Antibiogram of Diarrhoeagenic Escherichia coli Patients under Five Years Attending Selected Hospitals in Kaduna State, Nigeria

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Authors’ contributions

This work was carried out in collaboration between all authors. Author ROC designed the study and wrote the first draft of the manuscript. Author IM managed the analyses and literature searches of the study. Author JBO performed the statistical analysis and wrote the protocol. All authors read and approved the final manuscript.

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ABSTRACT

Escherichia coli infections and poor nutritional status have implications on the growth and development of children under five years, physically, mentally and health wise with consequences such as diarrhoea, stunting, wasting, underweight and often times leading to death, depending on their severity. This study evaluated the antibiogram of Escherichia coli O157 and Verocytotoxigenic Escherichia coli (VTEC) and the nutritional status of diarrhoeic children under five years in Kaduna State, Nigeria, using Conventional isolation methods, latex agglutination tests, VTEC-ELISA tests, Chi-square (SPSS Version 19) and WHO Antro (Version 3.2.2). Purposive sampling was used to select 350 children presenting with diarrhoea in six government hospitals within the three senatorial zones of Kaduna State. The results obtained revealed that 76(21.7%) of the 350 stool samples were positive for E. coli and 28(36.8%) were positive for E. coli O157:H7 serotype and 1(1.3%) verocytotoxigenic E. coli (VTEC) serotype. High susceptibility to ciprofloxacin, chloramphenicol and high resistance to sulphamethoxazole, cefotaxime, amoxicillin, gentamicin and tetracycline by the

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isolates were observed. The study concluded that antibiotics have not been very effective in the treatment of *E. coli*-related diarrhoea, with VTEC now emerging in this part of the world, making it a serious public health issue. The study therefore recommends the implementation of programmes geared towards good hygiene, good nutrition and good health.

**Keywords:** Antibiogram; O157:H7; verocytotoxigenic *E. coli*; five years; Kaduna.

1. **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 24 h period [1]. 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 [2]. An increase in stool mass, stool frequency or stool fluidity is perceived as diarrhoea by most patients [3]. 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 [3].

The World Health Organization defined diarrhoea as the voiding of more than two unformed watery stools within 24 h period, or any voiding of watery stool accompanied by fever, abdominal pain and / or vomiting [1,3]. Diarrhoea accounts for more deaths in childhood than any other disease in the developing world [3]. 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 disease [1,3-4]. 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 [3]. There are three major forms of diarrhoea namely: acute watery diarrhoea, acute bloody diarrhoea and persistent diarrhoea [4]. Diarrhoeal disease forms one of the two major killer diseases in children under five years of age in the developing world. *Escherichia coli* is one of the major bacterial causes of diarrhoeal diseases [5]. 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 intestine. External contact and subsequent ingestion of bacteria from faecal contamination can cause detrimental health effects [6]. Acute watery diarrhoea is that with a high volume of watery stool occurring over a period of less than 14 days. Persistent diarrhoea is usually associated with loose or watery stools with or without visible blood occurring in a period of more than 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 [7]. According to World health organization (WHO) Report, approximately 11 million children under the age of five, die because of *E. coli*-mediated gastroenteritis [8].

This study was aimed at determining the antiibiogram of diarrhoeagenic *Escherichia coli* in diarrhoeic patients under-five years with the following objectives

1. Isolate and characterize *Escherichia coli* O157, Verocytotoxigenic *E. coli* from diarrhoeal stools of children under 5 yrs in Kaduna State.
2. Determine the antibiotic susceptibility pattern of the *Escherichia coli* isolates.

2. **MATERIALS AND METHODS**

2.1 **Study Area**

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

2.2 **Study Population**

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

The sample size was determined using the formula of [9] which is as follows: $N = \frac{Z^2Pq}{L^2}$

Where $N$ is sample size $Z$ is the standard normal distribution at 95% confidence interval = 1.96 $P$ is the prevalence rate, which is taken as 34.1% [10] $q$ is 1 – $P$ $L$ is the allowable error, which is taken as 5% = 0.05 Therefore $N = (1.96)^2 \times 0.341 \times (1-0.341) (0.05)^2 = 3.8416 \times 0.341 \times 0.65 = 345.3 \ 0.0025$ The sample size calculated is 345.3 samples. A total of three hundred and fifty samples were collected from the diarrhoeic children for this study.

2.4 Collection of Samples and Processing

Stool samples were collected from children under 5 years of age visiting hospital due to acute diarrhoea and from healthy controls of similar age. Stool samples were collected using sterile stool containers and transferred to the microbiology laboratory on ice packs for laboratory analysis.

2.5 Preparation of Media

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

2.6 Isolation and Characterization of Escherichia coli

Bacterial isolates were identified according to the standard microbiological procedures as described [11], which includes examination of specimens to detect, isolate, and identify pathogens or their products using, Microscopy, Culture techniques, Biochemical tests, and Immunological (antigen) tests.

2.7 Isolation of Escherichia coli from Stool

Approximately 10 μl volumes of a micropipette of faecal samples were inoculated directly on to EMB [12] and CT-SMAC (differential media) [13]. The plate’s were incubated at 44.5 and 37°C respectively for 18 - 24 h. Non-sorbitol fermenting (NSF), colonies on CT-SMAC that appear as colourless colonies on the plates were stored on nutrient agar slants. A growth on EMB agar with greenish-metallic sheen was also transferred to nutrient agar slants for storage. All typical Escherichia coli isolates from EMB (selective media) and CT-SMAC stored on slants were confirmed by Gram- reaction, MRVP test, Citrate utilization, Indole, Motility tests.

2.8 Gram Stain Reaction

Smears of all stored isolates were made on slides and heat-fixed, covered with crystal violet for 1 min and rinsed immediately with clean water. The smears were then covered with Lugol’s iodine for another minute and rinsed again. Alcohol was used to decolorize it for a few seconds, rinsed and placed in a draining rack to air dry. The slides were examined microscopically with x100 objective lens to view the microscopic appearance of the gram negative rods [14].

2.9 Biochemical Characterization of Presumptive Escherichia coli Isolates

The basic microbiological characteristics of Escherichia coli were obtained when cultured on standard lactose-containing EMB agar. E. coli lactose-fermenters produce green-black colonies with metallic sheen and dark colonies for other fermenters, while non-lactose fermenters appear colourless [15].

2.10 Methyl red Voges-proskaeur (MRVP) Test

A colony of the isolate was inoculated into Methyl red Voges-Proskauer broth (MR-VP) and incubated for 24 h at 35-37°C. 0.6 ml of α-naphthol solution and 0.2 ml of 40% potassium hydroxide was added to 2.5 ml of the culture broth and shaken properly. Formation of a pink-red product within 5-15 min of dilution indicates a positive (acid) result which indicates the presence of Escherichia coli. [14]

2.11 Citrate Utilization Test

A light suspension of the organism was made in saline and then stab inoculated onto Simmon’s citrate agar using a sterile inoculating needle. Growth indicated by blue colour in Simmon’s agar, indicated positive result. This means that citrate has been utilized and hence the absence
of E. coli, hence it was considered citrate negative [14].

2.12 Indole Test

A colony of the E. coli isolate was inoculated into test tubes containing Sulphur Indole Motility medium using a sterile wire loop and incubated for 48 h at 37°C. Two drops of Kovac’s reagent were added to the medium and shaken. Formation of a pink layer indicated a positive result and hence, the presence of E. coli [14].

2.13 Serological Identification of Escherichia coli Isolates

Escherichia coli O157 isolates were identified serologically, using specific serological methods that are rapid. The method used was the Oxoid E. coli latex test which demonstrates by slide agglutination E. coli strains possessing the O157 sero-group antigen. A bacterial colony of pure growth was emulsified in physiological saline on a slide and antiserum containing specific antibody was added. The antibody binds to the bacterial antigen, resulting in the agglutination of the bacterial cells [16].

2.14 Latex Agglutination Test

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

2.15 Detection of E. coli Verotoxin

The detection of verocytotoxins produced by E. coli cultured from faecal samples was done by Enzyme Linked Immunosorbent Assay. The test is a double antibody (sandwich) ELISA using anti-verotoxin antibodies to capture the antigen from the stool supernatant. A second anti-verotoxin monoclonal antibody cocktail is then added, which binds to the complex. This reaction is visualized by the addition of anti-mouse antibodies conjugated to peroxidase. The resulting blue color following the addition of the chromogen indicates the presence of verotoxin being bound by anti-verotoxin antibodies. In this test, microtite wells are coated with rabbit anti-verotoxin antibodies and the antibody-enzyme conjugated is a monoclonal antibody chemically linked to the enzyme horse radish peroxidase (HRP). The conjugate binds specifically to the solid phase toxin [17].

The wells needed, which includes the number of samples plus two (2) for controls, were broken off and placed in a strip holder. 100ul each of the negative and positive (undiluted) controls were added to wells 1 and 2 respectively and then 100 ul of the stool supernatant added to the appropriate test wells and incubated for 30 minutes at room temperature and then washed (this involved using the distilled wash buffer to fill to the top of each well, shaking out the contents and refilling the wells for a total of 3 minutes). Then 2 drops of Reagent 1 (blue solution) was added to each well and incubated for 30 minutes and washed, thereafter, 2 drops of Reagent 2 (red solution) was also added to each well and incubated again for 30 minutes before washing and each well was rinsed three times with distilled water, then 2 drops of Chromogen was added to each well and it was incubated for 10 minutes also at room temperature. 2 drops of Stop solution was added to each well and mixed by tapping the strip holder. Results were read and interpreted using an ELISA reader on a spectrophotometer using bichromatic reading, with the filters set at 450nm-630nm.

2.16 Antibiotic Susceptibility Testing

The antibiotic susceptibility testing of the isolates were carried out using the disc diffusion technique according to the methods recommended by the [18] to determine the susceptibility or resistance profiles of the isolates. About 3-5 discrete colonies of the isolates from EMB agar were inoculated into 5ml of physiological saline and incubated overnight at 37°C. The overnight culture was then standardized with 0.5 MacFarland standard suspensions. Sterile cotton swab was used in inoculate the bacterial suspension to freshly prepared pre-dried Mueller-Hinton agar plates.
prepared according to manufacturer's instructions. The antibiotic sensitivity discs were aseptically and spaciously placed on the inoculated Mueller-Hinton agar plates. The antibiotic discs used were Amoxicillin (10 μg), Cefotaxime (30 μg), Ciprofloxacin (5 μg), Chloramphenicol (30 μg), Tetracycline (30 μg), Trimethoprim (25 μg), Gentamicin (10 μg) (Oxoid Ltd, UK). The reference standard strains used as control in this study include Escherichia coli ATCC 25922. It was obtained from the National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria.

2.17 Measurement of Diameter of the Inhibition Zone

After incubation, the test plates were examined for confluent growth and zone of inhibition. The diameter of each zone of inhibition was measured in millimetre (mm) using a ruler on the underside of the plate. The interpretation of the measurement as sensitive, intermediate and resistant was made according to Clinical Laboratory Standards Institute [18] manual.

2.18 Multiple-antibiotic Resistance Index

The multiple-antibiotic resistance (MAR) index was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested [19]. MAR index = Number of antibiotics the isolate is resistant to total number of antibiotics tested.

2.19 Statistical Analysis

Results obtained from the study, were subjected to the appropriate statistical analysis. Chi-square analysis was used to determine association between the observed and expected frequencies and infection at 95% confidence interval and at 0.05 significant levels (SPSS, version 19).

3. RESULTS

Table 1 show the distribution of E. coli isolates in the stool samples, with respect to the hospital location from where the samples were obtained. There was statistical difference observed in the prevalence obtained from the various hospitals (p>0.05). General Hospital Karfanchan, had the highest percentage prevalence of 40.74%, while Yusuf Danstho Memorial Hospital had a prevalence of 34.94%. The prevalence of E. coli infection on patients in Gambo Sawaba Memorial Hospital was 19.64%, General Hospital Makarfi (16.18%), General Hospital Kwoi had (15.79%) and Gomna Awan General Hospital had (11.34%).

Table 1 show the antibiotic susceptibility pattern of E. coli isolated from diarrhoeic stool samples of children less than five years in Kaduna state. The antibiotics with the highest antibacterial activity were chloramphenicol and ciprofloxacin with 100% activity against all the isolates. This

Table 1. Prevalence of Escherichia coli isolated from diarrhoeic stool samples according to hospital location

<table>
<thead>
<tr>
<th>Location</th>
<th>No of samples collected</th>
<th>Samples positive (%)</th>
<th>X2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSMH</td>
<td>56</td>
<td>11(19.64)</td>
<td>22.192</td>
<td>0.000**</td>
</tr>
<tr>
<td>GHM</td>
<td>68</td>
<td>11(16.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YDMH</td>
<td>83</td>
<td>29(34.94)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAGH</td>
<td>97</td>
<td>11(11.34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GHK</td>
<td>27</td>
<td>11(40.74)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KGH</td>
<td>19</td>
<td>3(15.70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>350</td>
<td>76(21.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X2=chi-square, p-value<0.05, (*)=Statistically significant, (%) =Prevalence, GSMH- Gambo Sawaba Memorial Hospital, GHM- General Hospital Makarfi, YDMH- Yusuf Danstho Memorial Hospital, GAGH- Gomna Awan General Hospital, GHK- General Hospital Kafanchan, KGH- Kwoi General Hospital
was followed by moderate resistance (50%) of the isolates to amoxicillin, tetracycline and gentamicin. High resistance was seen in the activity of the isolates to cefotaxime (97.5%) and sulphamethoxazole (88.9%).

Table 3, No resistance pattern was obtained for the single antibiotic used in the study rather the isolates were found to be resistant to more than one antibiotic combination. Two multiple resistance patterns were obtained with varying combinations of 4 and 5 antibiotic combinations. All the E. coli isolates from the faecal samples of the diarrhoeic children exhibited MAR.

4. DISCUSSION

A total of seventy six (76) isolates identified as E. coli were obtained from three hundred and fifty (350) diarrhoeal stool samples of children under five years of age, in Kaduna State, giving a prevalence of 21.7%. Data from other researchers showed 22.37% prevalence reported in Bosnia and Herzegovina [20], and the 22.6% reported by [21] in a study conducted in Mozambique. Higher prevalence of 44.74% was obtained in a study [22], 34.1% [23] in Tanzania and 60% reported by [24] in a previous study conducted in Kaduna. [25] reported a percentage prevalence of 51.5%. [22], reported the isolation of 119 out of 520 stool samples of children, with 22.88% prevalence in Southeast Nigeria, [26] also reported a 51% identification of E. coli from children with diarrhoea in Abeokuta. [27] reported a prevalence of 73.96% E. coli in India. [28], reported that 18.4% of diarrhoeagenic E. coli isolated from children with diarrhea in...
Table 3. Resistance patterns of *E. coli* isolated from diarrhoeic stool samples of children under 5-years (n=76)

<table>
<thead>
<tr>
<th>Single antibiotic resistance</th>
<th>Resistance pattern</th>
<th>Multiple antibiotic resistance (no. of antibiotic combinations)</th>
<th>No. of isolates (%) with pattern</th>
<th>Resistance pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>0(0)</td>
<td>NR</td>
<td>2</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>3</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>4</td>
<td>VTEC 1(1.3)</td>
<td>NG, TET, CEF, SX, SXT</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Non-O157</td>
<td>E. coli 47(61.8)</td>
<td>GN, TET, CEF, SX, SXT</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>O157:H7</td>
<td>28</td>
<td>AM</td>
</tr>
</tbody>
</table>

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

Gwagwalada Nigeria and 62.8% prevalence obtained from diarrhoeic children, also in Abuja, Nigeria by [29, 30] 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 [31] in Abuja, Nigeria. The isolates were further confirmed to be *E. coli* O157 by latex agglutination kit. A total of twenty eight (28) of the 350 stool samples gave positive cultures of *E. coli* O157: H7, and hence a prevalence of 8.0%, this is higher than the prevalence of 5.9% reported from a study conducted in Ondo State Nigeria [32] and 4.78% prevalence reported [30]. Studies carried out in Abuja [31] showed that the faecal samples did not yield *E. coli* O157. One(1) isolate (1.3%) produced the VTEC toxin also known as STEC, this agrees with the 1.9% recorded in Mozambique by [21] but was much lower than the 15.09% prevalence of VTEC reported [31].

This study is also consistent with previous studies conducted [33,34,35,36] in South Africa, Swaziland, sub-Saharan Africa and Tanzania with reports that there was low frequency of verotoxin-producing *E. coli* in the sample population. The 1.3% detection of VTEC in this study is similar to the 1.3% detected and reported [37] in India, but lower than the 3.6% prevalence reported by [23] and 5.1% incidence rate reported [38] in studies carried out in Lagos, Nigeria. [39] also from India reported a low incidence of 2% prevalence of STEC in humans, [27] also reported a low incidence of 1.4% prevalence of STEC from diarrheal stools. In this study, the prevalence of *Escherichia coli* aetiology of diarrhoea is 21.71% which is lower than the research conducted in Lagos, Nigeria which was reported to be 39.5% [38] and also in Kaduna State which was found to be 34.1% [10]. 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 [40]. *Escherichia coli* was the most encountered of all the organisms isolated in the study of aetiologic agents of diarrhoea, with a prevalence of 77.8% [41]. [42] reported a prevalence of diarrhoea aetiology in under five years old children in Ethiopia, to be 22.5%. Seventy eight percent (78%) of the three hundred and fifty diarrhoeal cases investigated had no *Escherichia coli* isolated from them, which suggests the possible presence of other bacterial pathogens, viral pathogens or non pathogenic factors, and this also shows relation to the work done [10]. It was observed in this study that Kafanchan General Hospital had the highest prevalence (40.74%) of *E. coli* isolated from the samples obtained 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.
The 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 [43] showing an increase in the rate of infection in the city, which could be tied to negligence on the part of the parents and caregivers as regards both personal and environmental hygiene. The antibiotic susceptibility patterns of the isolates indicate resistance of the isolates to cefotaxime, tetracycline, gentamicin, sulphamethoxazole and amoxicillin, and multiple antibiotic resistance in the isolates. [37], reported resistance to antibiotics such as tetracycline, ampicillin, streptomycin, nalidixic acid, neomycin, cefalothin and cotrimoxazole, and also showed multi-drug resistance in 14 strains. [31], 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 diarrhea [44], [24], 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 [45].

This study also agrees with that of [24], with respect to the limited sensitivity of the isolates to most of the commonly prescribed antibiotics. [26], 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 noticed 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 [27], were approximately more than half of the *E. coli* isolates tested displayed resistance to one or more antimicrobials. [26], reported antimicrobial resistance of the isolates to more than half of the antibiotics used. Regarding the gentamicin and tetracycline, low levels of intermediate resistance were found, corroborating data in the literature which suggest a good activity of these antimicrobials against enteric Gram-negative bacilli. Moreover, such drugs are considered as antimicrobials used in hospitals, and resistant bacteria originating from the community are not expected to thrive [46].

Multiple resistance patterns were obtained in this study, with varying combinations of 4 and 5 antibiotic combinations. [43], reported multidrug resistance to 8 and 9 antibiotic combinations in a study carried out in Zaria, Kaduna State. The VTEC isolate in this study was resistant to four (4) of the seven (7) antibiotics which includes tetracycline, gentamicin, cefotaxime and sulphamethoxazole. The *E. coli* O157 and non-O157 isolates were resistant to cefotaxime, tetracycline, sulphamethoxazole, gentamicin and amoxicillin indicating that chloramphenicol and ciprofloxacin were the most active antibiotics used. The high prevalence of multiple antibiotic resistance obtained in this study may be because *E. coli* acts as a reservoir for resistance available to enteric pathogens [26] 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 [47].

5. CONCLUSION

The dangers of diarrhoeal diseases in 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* O157:H7 (a re-emerging pathogen causing haemolytic-uremic syndrome) and Verocytotoxigenic *Escherichia coli* were isolated from the study population further confirming *E. coli* as an active causative agent of diarrhoeal diseases in Kaduna state, Nigeria.
2. The presence of antibiotic resistant strains of the *E. coli* isolates in the study population indicates the often unnecessary and uninformed use of these drugs in the treatment of most infantile diarrhoea cases.

6. RECOMMENDATIONS

The following recommendations are made from the findings obtained from this study:
I. Breastfeeding of children, which is known to help a lot in improving child’s immunity, should be encouraged for longer periods (1000 days) than the usual 6 months as this would help ensure that parents, especially the mothers, keep a watchful eye on the kind of things the child ingests.

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

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

IV. Hand washing practices should be inculcated and encouraged amongst preschool aged children as they are the most vulnerable group in the society.

V. Adequate diagnosis should be carried out at all times to ascertain the level of infection.

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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