CURRENT STUDIES IN COMPARATIVE EDUCATION, SCIENCE AND TECHNOLOGY, VOLUME 9, NUMBERS 1-2, 2022, PP. 121-135

SCIENCE PAPERS

PREVALENCE AND ANTIBIOGRAM OF MULTIDRUG RESISTANT ENTEROHEMORRHAGIC *ESCHERICHIA COLI* O157: H7 ISOLATED FROM SOME FECAL AND WATER SAMPLES IN PORT HARCOURT METROPOLIS

A. A. AKINTOLA, S. A. WEMEDO, S. A. & N. P. AKANI
Rivers State University, Port Harcourt, Nigeria

Abstract

Public health is at risk due to the prevalence of enterohemorrhagic *Escherichia coli* (EHEC) and the fact that EHEC is becoming resistant to most antibiotics demonstrates widespread antibiotics indiscrimination. The study was aimed at to conduct a survey of multidrug resistant EHEC isolated from some fecal and water samples in Port Harcourt, Rivers State. Eighty-four (84) samples of human, chicken, cow, and water were taken at random from four (4) distinct locations and put through standard microbiological tests. Results for Fecal Coliform Counts (FCC**)** showed that there was a significant difference ($p \leq 0.05$) in the FCC between locations sampled where chicken droppings had the highest mean count of $34.44 \pm 30.73 \times 10^7$CFU/g while water samples had the least mean count of $0.60 \pm 0.89 \times 10^7$CFU/ml. Results for Total Coliform Counts (TCC) showed no difference ($p \geq 0.05$) in the TCC between locations sampled where cow feces had the highest mean count of $2.77 \pm 1.87 \times 10^{10}$CFU/g while human squat had the least mean count of $0.77 \pm 0.81 \times 10^{10}$CFU/g. Eleven (11) *E. coli* isolates were identified as EHEC. The prevalence of EHEC showed human squat, chicken droppings, water sample had 27.27% while cow feces had 18.18%. Susceptibility pattern of EHEC showed resistance to cefuroxime, meropenem, cefotaxime, and ceftazidime antibiotics while susceptible to gentamicin, chloramphenicol, tetracycline, cotrimoxazole, and vancomycin antibiotics. The EHEC isolates showed multidrug resistance index above 0.2 with 90.91%. Conclusively, this study showed unacceptable levels and high prevalence of EHEC from fecal and water samples in the study area posing a serious threat to public health.

Keywords: enterohemorrhagic *e. coli* (ehec), fecal samples, water samples, multiple antibiotic resistance (mar) index

Introduction

Escherichia coli is one of the bacterial species that typically dwell in the intestines of healthy people and the majority of warm-blooded animals. It is a coliform, rod-shaped, Gram-negative, facultatively anaerobic bacterium (Tenaillon *et al*., 2010). The majority of *E. coli* strains are non-lethal, but some serotypes can seriously harm their hosts and occasionally result in food contamination incidents that force recalls. *E. coli* bacteria aid in preserving the equilibrium of beneficial and normal intestinal bacteria (Levine, 1987). According to Eckburg *et al.* (2005), *E. coli* and other facultative anaerobes make up about 0.1% of the microbiota in the gut, and fecal-oral

transmission is the main way that pathogenic strains of the bacterium spread disease. They are possible indicator organisms to check environmental samples for fecal contamination since they may survive outside the body for a very long time (Tortora, 2010).

However, there are a large number of diverse strains of *E. coli*, each with its own unique traits. The Enterohemorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E. coli* (STEC) family of *E. coli* is one of the *E. coli* strains that can cause serious intestinal infections in both people and domestic animals (CDC, 2012). They are among the most prevalent strains that lead to serious food-related illnesses in people. Its production of the powerful toxin called shiga toxin sets it apart from other strains of *E. coli*. Bloody diarrhea is the result of the toxin damaging the lining of the intestinal wall (CDC, 2012). Enterohemorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E. coli* (STEC) usually produces no symptoms in animals but causes severe human infections that might lead to hemorrhagic colitis, hemolytic uremic syndrome, or death (Shelton *et al*., 2006). Cattle are the primary source of EHEC, and a wide variety of other animals and birds also harbor it. EHEC causes disease in humans by producing one or more shiga-like toxins, which prevent host cells from making proteins, resulting in cell death.

These toxins are encoded by the genes stx1 and stx2, as well as their variations. Human sickness may result from eating as few as 1–10 EHEC cells. EHEC contamination of drinking water, both processed and untreated, has been linked to disease outbreaks in the past (Ashbott and Willets, 2000). Although the sickness is typically self-limiting, it can, especially in small children and the elderly, progress to a life-threatening condition like hemolytic uremic syndrome (HUS). *E. coli* O157:H7 is the most prevalent STEC, although other non-O157 STEC serotypes, especially O26, O45, O103, O111, O113, O121, O128 and O145, can also result in enterohemorrhagic disease. Serotype O104:H4 is reported to have caused a big, global outbreak in Europe in 2011 (CDC, 2012).

Humans who are infected with *E. coli* O157:H7 (EHEC) may have abdominal pain and bloody diarrhea. Toxins that are released by EHEC attach to endothelial cells that express globotriaosylceramide-3 (GS3), enabling their absorption and spread into the bloodstream and to other organs (Sandvig, 2001). Excessive antibiotic use is thought to be the primary cause of antibiotic resistance. It's possible for this antibiotic resistance to develop due to gene mutations or horizontal gene transfer (Laxminarayan and Brown, 2001). Multidrug resistant (MDR) bacteria may have a number of drug resistance genes and bacterial enzymes. Human morbidity and death rates have increased significantly as a result of the multidrug resistant *E. coli* strains that have emerged quickly (Nikaido, 2009). Beta-lactamases are bacterial enzymes that give *E. coli* resistance to beta-lactam antibiotics like penicillin and cephalosporins by hydrolyzing the beta-lactam ring. Extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases are two of the new varieties of beta-lactamase enzymes that have evolved (Babic *et al*., 2006; Bradford, 2001; Paterson, 2006). The following resistance genes are the most often discovered beta-lactamases in Gram-negative bacteria: TEM, SHV, OXA, CMY, and CTX-M beta-lactamases (Paterson, 2006). The majority of ESBLs and AmpCs are found on mobile genetic elements (plasmids, transporons or intergrons). Conjugation, transformation, and transduction have been found to contain mobile genetic components that facilitate horizontal gene transfer techniques used to spread bacterial cells to other organisms (Livermore *et al*., 2006). There is an increasing concern for public health over multi-drug resistance Enterohemorrhagic *E. coli,* which develops resistant genes and bacterial enzymes (CDC, 2012). As a result, this study examined the prevalence of entero-

hemorrhagic E. coli strains from fecal samples of human, cattle, poultry, and water samples and their resistant pattern to various antibiotics.

Materials and Methods

Collection and Transport of Samples

A total of 84 samples, including 21 samples of human squat, 21 samples of cow feces, 21 samples of chicken droppings, and 21 samples of water were collected from four (4) distinct sources. All samples were collected in sterile universal sample vials and transported aseptically to the bacteriological analysis facility at Rivers State University as soon as possible.

Serial Dilution

There was a sequential ten-fold dilution. To obtain stocks of the samples in various test tubes, one gram (1g) of each sample was weighed into nine milliliters (9ml) of sterile normal saline. One milliliter (1ml) was transferred from the stock into test tubes filled with 9ml of sterile normal saline before being serially diluted with dilution factors of 10^{-1} to 10^{-8} into the eighth test tube (Cheesbrough, 2005).

E. coli Plate Counting and Isolation

Each sample was diluted appropriately, and an aliquot (0.1 ml) of each was placed in duplicate on sterile MacConkey and Eosin Methylene Blue (EMB) agar plates. The spread plate technique was used to disperse it equally using a sterilized glass spreader. While the MacConkey agar plates were incubated at 37ºC for 24 hours, the EMB agar plates were incubated inverted at 44°C for 48 hours. The plates were examined after incubation, and the colonies that emerged were counted and noted (Karch *et al*., 1996; Zhou *et al*., 2002).

Purifying and Preserving of Bacterial Isolates

Following the bacterial isolation using a sterile wire loop, distinct colonies with specific characteristics were selected from the incubated agar plates and sub cultured using the streak plate method on sterile nutrient agar plates. The plates were then incubated at 37 °C for 24 hours to produce pure isolates. A single colony was moved aseptically from the subculture plates to the nutritional agar slants, where it was cultured for 24 hours at 37°C. Following incubation, the slants were housed in well-baffled vials of 10% glycerol storage media and kept in a refrigerator at -4°C. It has become vital to keep the pure cultures free of contamination in order to preserve the viability and purity of the isolates (Cheesbrough, 2006).

Characterization of Bacterial Isolates

Based on their colony appearance, microscopic analysis, and biochemical tests, bacterial isolates were characterized. For the purpose of identifying bacteria, references to Bergey's Manual of Determinative Bacteriology (1992) and the ABIS Online Identification tool were made (Holt *et al.,* 1994).

Colonial and Morphological Characterization

A colony of the isolate was selected and streaked on a freshly made nutrient agar plate, where it was cultured for 24 hours at 37°C. The following morphological characteristics of the isolate colony after incubation were seen visually using a hand lens: shape, size, coloration, edge, texture, and elevation (Cheesbrough, 2005). Gram

staining and other biochemical assays were utilized to examine the cell morphology of the overnight pure cultures of the bacterial isolates (Cheesbrough, 2006).

E. coli Enterohemorrhagic Strain Identification (E. coli O157:H7)

For some of the assays used to identify *E. coli* O157:H7, sorbitol MacConkey agar and the sorbitol fermentation test were used.

E. coli Enterohemorrhagic Strain Identification Using Sorbitol MacConkey Agar (SMAC)

The manufacturer's specifications were followed when preparing and sterilizing the sorbitol MacConkey agar. Twenty milliliters (20ml) was put into sterile petri dishes, which were then dried in a hot air oven after being given time to harden. Using the streak plate approach, single colonies of *E. coli* were selected from the nutrient agar subculture plate and inoculated on the SMAC for 24 hours at 37°C. Because sorbitol is not fermented by *E. coli* O157:H7, it stayed colorless on the sorbitol MacConkey agar (Osazee and Shadrach, 2020; Wells *et al*., 2005).

E. coli Enterohemorrhagic Strain Identification Using Sorbitol Fermentation Test

One (1) gram of sorbitol was added to 80ml of peptone water and vigorously mixed. The sugar-peptone water solution was then given 1ml of phenol red indicator, which contains 0.2% (w/v) of phenol. A crystal violet drop of around 0.1 ml was added to the mixture. Without using Durham's tubes, five milliliters of the sugar-peptone solution were distributed into test tubes and autoclaved at 121°C for 15 minutes. The test tubes were given time to cool before being infused with a 24-hourold overnight growth culture. After that, the tubes were incubated for 24 hours at 37°C.An orange or yellow tint indicated a good result for fermentation, whereas *E. coli* O157:H7 was identified by a negative result where the isolate could not ferment the sugar (Thompson *et al*., 1990).

Preparation of Standard Bacterial Suspension and 0.5 McFarland Turbidity Standard

A pure culture of the test organism that had been grown for 24 hours was emulsified in sterile nutrient broth. By appropriately mixing 1ml of concentrated sulfuric acid w ith 99ml of distilled water, an approximately 1% weight-to-volume solution of sulfur ic acid was created. An amount of 0.5g of dehydrated barium chloride ($BaCl_2.2H_2O$) was dissolved in 50ml of distilled water to create a 1% w/v barium chloride solution. 99.4 ml of the sulphuric acid solution and approximately 0.6 ml of the barium chlori de solution were combined and thoroughly homogenized. A produced turbid solution was transferred to a sealed tube and stored at room temperature (25 to 28°C) in a wel l-covered, dark environment (CLSI, 2017). An inoculum of 1.0 x 10^8 was calibrated into 0.5 McFarland Turbidity Standard (Cheesbrough, 2005).

Mueller-Hinton Agar Preparation

According to the manufacturer's instructions, the Mueller-Hinton agar preparation w as sterilized in an autoclave at 121°C psi for 15 minutes. To prevent inaccurate readi ngs of the zones of inhibition, the pH of the medium was validated to be 7.2 and put into the petri dish to the proper depth.

Agar Disk Diffusion Method (Kirby Bauer's Method)

The tube containing the bacterial suspension, whose turbidity is equivalent to 0.5 Mc

Farland Turbidity Standard, was dipped into with a sterile swab stick. To ensure that the organism was distributed evenly, swab ticks were used to swab the surface of the petri dish, which was filled with Mueller Hinton agar that had already been prepared. The agar was left for three to five minutes to dry. The impregnated antimicrobial dis cs were equally distributed on the surface of the inoculation plate using sterile forcep s, 15 mm from the plate's edge. Each disc was pressed down slightly to establish con tact with the agar using the forceps' head. The plates were incubated in an inverted p osition aerobically at 35°C for 24 hours after the discs were applied. The test plates were checked for growth after incubation. Each plate's zone of inhibition was measur ed and reported in terms of its diameter (mm) (CLSI, 2017).

Analytical Statistics

Percentages or frequency of the *E. coli* isolates were used in the statistical analysis of the data that was gathered. The prevalence of resistance to the chosen antibiotics was compared between the four host samples using a two-way analysis of variance (ANOVA) without replication to identify any significant differences. To determine whether there was a significant difference between the data presented as means with standard deviations and plotted graphically using Microsoft Excel 2016 and to all data collected during the study using the IBM SPSS (Statistical Package for the So-cial Sciences) software, a p-value of 0.05 was considered at the 95% level of signifi-cance.

Results

Results of bacterial population of samples obtained from diverse sources in the resea rch area as presented where Fecal Coliform Counts (FCC) results showed a significa nt difference ($p \leq 0.05$) in the FCC across the studied locations, with water samples ha ving the lowest FCC of $0.60 \pm 0.89 \times 10^7$CFU/ml and chicken droppings having the hi ghest FCC of $34.44 \pm 30.73 \times 10^7$CFU/g. The Total Coliform Counts (TCC) results sh owed that there was no significant difference ($p > 0.05$) in the TCC across the sample d locations, with Cow faeces having the highest count of $2.77 \pm 1.87 \times 10^{10}$CFU/g and human squat having the lowest of $0.77 \pm 0.81 \times 10^{10}$CFU/g.

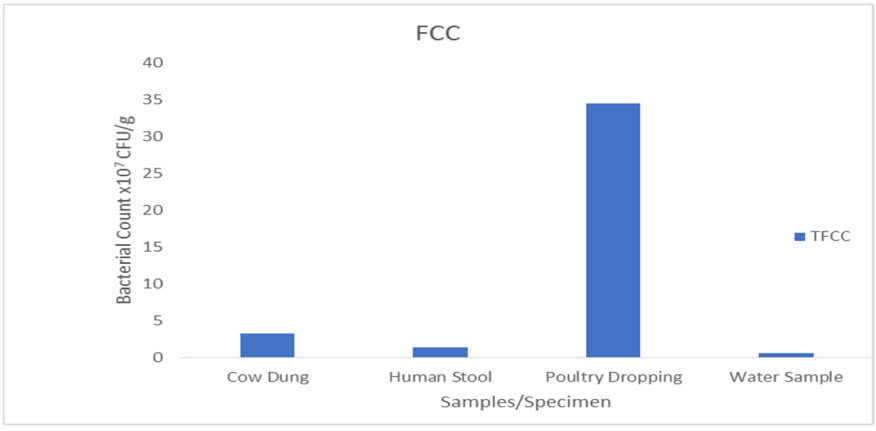

Figure 1: Fecal Coliform Counts of Fecal and Water Samples (Cfu/ml) from different Sources

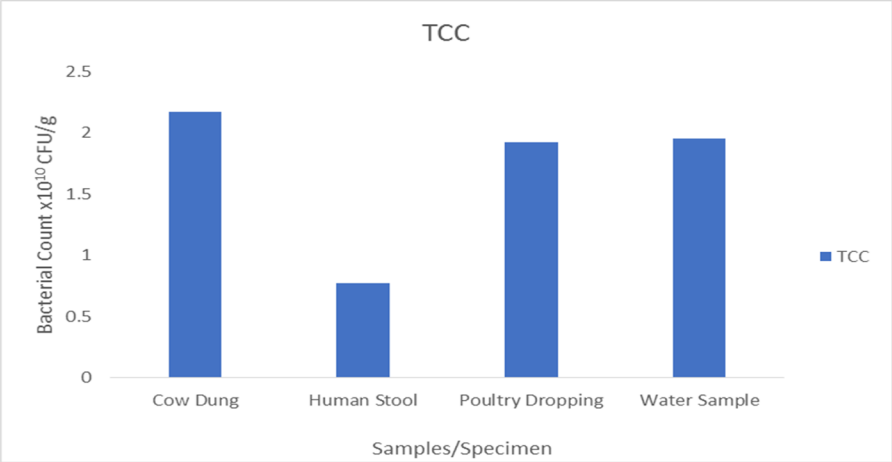

Figure 2: Total Coliform Counts of Fecal and Water Samples (Cfu/ml) from different Sources

Results of the percentage occurrence of *E. coli* and EHEC isolates (Table 1) revealed that 31 *E. coli* isolates with chicken droppings having the highest prevalence (32.26%) and water samples having the lowest prevalence (9.68%). Eleven (11) of the *E. coli* isolates were identified to be EHEC, with cow feces having the least prevalence (18.18%) and human squat, chicken droppings, and water samples having the highest percentages of prevalence (27.27%).

Table 1: Percentage Occurrence of *E. coli* and EHEC across all samples analyzed

Samples/ Specimen	***E. coli* N=31**		**EHEC N=11**	
	Occurrence	**% Occurrence**	**Occurrence**	**% Occurrence**
Human Squat	9	29.03	3	27.27
Chicken Droppings	10	32.26	3	27.27
Cow Feces	9	29.03	2	18.18
Water Sample	3	9.68	3	27.27

KEY: EHEC (Enterohemorrhagic *E. coli*), N (Total Number of Isolates), % (Percentage).

Results of the susceptibility pattern of *E. coli* (Table 2) revealed the majority of the *E. coli* isolates were sensitive to chloramphenicol (93.55%), followed by gentamicin (90.32%) and tetracycline (70.97%). Cefuroxime (80.65%), Meropenem (70.97%), Cefotaxime (64.52%), Ceftazidime (61.29%), Vancomycin (54.84%),

Ceftriaxone (38.71%), Ciprofloxacin (32.26%), Cotrimoxazole (29.03%), and Amikacin (25.81%) were the drugs with the lowest resistance in *E. coli*.

Table 2: Susceptibility Profile of *Escherichia coli* as collected from all samples analyzed

Antibiotics Conc. (µg)	**Resistant n (%)**	**Intermediate n (%)**	**Susceptible n (%)**
Tetracycline (10)	0(0.00)	9(29.03)	22(70.97)
Cotrimoxazole (25)	9(29.03)	8(25.81)	14(45.16)
Gentamycin (10)	0(0.00)	3(9.68)	28(90.32)
Cefuroxime (30)	25(80.65)	1(3.22)	5(16.13)
Chloramphenicol (10)	2(6.45)	0(0.00)	29(93.55)
Ceftriaxone (30)	7(22.58)	12(38.71)	12(38.71)
Cefotaxime (30)	20(64.52)	9(29.03)	2(6.45)
Ciprofloxacin (5)	10(32.26)	15(48.39)	6(19.35)
Amikacin (30)	8(25.81)	12(38.71)	11(35.48)
Vancomycin (30)	17(54.84)	1(3.22)	13(41.94)
Ceftazidime (30)	19(61.29)	5(16.13)	7(22.58)
Meropenem (10)	22(70.97)	2(6.45)	7(22.58)

Results of EHEC susceptibility pattern (Table 3) revealed that a greater proportion of EHEC isolates were resistant to cefuroxime (90.91%), meropenem (90.91%), cefotaxime (72.73%), and ceftazidime (54.55%), while susceptibility to the following drugs decreased in that order: gentamicin (100%), chloramphenicol (100%) > tetracycline (63.64%), cotrimoxazole (63.64%) > vancomycin (54.55%).

Table 3: Susceptibility Pattern of Enterohemorrhagic *Escherichia coli* (EHEC) isolated from all sources in the study area

Antibiotics Conc. (µg)	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Tetracycline (10)	0(0.00)	4(36.36)	7(63.64)
Cotrimoxazole (25)	1(9.09)	3(27.27)	7(63.64)
Gentamycin (10)	0(0.00)	0(0.00)	11(100)
Cefuroxime (30)	10(90.91)	0(0.00)	1(9.09)
Chloramphenicol (10)	0(0.00)	0(0.00)	11(100)
Ceftriaxone (30)	3(27.27)	4(36.36)	4(36.36)
Cefotaxime (30)	8(72.73)	3(27.27)	0(0.00)
Ciprofloxacin (5)	4(36.36)	7(63.64)	0(0.00)
Amikacin (30)	0(0.00)	10(90.91)	1(9.09)
Vancomycin (30)	5(45.45)	0(0.00)	6(54.55)
Ceftazidime (30)	6(54.55)	3(27.27)	2(18.18)
Meropenem (10)	10(90.91)	0(0.00)	1(9.09)

Results of MAR indices of *E. coli* and EHEC (Table 4) revealed the multi-drug resistance index of 30 (96.77%) of the 31 *E. coli* isolates was equal to or more than 0.2, while the index of 1 (3.23%) strain was less than 0.2. The Multiple Antibiotic Resistance Index for EHEC isolated from all samples. Ten (90.91%) of the 11 EHEC isolates exhibited a multidrug resistance index of more than 0.2, whereas one (9.09%) was lesser than 0.2.

Table 4: MAR Indices of *Escherichia coli* and Enterohemorrhagic *Escherichia coli* (EHEC) Isolated during the study

MAR Index	*Escherichia coli* N=31	Enterohemorrhagic *Escherichia coli* (EHEC) N=11
0.0	1(3.23)	1(9.09)
0.1	0(0.00)	0(0.00)
0.2	3(9.68)	0(0.00)
0.3	12(38.71)	5(45.45)
0.4	7(22.58)	3(27.27)
0.5	3(9.68)	1(9.09)
0.6	2(6.45)	1(9.09)
0.7	2(6.45)	0(0.00)
0.8	1(3.23)	0(0.00)

KEY: Multiple Antibiotic Resistance (MAR)

Discussion

The presence of *E. coli* O157:H7 and other enteric organisms as common pathogens in the environment or community, causing bacterial infections, particularly food-borne or waterborne infections cannot be overemphasized (Kumar *et al*., 2009). The research observed that out of the eighty-four (84) samples, higher fecal coliform counts were obtained in the samples, demonstrating a significant increase ($p \leq 0.05$) in the bacterial load analyzed where high mean counts were observed in chicken droppings while water samples had the lowest mean counts and probably attributed to environmental conditions, ingestion of microorganisms that reside in their natural habitat during consumption of contaminated food and water (Takeda, 2011). Cow feces had the highest total coliform counts in this study, while human squat had the lowest mean total coliform counts, which is consistent with similar counts found by Galvin *et al.* (2010), and there was no significant difference ($p \geq 0.05$) in the Total Coliform Counts (TCC) of the samples across the locations. The most common water source for human food preparation, the distance between the localization and dispersal of microorganisms within the study area, and wastes (human and animal feces) where anthropogenic activities are predominating, as previously stated by Takeda. (2011), could all contribute to the detection of coliforms.

Thirty-one (31) *E. coli* isolates were found in all of the samples taken from the four distinct locations, making a total of fifty (50) isolates from the samples. The prevalence of *E. coli* in this study was high across all samples, at 62%. This finding is comparable to that of Azuonwu *et al*. (2019), who also found a high prevalence of *E. coli* at 74%. Chicken droppings revealed a significant prevalence of *E. coli* (32.26%), while water samples had a low prevalence (9.68%). Among the other strains of *E. coli* found the prevalence of *E. coli* O157:H7 was 35.48% in this study. This finding is consistent with Enabulele and Uraih's (2009) report, which claimed that the high rate of *E. coli* O157:H7 occurrence is a sign of the unhygienic conditions in which animals are slaughtered and sold. Human feces, bird droppings, and water samples all had the same prevalence of EHEC, which was 27.27%, however cow feces had the lowest incidence of EHEC, which was 18.18%.Evidently, *E. coli* 0157:H7 has been found in the feces or gastrointestinal system of a range of wild species, including cattle, sheep, horses, pigs, turkeys, dogs, and sheep (Hancock *et al.*, 1998). This is similar to Smith *et al*. (2009)'s study, though, which demonstrated a high prevalence of *E. coli* O157:H7 isolates from human and environmental samples from Lagos and Zaria, Nigeria. The high frequency of EHEC in this study is probably due to unsanitary and unhygienic settings, cross contamination, the area's high population density, anthropogenic activities, and the discharge of feces into the water body (Kibret and Abera, 2011).

Antibiotic resistance in bacteria, especially *E. coli* linked to water and feces, has been a worry worldwide, and its susceptibility patterns revealed significant variance as well as differences in population and environment. Antimicrobial drugs and the development of resistance are now generally acknowledged to be related (Kibret and Abera, 2011). The results of the antibiotic sensitivity patterns, as interpreted using the Clinical Laboratory Standard Institute Guideline (2017), showed that a significant amount of *E. coli* was susceptible to chloramphenicol (93.55%) and gentamicin (90.32%). The antibiotic sensitivity pattern of the *E. coli* and EHEC found in this study has a significant impact on the public health implications of these organisms because it affects the clinical treatment option(s) that are accessible for therapy. As a result, the antimicrobials put organisms under selective pressure, which is a major problem in epidemiological investigation. Additionally, the EHEC

isolates revealed 100% susceptibility to the antibiotics mentioned, demonstrating that they are the most effective drugs on *E. coli* from this work and it is consistent with the work of Kibret and Abera (2011), which showed that *E. coli* is most sensitive to Gentamicin, Ciprofloxacin and Chloramphenicol isolated from clinical samples.

By adhering to ribosomes, these medications prevent bacteria from producing proteins (Schultsz and Geerlings, 2012). Tetracycline (70.97%) followed this susceptibility pattern in *E. coli*, while Tetracycline, Cotrimoxazole (63.64%), and Vancomycin (54.55%) followed this susceptibility pattern in EHEC. The effects of the antibiotic tetracycline, which also inhibits bacterial protein synthesis by attaching to ribosomes. Cotrimoxazole, a drug made of trimethoprim and sulfamethoxazole, inhibits either DNA gyrase or topoisomerase IV by interfering with the synthesis of nucleic acids during DNA replication. The unusual glycopeptide structure of Vancomycin also inhibits the peptidoglycan layer of bacterial cell walls, which prevents bacteria from correctly performing cell development and division (Schultsz and Geerlings, 2012). This research is comparable to that of Zhou *et al*. (2015), who showed how Vancomycin interacts synergistically with a variety of antibiotics, including Trimethoprim, Sulfamethoxazole, Ciprofloxacin, Tetracycline, Streptomycin, and others. *E. coli*'s susceptibility pattern revealed resistance to the following antibiotics which include; Vancomycin, Cefuroxime, Cefotaxime, Ceftazidime and Meropenem while antibiotics such as Cefuroxime, Meropenem, Cefotaxime and Ceftazidime were the drugs with the highest resistance in EHEC's susceptibility pattern. As a result, a significant portion of these organisms were shown to be resistant to cefuroxime, cefotaxime, ceftazidime, and meropenem. These microorganisms had higher levels of resistance to Meropenem, an intravenous beta-lactam antibiotic that works by preventing the synthesis of bacterial cell walls. Meropenem resistance can develop as a result of mutations in penicillin-binding proteins, production of metallo-β-lactamases, or resistance to diffusion across the bacterial outer membrane (Martha *et al.,* 2014). In the family of beta-lactam antibiotics, Cefuroxime is a second-generation cephalosporin, whereas Cefotaxime and Ceftazidime are third-generation cephalosporins. Gram-negative bacterial resistance to third-generation cephalosporin and carbapenem within a community is exceedingly challenging to handle, as suggested by Park (2014). The high resistance of these organisms to the beta-lactam antibiotics (Cefuroxime, Cefotaxime, and Ceftazidime) in this study can be explained by the extensive and uncontrolled use of these antibiotics as well as their affordability and the acquisition of the bla_{CTX-M}, bla_{SHV}, and bla_{TEM} genes. However, the resistance to beta-lactam drugs is consistent with work by Bedasa *et al.,* (2018).

E. coli is evolving new ways to resist antibiotics, which is a serious public health concern. The existence of multi-drug resistant strains demonstrates how *E. coli* is generating these new strategies, which are limiting and expensive therapeutic choices. As a result, the Multiple Antibiotic Resistance (MAR) index of the *E. coli* isolates used in this investigation showed that 96.77% of them had a MAR score above 0.2. The Multiple Antibiotic Resistance (MAR) index of the EHEC isolates included in this investigation likewise showed that 90.91% of them had a MAR value above 0.2. It is crucial to be aware that sources of contamination where antibiotics are often used have MAR index values larger than 0.2 (Davis *et al*., 2016; Krumperman, 1985). However, a significant portion of the MAR indices of the *E. coli* and EHEC identified in this investigation demonstrated various antibiotic resistances and indiscriminate use of these antibiotics for infections (Davis *et al*., 2016).

Conclusions and Recommendations

The EHEC was discovered in water and fecal samples that revealed the presence of fecal coliforms in this study and suggested that food poisoning is a likely result, which is a significant reason for public health action. When compared to other samples evaluated during this investigation, the results of this study revealed significant fecal coliform level in chicken droppings, and the prevalence raised public health concerns. This study has confirmed that EHEC is resistant to several classes of antibiotics showing a varying increase in resistance of the EHEC isolates of 90.91% with a multiple antibiotic resistant index greater than 0.2. Chloramphenicol, Gentamicin, Tetracycline, Cotrimoxazole, and Vancomycin can be used as first-line medications for EHEC-related foodborne and waterborne diseases from these sources. Surveillance systems should be increased for assessing risk factors of diseases and to provide strategies to prevent and protect public health. To stop the spread of antibiotic resistance strains, public awareness campaigns about the risks associated with the indiscriminate use of antibiotics are advised.

Correspondence
Professor Nedie Akani
Department of Microbiology
Faculty of Science
Rivers State University
P. M. B. 5080, Port Harcourt
Nigeria
Email: nedieakani@gmail.com

References

Azuonwu, O. A., Umasoye, U. and Ekong, I. U. (2019). Investigation of Prevalence of *Escherichia coli* in Public Drinking Water sources randomly collected in and around Diobu Residential Area of Port Harcourt, Niger Delta. *International Journal of Research Studies in Microbiology and Biotechnology.* 5(4): 6-11.

Ashbott, N. J., and Willetts, J. R., (2000). Understanding anaerobic decolonization of textile dye wastewater: mechanism and kinetic. *Water Science Technology*, 42(1-2):409-415.

Babic, M., Hulzer, A. M., and Bonomo, R. A., (2006). What's new in antibiotic resistance? Focus on beta-lactamases. *Drug Resistance Updates,* 9(3):142-156.

Bedasa, S. Shiferaw, D., Abraha, A. and Moges, T. (2018). Occurrence and antimicrobial susceptibility profile of Escherichia coli O157:H7 from food of animal origin in Bishoftu town, Central Ethiopia. *International. Journal of Food Contamination*. 5: 2-3.

Bradford, P. A., (2001). Extended-spectrum beta-lactamases in 21st Century: Characterization, epidemiology and detection of this important resistance threat. *Clinical Microbiology, Revise*, 14(4):933-951.

CDC (Centers for Disease Control and Prevention), (2012). National center for Emerging and Zoonotic Infectious Diseases. US.

Cheesbrough, M., (2000). Microbiological test District Laboratory Practice in Tropical Countries. In: Cremer, A., and Evan, G., (eds). *Cambridge University Press*, UK. Pp: 1-226.

Cheesbrough, M., (2005). District Laboratory Practice in Tropical Countries, part 2. *Cambridge University Press, Cambridge.* Pp: 159-162.

Cheesbrough, M., (2006). District Laboratory Practice in Tropical Countries. *Cambridge University Press*. Pp: 62

Clinical and Laboratory Standard Institute. (2017). *Performance Standards for Anti microbial Susceptibility Testing, Twenty-first Informational Supplement.* CLSI docu ment M100-S21 (ISBN1-56238-742-1) Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 USA, 30(1): 68 -70.

Davis, R. and Brown, P. D. (2016). Multiple Antibiotic Resistance index, Fitness an d Virulence Potential in Respiratory *Pseudomonas aeruginosa* from Jamaica. *Journa l of Medical Microbiology.65*: 261 – 271.

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefoen, L., and Sargeant, M., (2005). Diversity of the human intestinal microbial flora. *Science,* 308 (5728):1635-1638.

Enabulele, S. A. and Uraih, N. (2009). Enterohaemorrhagic *Escherichia coli* 0157:H7 Prevalence in meat and vegetables sold in Benin City, Nigeria. *African Journal of Microbiology.* 3(5): 276-279.

Galvin, S., Boyle, F., Hickey, P., Vellinga, A., Morris, D., and Cormican, M., (2010). Enumeration and characterization of antimicrobial-resistant *Escherichia coli* bacteria in effluent from municipal, hospital, and secondary treatment facility sources. *AppliedEnvironmental Microbiology,* 76*:* 4772-4779.

Hancock, D. D., Besser, T. E. and Rice, D. G. (1998). Multiple source of *Escherichia coli* 0157 in feed lots and dairy farms in the northrestorn USA. *Preventive Veterinary Medicine*. 35:11-19.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Stanley, J. T., and William, S. T., (1994). Bergey's Manual of Determinative Bacteriology. Williams and Wilkins, Baltimore, 786-788.

Karch, H., Mellman, A., and Bielaszewska, M., (2009). Epidemiology and pathogenesis of enterohemorrhagic *Escherichia coli. Journal of Clinical Microbiology*, 122: 417-424.

Kibret, M. and Abera, B. (2011). Antimicrobial susceptibility patterns of *E. coli* from cliical sources in northeast Ethiopia. *African Health Sciences*. 11(S1): S40-S45.

Krumperman, P. H. (1985). Multiple Antibiotic Indexing of E. coli to Identify High Risk Sources of Fecal Contamination of Foods. *Applied and Environmental Microbiology* 46:165–170.

Kumar, S., Otta, S. K. and Karunasagar, I. (2009). Detection of Shiga-toxigenic Escherichia coli (STEC) in fresh seafood and meat marketed in Mangalore India by PCR. *Letters in Applied Microbiology* 33(5): 334–338.

Laxminarayan, R., and Brown, C. M., (2001). Economics of antibiotic resistance: a theory of optimal use of *Escherichia coli. Journal on Environmental Economics Management.* 42(2):183-206.

Levine, M. M., (1987). *Escherichia coli* that cause diarrhea: Enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. *Journal on Infectious Diseases*, 155(3):377-389.

Livermore, D. M., and Woodford, N. (2006). The beta-lactamases threat in *Enterobacteriacae*, *Pseudomonas,* and *Acinetobacter. Trends Microbiology,* 14(9): 413-420.

Martha, F. M., Stephen, E. M., Can, I. and Freddie, B. (2014). Carbapenemase Genes among Multidrug Resistant Gram-Negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. *Biomed Research International*. 30: 31-34.

Nikaido, H. (2009). Multidrug resistance in bacteria. *Annual Revise Biochemistry,* 78:119-146.

Osazee, E. I. and Shadrach, O. O. (2020). Occurrence of *Escherichiacoli* O157:H7 from meat products sold in Obinze abattoir, Imo State, Nigeria. *International Journal of Applied Biology*, 4(2): 2580.

Park, S. H. (2014). Third-generation cephalosporin resistance in gram-negative bacteria in the community: a growing public health concern. *Korean Journal International*. 29(1): 27-30.

Paterson, D. C., (2006). Resistance in Gram-negative bacteria – Enterobacteriaceae. *Amateur Journal Medicine.* 119(6 Suppl 2), S20-S28.

Sandvig, K. (2001). Shiga toxins. *Toxicon*, 39;1629-1635.

Sauvage, E., Kerff, F., Terrak, M., Ayala, J.A. and Charlier, P. (2008). The penicillin -binding proteins: structure and role in peptidoglycan biosynthesis. *Journal of Federation of European Microbiological Societies Microbiology Reviews*.32:234-258.

Schultsz, C. and Geerlings S. (2012). Plasmid-mediated resistance in Enterobacteriaceae. Changing Landscape and Implications for Therapy. Drugs. 72:1-16.

Shelton, D. N., Sandoval, I. T., Eisinger, A., Chidester, S., Ratnayake, A., Ireland, C. M., and Jones, D. A., (2006). Up-regulation of CYP26A1 in Adenomatous Polyposis Coli-Deficient vertebrates via a WNT-Dependent Mechanism: Implications for Intestinal Cell Differentiation and colon Tumor Development. *Cancerresearch,* 66 (15): 7571-7577.

Smith, S. I., Bello, O. S., Goodluck, H. A., Omonigbehin, E. A., Agbogu, V. N. and Odeigah, P. (2009). Prevalence of EHEC O157:H7 from human and environmental samples from Lagos and Zaria. *Pakistan Journal of Medical Sciences.* 25(3): 398-403.

Takeda, Y. (2011) Vibrio parahaemolyticus, enterotoxigenic *Escherichia coli*, enterohemorrhagic *Escherichia coli* and *Vibrio cholerae*. Proceedings of the Japan Academy, Series B. *Physical and Biological Sciences*. 87: 1- 12.

Tenailon, O., Skurnik, D., and Picard, B., (2010). The population genetics of Commensal *Escherichia coli. Nature Review Microbiology,* 8(3): 207-217.

Thompson, J. S., Hodge, D. S., and Borczyk, A. A. (1990). Rapid biochemical test to identify verocytotoxin-positive strains of *Escherichia coli* serotype O 157. *Journal of Clinical Microbiology.* 28(2): 165-168.

Tortora, G. (2010). Microbiology: An introduction. San Franscisco CA: *Benjamin-Cummings*, pp:85-87,161-165.

Wells, J. E., Barry, E.D. and Varel, V. H. (2005). Effects of common forage phenolic acids on *Escherichia coli* 0157:H7 viability in bovine faeces. *Journal of Applied and Environmental Microbiology*, 71(12):7974-7979.

Zhou, Z., Nishikawa, Y., and Zhu, P., (2002). Isolation and Characterization of Shiga toxin- producing *Escherichiacoli* O157:H7 from beef, pork and cattle fecal sam-

ples in Changchun, China. *Journal of Veterinary Medical Science*, 64(11): 1041-1044.