peptone water (APW, Pronadisa, Madrid, Spain) and incubated aerobically at 37°C for 18 to 24 hours. Turbid cultures were streaked onto thiosulphate citrate bile salts sucrose (TCBS) agar (Pronadisa, Madrid, Spain) and incubated at 37°C for 24 hours. Suspected Vibrio species appear as green or yellow colonies on TCBS. Colonies per plate were randomly picked from each sample and sub-cultured onto fresh TCBS agar plates. The pure isolates were subjected to preliminary identification using standard cultural and biochemical methods as described by Kaysner and DePaola (2004).

## Results:-

Stool samples collected from all of the children enrolled in the study were tested for *E coli* and *Vibrio cholera* during the period of the study. There were altogether 50 stool samples processed for *Vibrio cholerae*and *Escherichia coli*. Out of 50 diarrhoeal patients (children) studied, 22 (44%) were male and 28 (56%) were female (Table 1). Majority of the children with diarrhea were between 13 – 24 months 16 (32%), followed by 25 - 36 months 12 (24%), 0 – 12 months 10 (20%), 37 – 48 months 8 (16%) and 49 – 60 months 4 (8%) (Table 2).

Table 3 shows the prevalence of *E. coli* and *V. cholera*in the study according to age. Out of the fifty (50) stool samples examined, four (4) were positive to *E. coli*. Among the 4, children within the age of 0 – 12 months had a prevalence of 1 (5.6%), those within the age range of 13 – 24 months had a prevalence of 2 (20%) while those within the age range of 25 – 36 months had a prevalence of 1 (16.7%). While for Vibrio, out of the fifty (50) stool

samples examined, three (3) were positive to *Vibrio cholerae*. Among the 3, one (1) child from within the age of 13 – 24 months, 25 – 36 months and 37 – 48 months. None was found among children within the age range of 0 – 12 months and 49 – 60 months.

Table 4 shows the prevalence of *E. coli* and *V. cholera*in the study according to gender. Out of the 22 stool samples examined for male subjects, 2 (9.1%) were positive to *E. coli* and *V. cholera* each. Among the 28 female stool samples examined, 2 (7.1%) were positive to *E. coli* and 1 (3.6%) was positive to *V. cholera*.

Table 5 and 6 shows the antibiotic susceptibility pattern of *E. coli* and *V. cholerae*isolated from diarrhoeic stool samples of children under five years in Ekpoma. The E. coli and isolates were more resistant to amoxicillin 100% followed by tetracycline 89.7% and were least resistant to nitrofurantoin 58.6% and ciprofloxacin 50.9%. All isolates of *V. cholerae*(100%) were found sensitive to Tetracycline followed by Ofloxacin (42.9%) and Ciprofloxacin (34.3%).

**Table 1:-** Distribution of gender in the study.

| Gender | Number examined | Percentage prevalence (%) |
|---|---|---|
| Male | 22 | 44 |
| Female | 28 | 56 |
| TOTAL | 50 | 100 |

**Table 2:-** Distribution of diarrhoeal children by age.

| Age range (months) | Number examined | Percentage prevalence (%) |
|---|---|---|
| 0 – 12 | 10 | 20 |
| 13 – 24 | 16 | 32 |
| 25 – 36 | 12 | 24 |
| 37 – 48 | 8 | 16 |
| 49 – 60 | 4 | 8 |
| TOTAL | 50 | 100.0 |

**Table 3:-** Prevalence of *Escherichia coli* and *Vibrio cholerae*in the study according to age.

| Age range (months) | Number examined | Number positive (%) for *Escherichia coli* | Number positive (%) for *Vibrio cholerae* |
|---|---|---|---|
| 0 – 12 | 10 | 1 (10) | 0 (0) |
| 13 – 24 | 16 | 2 (12.5) | 1 (6.25) |
| 25 – 36 | 12 | 1 (8.3) | 1 (8.3) |
| 37 – 48 | 8 | 0 (0) | 1 (12.5) |
| 49 – 60 | 4 | 0 (0) | 0 (0) |
| TOTAL | 50 | 4 (30.8) | 3 (27.05) |

**Table 4:-** Prevalence of *Escherichia coli* and *Vibrio cholerae*in the study according to gender.

| Gender | Number examined | Number positive (%) for *Escherichia coli* | Number positive (%) for *Vibrio cholerae* |
|---|---|---|---|
| Male | 22 | 2 (9.1) | 2 (9.1) |
| Female | 28 | 2 (7.1) | 1 (3.6) |
| TOTAL | 50 | 4 (16.2) | 3 (12.7) |

**Table 5:-** Antibiotics sensitivity of the *Escherichia coli*(Gram Negative Disc).

| Antibiotics | Code | Zone of Inhibition (mm) | No of Sensitive (%) | No of Resistant (%) | Sensitive | Resistance |
|---|---|---|---|---|---|---|
| Cloxacillin | CLX | 3 | 1 (25) | 3 (75) | S (1) | R (3) |
| Ofloxacillin | OFX | 3 | 2 (50) | 2 (50) | S (2) | R (2) |
| Tetracyclin | TET | 5 | 1 (25) | 3 (75) | S (1) | R (3) |
| Cefuroxin | CRM | 15 | 1 (25) | 3 (75) | S (1) | R (3) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Clotrimoxazole | COT | 13 | 1 (25) | 3 (75) | S (1) | R (3) |
| Amoxycillin | AMX | 13 | 0 (0.0) | 4 (100) | S (0) | R (4) |
| Erythromycin | ERY | 3 | 1 (25) | 3 (75) | S (1) | R (3) |
| Nitrofuratoin | NIT | 15 | 2 (50) | 2 (50) | S (2) | R (2) |
| Gentamycin | GEN | 5 | 1 (25) | 3 (75) | S (1) | R (3) |
| Ciprofloxacin | CIP | 3 | 2 (50) | 2 (50) | S (2) | R (2) |

KEY:
S = Sensitive; R = Resistance

**Table 6:-** Antibiotics sensitivity of the *Vibrio cholera* (Gram Negative Disc).

| Antibiotics | Code | Zone of Inhibition (mm) | No of Sensitive (%) | No of Resistant (%) | Sensitive | Resistant |
|---|---|---|---|---|---|---|
| Cloxacillin | CLX | 3 | 2 (66.7) | 1 (33.3) | S (2) | R (1) |
| Ofloxacillin | OFX | 3 | 2 (66.7) | 1 (33.3) | S (2) | R (1) |
| Tetracyclin | TET | 5 | 3 (100) | 0 (0.0) | S (3) | R (0) |
| Cefuroxin | CRM | 15 | 1 (33.3) | 2 (66.7) | S (1) | R (2) |
| Clotrimoxazole | COT | 13 | 1 (33.3) | 2 (66.7) | S (1) | R (2) |
| Amoxycillin | AMX | 13 | 1 (33.3) | 2 (66.7) | S (1) | R (2) |
| Erythromycin | ERY | 3 | 1 (33.3) | 2 (66.7) | S (1) | R (2) |
| Nitrofuratoin | NIT | 15 | 1 (33.3) | 2 (66.7) | S (1) | R (2) |
| Gentamycin | GEN | 5 | 1 (33.3) | 2 (66.7) | S (1) | R (2) |
| Ciprofloxacin | CIP | 3 | 2 (66.7) | 1 (50) | S (2) | R (1) |

## Discussion:-

Diarrhoea is still the most common illness among children causing highest number of morbidity and mortality in the developing countries including Nigeria. Due to lack of hygiene knowledge, unsafe drinking water and sanitation, illiteracy, lack of health education, under nutrition, incorrect feeding practice, wide spread faecal contamination of the environment, underlying disease, dense population in the region, gastric hypoacidity, immunodeficiency, superstition in rural areas are associated with the risk of children diarrhoea (WHO, 1993). Although the diarrhoea is caused by numerous microflora, but in this study, we considered *Vibrio cholerae*, which is major pathogen as also suggested by Pokharel et al. (1997) in Kathmandu, Basak et al. (1992) in Calcutta and Shakya (1999) in Kathmandu. This study was also carried out with special interest to isolate Enterohaemorrhagic *Escherichia coli*which is now recognized as an important human pathogen.

Out of 50 diarrhoeal children, 22 (44%) were male and 28 (56%) were female which shows the numbers of female patients were higher than the male patients (Table 1). This indicates that females are more susceptible than male. Similar results were observed by Aggrawal et al. (1989) in India, Huilan et al. in 1991, Aryal (1996), Shakya (1999), Gurung (1997), and Piya (2000) in Kathmandu. Diarrhoea occurs among all age group children. In this study, the diarrhoea was mostly seen in the age group 13 – 24 months, ie 16 (32%) followed by age group 25 – 36 months, 12 (24%), then age group 0 – 12 months, 10 (20%), age group 37 – 48 months, 8 (16%) and age group 49 – 60 months, 4 (8%) in (Table 2). The increment of diarrhoea among the above age group might be due to lack of maternally acquire antibodies, the lack of active immunity in the infant, the introduction of food that may be contaminated with faecal bacteria, and direct contact with human or animal faeces, when infant starts to crawl. Most enteric pathogens stimulate at least partial immunity against repeated infection or illness, which helps to explain the declining incidence of disease in elder children and adults. Similar result was observed in the study conducted by Rajkarnikar (2000) where majority of patients (37.20%) belong to age group 13 – 36 months. In a study carried out in Kathmandu by Shakya (1999), reported higher number 60.85% of diarrhoeal children in the age group 0 – 3 years (0 – 36 months).

*E. coli*has been established as an important etiologic agent of human diarrhea illness. This organism, while being primarily associated with food-borne outbreaks (Chapman et al., 1993) has become an important public health concern, being also transmissible through contaminated drinking water. Routine surveillance for diarrhoegenic *E. coli*has never been carried out in Ekpoma, Edo State. The only report, documented to our knowledge, is that of Omoigberale et al., (2002) which reported a zero percent prevalence rate for *E. coli*in children presenting with

diarrhea at the University of Benin Teaching Hospital, Benin City. In this study the presence of *E. coli*was detected, with a prevalence rate of 8%. Our main focus was to establish the presence of this organism in this area.

A total of fifty (50) stool samples were analysed for this study and isolates identified as *E. coli*were obtained from four (4) diarrhoeal stool samples of children under five years of age, in Ekpoma, giving a prevalence of 8%. Data from other researches showed 22.37% prevalence reported in Bosnia and Herzegovina (Dedeic-Ljubovic et al., 2009), and the 22.6% reported by Mandomando et al., (2007) in a study conducted in Mozambique. Higher prevalence of 44.74%was obtained in a study by Nweze (2010), 34.1% by Raji et al., (2008) in Tanzania and 60% reported by Eseigbe et al. (2013) in a study conducted in Kaduna. Behiry et al.(2011) reported a percentage total of *E. coli*isolates in children under five years as 51.5%. Nweze (2010), reported the isolation of 119 out of 520 stool samples of children, with 22.88% prevalence in Southeast Nigeria, Akingbade et al. (2013) also reported a 51% identification of *E. coli*from children with diarrhoea in Abeokuta. Dhanashree and Shrikar Mallaya, (2008) reported a prevalence of 73.96% *E. coli*in India. The reason(s) for the low (8%) prevalence rate recorded compared to these reports cannot be immediately hazard. In a study done in Lagos by Olorunshola et al., (2000) a detection rate of 6% was recorded for enterohaemorrhagic *E. coli*, while 3.1% prevalence rate was recorded for diarrhea patients in Jos, Nigeria (Ngbede et al., 2006). A prevalence rate of 8% recorded in our study was relatively higher than these reports.

Onanuga et al. (2014), reported that 18.4% of diarhoeagenic *E. coli*isolated from children with diarrhoea in Gwagwalada Nigeria and 62.8% prevalence obtained from diarrhoeic children, also in Abuja, Nigeria by Ifeanyi et al.(2010). Al-Dawmy and Yousif (2013) in a study carried out in Baghdad reported a prevalence of 54.7%. Twenty-eight 28(36.84%) of the 76 isolates obtained in this study, were found to be non-sorbitol fermenting, and this prevalence obtained was found to be higher than the 22.4% prevalence reported by Asamole-Osuocha (2006) in Abuja, Nigeria. The isolates were further confirmed to be *E. coli*by latex agglutination kit. A total of four (4) of the 50 stool samples gave positive cultures of *E. coli*, and hence a prevalence of 8.0%, this is higher than the prevalence of 5.9% reported from a study conducted in Ondo state Nigeria (Ademokoya et al., 2014) and 4.78% prevalence reported by Al-Dawmy and Yousif, (2013). Studies carried out in Abuja by Asamole-Osuocha in (2006) showed that the faecal samples did not yield *E. coli*.

A study conducted in Dhaka, Bangladesh reported that among 200 stool samples collected from patients aged, 0 to 60 months, *E. coli*was isolated in 135 (67.50%), as reported by Roy et al.(2013). *E. coli*was the most encountered of the organisms isolated in the study of aetiologic agents of diarrhoea, with a prevalence of 77.8% (Olowe *et al.*,2003). Mengistie et al. (2013) reported a prevalence of diarrhoea aetiology in under five years old children in Ethiopia, to be 22.5%. Seventy eight percent (78%) of the three hundred and fifty diarrhoeal cases investigated had no *E. coli*isolated from them, which suggests the possible presence of other bacterial pathogens, viral pathogens or non-pathogenic factors, and this also shows relation to the work done by Sule et al.(2011).It was observed in this study that Kafanchan General Hospital had the highest prevalence (40.74%) of *E. coli* isolated from the samples obtained therefrom and this could be as a result of the insurgencies and unrest ongoing at the time of sampling which could have led to displacement of the people, rendering them homeless and having to rely on refugee camps which may not be very hygienic, as they are too crowded and do not possess the necessary social amenities and facilities to satisfy the need of the people housed therein. A prevalence by location (in this case, hospitals) showed Gambo Sawaba Memorial Hospital, Zaria having 19.64% as percentage of occurrence of *E. coli*in stools as against 5.4% reported by Vincent et al. (2010) showing an increase in the rate of infection in the city, which could be tied to negligence on the part of the parents and care-givers as regards both personal and environmental hygiene.

In this study, the incidence of cholera was found 27.05 percent**.** Close result (41.7 percent) was reported by Ise et al., in (1994). Out of 3 *V. cholerae*, 2 (9.1%) were from male and 1 (3.6%) were female (Table 4). Equal number of isolates 1 (6.25%), 1 (8.3%) and 1 (12.5%) was found in age group 13 – 24 months, 25 – 36 months and 37 – 48 months respectively. This might be due to unhygienic practices of children and their parents and also children of this age group were more likely to eat street foods. Cholera rarely occurs in children under 2 years of age (Khan et al., 1996). However in this study, 1 case (6.25%) of cholera was observed in this age group, which is remarkable feature of our study and clearly showing the eradication of cholera in this region of the state and country.

*Vibrio cholerae*produce an enterotoxin, which causes the intense diarrhoea which is so typical of the disease cholera. The detection of cholera toxin (CT) production is an important indicator of virulence. In this study, out of 3 *V. cholerae*, which were identified by biotyping and serotyping, 2 showed the toxin production. However, epidemic

strain must produce enterotoxin. There might have low concentration of toxin produced, which couldn't be detected in the experiment. In a study conducted by Shrestha (1995), she found cholera toxin both on *V. cholerae*O139 (5) and *V. cholerae* O1 (1) by colony hybridization technique using oligonucleotide probe against ctx gene.

The antibiotic susceptibility patterns of the isolates as shown on table 5 indicates resistance of the *E. coli* isolates to amoxicillin 25(100%) which is the highest followed by tetracycline 23 (89.7%) while the least resistance is nitrofurantoin 10 (41.3%). Verma et al. (2013) in India, reported resistance to antibiotics such as tetracycline, ampicillin, streptomycin, nalidixic acid, neomycin, cefalothin and cotrimoxazole, and also showed multi-drug resistance in 14 strains. Asamole-Osuocha (2006) reported a 100% resistance of isolates to all the six antimicrobials such as ciprofloxacin, sulphamethoxazole, tetracycline, amoxicillin, chloramphenicol and streptomycin used in the study. The level of resistance observed in this study for trimethoprim-sulfamethoxazole reflects the results from several studies by other authors who demonstrated high rates of resistance towards enteric *E. coli*against this drug. One explanation for this could be its widespread use in the treatment of diseases associated with Gram-negative bacteria, especially in children under two years of age with acute infectious diarrhoea (Aranda et al., 2004). Eseigbe et al.(2013), in a study in Kaduna, reported that the *E. coli*isolates were more sensitive to cefuroxime, ceftriaxone and streptomycin than ciprofloxacin, and this was in contrast to the more commonly used drugs, in the treatment of diarrhoea and this disagrees with the level of sensitivity of ciprofloxacin, observed in this study. However, there has been limited use of the drug, except in very rare cases due to its reported effect on growth (Oshikoya, 2006).

*Escherichia coli* was more sensitive to nitrofurantoin 15 (58.6%) followed by ciprofloxacin 13 (50.9%) while the least sensitive been amoxicillin 0(0.0). This study also however, agrees with that of Eseigbe et al. (2013), with respect to the limited sensitivity of the isolates to most of the commonly prescribed antibiotics. Akingbade et al. (2013) also reported that most of the *E. coli*isolates were resistant to cotrimoxazole, erythromycin, ampicillin and tetracycline, due to widespread and indiscriminate use of these antimicrobials. No resistance pattern was obtained with the single antibiotic used in this study, rather they were found to be resistant to more than one antibiotic. This data is in accordance with earlier reports by Dhanashree and Shrikar Mallaya, (2008), were approximately more than half of the *E. coli*isolates tested displayed resistance to one or more antimicrobials. Akingbade et al. (2013) also reported antimicrobial resistance of the isolates to more than half of the antibiotics used. Schroeder et al. (2002) and Bettleheim et al. (2003) also had similar data reports on the susceptibility pattern of *E. coli*isolates. Moreover, such drugs are considered as antimicrobials used in hospitals, and resistant bacteria originating from the community are not expected to thrive (Usein *et al.*, 2009).

All isolates of *V. cholerae*(100%) were found sensitive to Tetracycline followed by Ofloxacin (42.9%) and Ciprofloxacin (34.3%) (Table 5). Tetracycline seemed to be most effective antibiotic which was sensitive in 3 (100%) *V. cholerae*. Similar results were reported by Aryal (1996), Shakya (1999) and Gurung (1997).

Multiple resistance patterns were obtained in this study, with varying combinations of 4 and 5 antibiotic combinations. Vincent et al. (2010) reported multidrug resistance to 8 and 9 antibiotic combinations in a study carried out in Zaria, Kaduna state. The *E. coli*and isolates were more resistant to amoxicillin 100% followed by tetracycline 89.7% indicating that nitrofurantoin 58.6% and ciprofloxacin 50.9% were the most active antibiotics used. The high prevalence of multiple antibiotic resistance obtained in this study may be because *E. coli*acts as a reservoir for resistance available to enteric pathogens (Akingbade *et al.,* 2013) or may be due to the fact that antimicrobial resistance in *E. coli*has increased worldwide and its susceptibility patterns show substantial geographic differences and variations (von Baum & Reinhard, 2000). Kibret & Abera (2011) in a study carried out in Ethiopia, reported high resistance rates of *E. coli*to amoxicillin and tetracycline and also high rates of multiple antibiotic resistance, and this agrees with the findings obtained in this study. The overall resistance to antimicrobials was found to be high in this study, just as was observed in previous studies carried out in Trinidad. The *Escherichia coli*isolates obtained from this study showed resistance to more than half of the antimicrobials used in this study and this is in agreement with previous studies, this could either be due to the fact that antimicrobials are unnecessary in the treatment of majority of infantile diarrhoea cases but are often misused in this condition, non-adherence to duration of antibiotics prescribed or that the use of these drugs has been a key factor in the emergence of antimicrobial-resistant *E. coli*(Schroeder *et al.*,2002; Raji *et al.*,2008; Ifeanyi *et al.*,2010). It could also be because the misuse of antimicrobials generally impacts resistance in enteric organisms heavily and directly because they readily acquire resistance genes from the faecal flora. This is in agreement with work done by Vincent et al. (2010) in Kaduna. Poor sewage disposal with the rains washing these surface waters into rivers and streams.

The 30.8% prevalence of *E. coli*isolated from the stools of diarrhoeic children used in the study, though higher than the 5.4% prevalence obtained by Vincent et al.(2010) further suggests that the epidemiological situation in Nigeria still needs to be looked into properly. Omoikhodon et al. (1998) in Ibadan, reported that 33% of children of market women had diarrhoea and this was low in comparison with other occupations. This however, shows a similarity with the low rate of the bacteria recorded in this study with regards to trading as an occupation, and this could be attributed to the fact that most markets these days have received government attention and unions are established to ensure cleanliness and orderliness.

Diarrhoeal diseases are major public health concern in most developing countries, being one of main cause of childhood morbidity and mortality. This disease has also been accepted as part of life in some countries. It prevails throughout year and become epidemic in rainy season. It is mostly seen to occur in places with an unsanitary environmental condition, unhygienic disposal of faeces, use of contaminated open water source, overcrowding, malnutrition and low socioeconomic status, particularly that of women. High diarrhoeal mortality and morbidity in low socioeconomic countries are related to the availability of safe water for personal and domestic purpose and environmental sanitation (Gracey, 1997). Majorities of diarrhoea last for 1-2 weeks and onset after oral rehydration therapy, but proportion of case lack of compensation to death of patient. An estimated diarrhoeal death due to dehydration is 60-70%,most of them are young children (Aggarwal etal.,1983).

## Conclusion:-

Childhood diarrhea is very prevalent in the world and is the second leading cause of death in children. Watery diarrhea causes a rapid depletion of water and electrolytes in the body while bloody diarrhea may cause substantial blood loss leading to hypovolemic shock. Left untreated these situations can lead to death. Diarrhea, which is a symptom of intestinal infection, is still a burden on the world today due to inadequate access to water, sanitation, and treatment.

Most of the pathogens that can cause diarrhea in children are transmitted via the fecal oral route and are found in drinking water. In less developed countries that are uneducated on the importance of water sanitation, there is an increased risk of contracting a diarrheal disease through ingestion of water. Better education about sanitation and the importance of proper hygiene can lower the number of infections caused by waterborne pathogens.

This study suggests that *E. coli*are an important diarrhoea pathogen in subjects in Ekpoma. The implication of this is that our health inspectors in the local government areas of the state must be vigilant and ensure that campaigns are mounted to educate our citizens on ways of improving on the unsanitary environment. Potential sources of infection as well as food and meat inspection must be followed up. In addition, our physicians should query *E. coli*infection whenever patients present at their clinics with bloody or non-bloody diarrhea, so as to curb possible outbreaks early enough. Also of worthy note is the little or no prevalence of *Vibrio cholera* outbreak in Ekpoma and entire southern region of the country unlike in the Northern region of Nigeria were about 28 death cases of cholera was recorded in Kaduna by daily news on the 15$^{th}$ of August 2018. Therefore the recorded cases in this study were treated with utmost attention. The dangers of diarrhoeal diseases and its effect on the nutritional status of children under five years of age have long been established, to this end, the following conclusion have been drawn from this study.

1. *Escherichia coli*(a re-emerging pathogen causing haemolytic-uremic syndrome) were isolated from the study population further confirming *E. coli*as an active causative agent of diarrhoeal diseases in Ekpoma, Edo state, Nigeria.
2. The presence of antibiotic resistant strains of the *E. coli* and *V. cholerae*isolates in the study population indicates the often unnecessary and uninformed use of these drugs in the treatment of most infantile diarrhoea cases.

## Recommendations:-

1. Breastfeeding of children, which is known to help a lot in improving child‘s immunity, should be encouraged for longer periods (1000 days) than the usual 6months as this would help ensure that parents, especially the mothers, keep a watchful eye on the kind of things the child ingests.
2. Children should be monitored more closely by their parents and care-givers, as negligence puts them more at risk of infection.
3. Personal and environmental hygiene strategies should be embarked upon and maintained by parents and society in general.

4. The Government should improve on existing infrastructural facilities, make provision for new ones, especially in rural areas and establish programs that are centered around the fortification and supplementation of foods and making them readily available especially in rural areas, and if possible free of charge for the low income earners who reside more in these rural areas. This would help improve the nutritional status of children, thereby reducing the incidences of diarrhoeal diseases which have so far been established to be bidirectional.
5. Handwashing practices should be inculcated and encouraged amongst pre-school aged children as they are the most vulnerable group in the society.
6. Care-givers in schools such as daycare centres and nursery schools were we have these children enrolled should be educated on proper hygienic practices as these children cannot handle such things on their own.
7. Antimicrobials should be conserved for use only when safe and necessary.

**Conflict of interest**

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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