

## Antimicrobial Susceptibility Pattern of Enterohaemorrhagic *Escherichia coli* (EHEC O157:H7) in Raw Beef samples collected from abattoirs and Meat Vendors in Ile Ife and its environs, South-West, Nigeria

Fashina Christiana Dunah<sup>1\*</sup>, Ayemoba Iyanuoluwa Oluwanifemi<sup>1</sup> and Oladosu Samuel Aanuoluwa<sup>2</sup>

<sup>1</sup>Department of Microbiology, Obafemi Awolowo University, Ile Ife.

<sup>2</sup>Pathology Unit, Medical and Health Services, Obafemi Awolowo University, Ile Ife.

### \*Correspondence:

Fashina Christiana Dunah. Department of Microbiology, Obafemi Awolowo University, Ile Ife, E-mail: chrisfashd64@gmail.com.

Received: 15 Nov 2022; Accepted: 17 Dec 2022; Published: 23 Dec 2022

**Citation:** Fashina C.D, Ayemoba I.O, Oladosu S.A. Antimicrobial Susceptibility Pattern of Enterohaemorrhagic *Escherichia coli* (EHEC O157:H7) in Raw Beef samples collected from abattoirs and Meat Vendors in Ile Ife and its environs, South-West, Nigeria. American J Pathol Res. 2022; 1(1): 1-9.

### ABSTRACT

Nigeria bears a large burden of foodborne diseases in Africa, and the second leading causes of premature deaths in Africa is linked to diarrheal diseases. Enterohemorrhagic *Escherichia coli* O157 (EHEC O157:H7) causes an asymptomatic infection to severe diarrhea and/or hemolytic-uremic syndrome in humans which are serious health conditions that calls for serious health concern. The present investigation was done to study the prevalence and antibiotic resistance of Enterohaemorrhagic *Escherichia coli* O157:H7 strains isolated from raw meat samples. Raw beef samples were collected from abattoirs and retail outlets in three local government areas (LGAs) in Ile-Ife axis namely: Ife North, Ife Central and Ife East for isolation and identification of EHEC O157:H7. Standard microbiological methods were used to isolate and identify EHEC O157:H7 and antibiotics susceptibility pattern was carried out on the isolates to determine their susceptibility or otherwise. EHEC O157 was detected in 16% of meat samples from Abattoirs slabs and 33% from meat vendors and retailers. EHEC O157 was not detected in one of the beef samples obtained from a particular Abattoir. The EHEC O157:H7 isolates were found to be most susceptible to meropenem (91.50%) followed by vancomycin (90.80%) and ceftazidime (88.8%). Isolates were found to be most resistant to ampicillin (90.85%), followed by chloramphenicol (86.93%) and cefixime (85.63%). Furthermore, isolates were resistant to a range of more than five antibiotics and three classes of antibiotics, depicting multi drug resistance pattern. Five isolates were resistant to ampicillin, two isolates to streptomycin and three isolates to chloramphenicol. One isolate was resistant to two drugs and another to three drugs. The present study shows a bit higher prevalence of EHEC O157 in beef sold directly at abattoirs. Considering, the high infective dose of this pathogen and the deep-rooted tradition of consuming raw or undercooked beef, the current prevalence should not be considered lightly from a public health perspective.

### Keywords

Antimicrobial susceptibility, Beef, Abattoirs, Enterohaemorrhagic, EHEC O157, Meat vendors.

### Introduction

Foodborne diseases have become one of the important health concerns all over the world especially in this 21<sup>st</sup> century. However, in developing countries, due to poor infrastructure and low level of awareness, this problem is becoming worse and, on the increase, [1].

Enterohemorrhagic *Escherichia coli* O157 is a major pathogenic microorganism that frequently have been closely associated with foods of animal origin [2]. This pathogen in Humans causes asymptomatic infection to severe diarrhea and/or hemolytic-uremic syndrome (HUS) [30]. Human infections with EHEC O157 have been mostly linked with the consumption of improperly cooked or contaminated beef and unpasteurized cow milk [4]. Abattoirs / slaughter-houses are frequently incriminated as sources of EHEC O157 for human infections [5]. Nigeria, however ranks first in the health burden of zoonotic diseases in Africa. This country located

---

in a sub-region that experiences the second highest foodborne disease burden in the world, where EHEC O157:H7 is one of the leading causes of foodborne disease disability adjusted life years [6]. In Ethiopia, years of life lost due to diarrheal diseases was 2.6 million in 2010, and diarrheal diseases are the second leading cause of premature death after lower respiratory infections [7].

One of the factors that exacerbate the transmission of foodborne pathogens including EHEC O157:H7 is the habit of consuming raw and/or undercooked meat which is on the rise. Proper cooking/heating of meat at a very high temperature for a long time inhibits these organisms [8]. However, in Nigeria particularly in Ile-Ife, most consumers prefer to eat undercooked beef as a result of the increase in the price of fuel because cooking meat to high temperature consumes a lot of fuel. Also, some consumers of meat prefer cooking it shortly because it taste nicely than meat that are properly or well cooked. In Nigeria however in Ile-Ife City to be precise, only few small-scale studies estimating the prevalence and/or assessing the antimicrobial sensitivity profile of Enterohemorrhagic *Escherichia coli* O157:H7 has been done. Also studies at the level of consumption of meat particularly sold in abattoirs shops and meat vendors is lacking. This study consequently was designed to address the information gap pertaining to the prevalence and antibiotic susceptibility profiles of EHEC O157 in beef samples and retail beef at Abattoirs and Meat vendors respectively in Ile-Ife City, Osun State.

## Research Methodology

### Study Area

This study was conducted in Ile-Ife metropolis namely Ife east, Ife north Ife Central metropolis. Twenty-five wards spanning across the entire region; seven (7) wards from Ife-East, twelve (12) wards from Ife central and six (6) from Ife-North. Ile-Ife central particularly is a Local Government Area and a major city in Osun State in south-western Nigeria. Ile-Ife axis economy are primarily based on agriculture and small-scale businesses.

### Study Design

A cross-sectional study was carried out and three hundred and eight (308) samples were collected in this study, eighty-six samples (86) from Ife east, one forty-eight (148) samples from Ife central and seventy-four samples from Ife north. Twenty-five wards spanning across the entire region; seven (7) wards from Ife-East, twelve (12) wards from Ife central and six (6) from Ife-North from each Local Government Area using simple random sampling.

### Sample Size

The sample size was determined according to the formula described by Thrusfield using a 95% level of significance. A 50% expected prevalence was employed in the determination of the sample size.

### Sample Collection

Samples were collected in rain (April-November) and dry season (December-March). Samples (retailed meat samples) were purchased in sterile packs and were transported on ice packs with all aseptic

precautions observed. All the samples were labelled accordingly after collection, stored at 4°C until examination and transported to the postgraduate laboratory of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife for analysis. The time lapse between sample collection and analysis was within 2 hours after the collection.

### Isolation and Identification of Enterohemorrhagic *Escherichia coli* O157:H7

Enterohemorrhagic *Escherichia coli* colonies were isolated and enumerated using Hi-crome agar using Hi-Crome Media (Mumbai India). Twenty-five-gram (25g) of fresh beef samples was aseptically cut out and placed in a sterile blender for proper blending and homogenized in 9 ml of (1%) peptone water for 1 Minute to obtain a homogenate. The homogenate was further serially diluted up to  $10^{-4}$  to obtain discrete colonies according to Paton in 1998.

### Biochemical tests were carried out on the isolates to further confirm, the tests include:

#### a. Indole test

The indole test screened for the ability of an organism to degrade the amino acid tryptophan and produce indole. It was used as part of the IMViC procedures, a battery of tests designed to distinguish among members of the family Enterobacteriaceae in which EHEC O157:H7 is part. A tube of tryptone broth with a small amount of a pure culture of EHEC O157:H7 was incubated at 35°C for 24 hours. Five drops of Kovács reagent were added directly to the tube. A positive indole test was indicated by the formation of a pink to red colour ('cherry-red ring') in the reagent layer on top of the medium within seconds of adding the reagent.

#### b. Methyl red test

The basic principle of MR test was to check the ability of the organism to produce and maintain sufficient amount of stable acid as end product from glucose fermentation and to overcome the buffering capacity of the system. Among different fermentation pathway followed by bacteria, MR test works upon the mixed acid fermentation pathway. Two tubes containing prepared MR-VP Broth with a pure culture of the EHEC O157:H7 was incubated at 35°C for up to 4 days. Five drops of the methyl red indicator solution was added to the first tube (for Voges-Proskauer test, Barrit's reagent is added to another tube). A positive reaction was indicated because the colour of the medium changes to red.

#### c. Voges-proskauer Test

A tube of MR/VP broth with a pure culture of EHEC O157:H7 was incubated for 24 hours at 35°C. Aliquot 1 mL of broth was dispensed into clean test tube and 0.6mL of 5% anaphthol, followed by 0.2 mL of 40% KOH was added (Note: It was essential that the reagents be added in this order). The tube was gently shaken to expose the medium to atmospheric oxygen and allow the tube to remain undisturbed for 10 to 15 minutes. A positive test was indicated by the presence of a red color 15 minutes or more after the addition of the reagents indicating the presence of diacetyl, the oxidation product of acetoin.

#### d. Citrate test

This test is among a suite of IMViC Tests (Indole, Methyl-Red, Vogues-Proskauer, and Citrate) that are used to differentiate among the Gram-Negative bacilli in the family Enterobacteriaceae. In a beaker, 24.28 grams of the dehydrated powder or lab-prepared media was added to 1000 milliliters of pure distilled or deionized water. The solution was then heated to bring it to a boil in order to dissolve the medium completely. The dissolved medium was then dispensed into tubes and sterilized in an autoclave at 15 lbs pressure (121°C) for 15 minutes. Once the autoclaving process was complete, the tubes were taken out and cooled at a slanted position to a temperature of about 40-45°C. The position was maintained in order to obtain butts of 1.5 – 2.0 cm depth. A well-isolated colony of EHEC O157:H7 was taken from an 18-24 hour culture with a sterile inoculating needle. The citrate agar tubes were inoculated by streaking the surface of the slant. The slant was streaked back and forth with the loop or the inoculating stick. The cap of the test tubes was left loosened to ensure adequate aeration. The tubes were incubated aerobically at 35-37°C for up to 4 days. The test tubes were examined daily for 4 days before discarding the result as a negative. The change in color, if present, is observed. A positive test is demonstrated by growth with a color change from green to intense blue along the slant. A negative test is demonstrated by no growth and no colour change, and the color of the slant remains green. For *Escherichia coli*, the colour remains green because its growth was been inhibited.

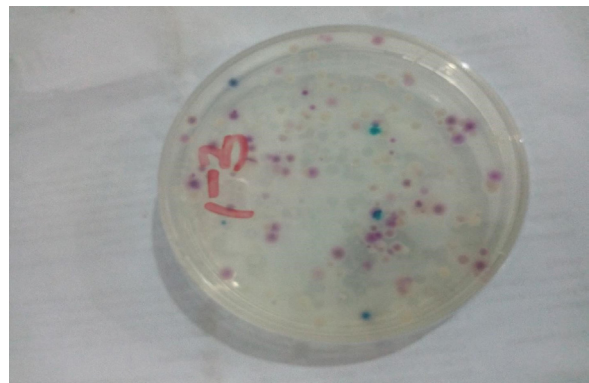
Afterwards, the homogenate was cultured on HiCrome EC O157:H7 Agar using the streaking method. This agar is a chromogenic medium recommended for the isolation and differentiation of *E.coli* O157:H7 from food and environmental samples distinctively. The medium contains sorbitol and a propriety chromogenic mixture instead of lactose and indicator dyes respectively, as is conventionally used. The chromogenic substrate is specifically and selectively cleaved by *E.coli* O157:H7 resulting in dark purple to magenta coloured moiety which distinguishes EHEC O157:H7 from other strains of *Escherichia coli*. The working principle of this media is that it is specific for isolating *E. coli* O157:H7 because it has both selective and chromogenic properties. This makes it easy to differentiate the target strain '*E.coli* O157:H7' from other *E.coli* strains as shown in figure 1, making the lab work much easier, faster and accurate than when the conventional Biochemical which is time consuming and not much reliable because of the unsterile laboratory conditions is used. Results are obtained distinctively within a short period of time, thereby enabling the researcher to isolate from many samples within a short period of time.

#### Antimicrobial Susceptibility Testing of Isolates

##### Preparation of McFarland standard

The turbidity standard solution was prepared by adding 0.5 ml of 0.048 M BaCl<sub>2</sub> to 99.5 ml of 0.36 N H<sub>2</sub>SO<sub>4</sub> (1% w/v). This solution is equal to half the density of McFarland standard solution. This solution was taken into the glass tube, sealed tightly and kept in the dark, at room temperature for further use.

The tube was vigorously agitated just before each use [9].



**Figure 1:** EHEC O157:H7 (Purple colonies) Showing on HiCrome Chromogenic Media.

##### Preparation and standardization of inoculum

From a pure culture, four isolated distinct colonies were selected and transferred into a sterile nutrient broth and incubated at 37°C. The turbidity of the broth culture was adjusted using solution of the prepared McFarland standard solution. When the broth culture was found to be more turbid than expected, it was diluted with nutrient broth and when the turbidity was found to be less, the culture was incubated for more time to achieve the required turbidity [9].

##### Inoculation

A swab stick was dipped into standardized inoculum and excess inoculum was removed from the swab by rotating it several times with a firm pressure on the inside wall of the test tube, above the fluid level. Within 15 minutes after adjusting the density of inoculum, swabbing over its entire surface. The swabbing procedure was repeated two more times, rotating the plates to ensure an even distribution of inoculum. The inoculated plates were allowed to dry for 15 min [10].

##### Application of antibiotic discs

The inoculated plate was left for not more than 15 minutes at room temperature to absorb any excess surface moisture before applying the drug impregnated discs. With a sterile forceps, the discs were applied to the surface of the inoculated agar. With the tip of the forceps, each disc was gently pressed down to ensure complete contact with the agar surface. During the application of discs, proper care was taken not to place it closer than 15mm from the edge of the plate and the distance between the centers of two such discs was not less than 24mm. The inoculated plate was inverted and incubated at 37°C for 18 hours after the application of the discs. In this study, more than five available antibiotic class were used in the antibiotic's susceptibility test [11].

##### Result and Interpretation

The plates were examined at the end of the incubation period, and the diameter of the zones of complete inhibition was measured to the nearest whole millimeter with a caliper held at the back of the Petri plate, which was made more visible with a reflected

light as shown in figure 2. The zone of inhibition of each disc was measured in three different directions keeping the midpoint of the disc as the center of the zone. The mean of the measurement of inhibition was used for the interpretation of the results. The interpretation of the result was made by comparing diameter of the zone of inhibition with standard zone of inhibition chart provided by the disc manufacturer (Biomark Laboratories, India.). The isolates were grouped as sensitive, intermediate, and resistant, against each antibiotic according to the interpretation of results standard from CLSI [12] as shown in table 2. The list of antibiotics used for the study and their concentrations are: Ciprofloxacin (5ug), Cotrimoxazole (25ug), Augmentin (30ug), Ampicillin (30ug), Chloramphenicol (10ug), Tetracycline (30ug), Gentamicin (30ug), Cefuroxime (30ug), Ceftazidime (20ug), Gentamicin (100ug), Cefixime (5ug), Ofloxacin (5ug), Nitrofurantion (300ug) and Ceftriaxone (30ug) and Meropenem (5ug).

**Table 1:** List of Antibiotics Used for Susceptibility Study and their Concentrations.

S/N	Antibiotics	Abbreviations	Concentration	Class
1	Ciprofloxacin	CIP	5ug	Fluoroquinolones
2	Cotrimoxazole	COT	25ug	Cephems
3	Augmentin	AUG	30ug	Penicillin
4	Ampicillin	AMP	30ug	Penicillin
5	Chloramphenicol	CHL	10ug	Phenicol
6	Tetracycline	TET	30ug	Tetracycline
7	Gentamicin	GEN	30ug	Aminoglycosides
8	Cefuroxime	CEFU	30ug	Cephems
9	Ceftazidime	CEFT	20ug	Cephems
10	Vancomycin	VAN	100ug	Cephems
11	Cefixime	CEFI	5ug	Cephems
12	Ofloxacin	OFL	5ug	Fluoroquinolones
13	Nitrofurantoin	NIT	30ug	Nitrofurans
14	Ceftriaxone	CEF	30ug	Cephems
15	Meropenem	MER	5ug	Cabapenam

**Table 2:** Antibiotic disk used to test EHEC O157: H7 and their respective concentrations and corresponding zones of inhibition according to (CLSI 2019) [12].

No	Antibiotics	Resistant (mm)	Intermediate (mm)	Suceptible (mm)
1	Tetracycline (10ug)	≤12	15-18	≥19
2	Cotrimoxazole (25ug)	≤12	13-15	≥16
3	Gentamicin (10ug)	≤12	13-15	≥16
4	Cefuroxime (30ug)	≤14	15-22	≥23
5	Choramphenicol (10ug)	≤14	15-20	≥21
6	Ceftriaxone (30ug)	≤14	15-20	≥21
7	Ciprofloxacin (5ug)	≤14	16-20	≥21
8	Ampicillin (30ug)	≤13	14-17	≥18
9	Ceftazidime (30ug)	≤12	13-17	≥18
10	Cefixime (5ug)	≤12	13-17	≥18
11	Ofloxacin (5ug)	≤13	14-16	≥17
12	Augmentin (30ug)	≤14	15-17	≥18
13	Nitrofurantoin (30ug)	≤13	14-17	≥18
14	Vancomycin (30ug)	≤11	12-14	≥15
15	Meropenem (30ug)	≤11	20-22	≥23

## Results

### Standard Plate Count (SPC) of Non-EHEC O157:H7 and EHEC O157:H7 in Beef Samples

The results of standard plate count of raw beef revealed the average mean value to be  $9.12 \pm 0.08$ . The highest SPC for non-EHEC O157:H7 was found in meat samples from sqbo area (Ife central) with mean value of  $9.43 \pm 0.05$  and the lowest count was found to be in meat samples from modakeke 2 (Ife east) with mean values of  $4.84 \pm 0.24$  (Table 3). The highest SPC count for EHEC O157:H7 was found in meat samples from old ife market (Ife central) with mean value of  $9.43 \pm 0.15$  and the lowest count was found to be in meat samples from modakeke 2 (Ife east) with mean values of  $4.34 \pm 0.21$  (Table 4).



**Figure 2:** Plates Showing the Antibiotics Suceptibility Pattern of Isolates.

Table 3 to Table 5 shows the occurrence pattern of EHEC O157:H7 in meat samples from different locations in the local governments in Ile- Ife axis. The highest SPC colony count of EHEC O157:H7 in ife east LGA was found in meat samples from moore market area with mean value of  $6.91 \pm 0.26$  while the lowest count was found in meat samples from ita Osa (Ife east) with  $4.34 \pm 0.21$  (Table 3). In Ife central LGA, the highest SPC colony count of EHEC O157:H7 was found in meat samples from Ife market with mean value of  $9.43 \pm 0.15$  while the lowest count was found in meat samples from the meat republic (1) with  $4.61 \pm 0.13$  as mean value (Table 4).

**Table 3:** Standard Plate Count of non-EHEC O157:H7 and EHEC O157:H7 in Meat samples from Ife East LGA Location.

Location	No of Samples	SPC of non-EHEC O157:H7	SPC of EHEC O157:H7	
Modakeke 1	16	$6.19 \pm 0.19^a$	$5.91 \pm 0.19^b$	Values are the mean ( $\pm$ ) standard deviation where n=86. Mean with different superscript along the row (each location) are significantly different at $p < 0.05$ .
Ondo Road	8	$6.36 \pm 0.08^a$	$6.26 \pm 0.08^b$	
Ita Osa	8	$5.80 \pm 0.24^a$	$4.34 \pm 0.21^b$	
Modakeke 2	16	$4.84 \pm 0.24^b$	$5.34 \pm 0.17^b$	
Ilode	16	$7.00 \pm 0.27^a$	$6.00 \pm 0.17^a$	
Oke Ogbo	14	$6.12 \pm 0.15^a$	$6.42 \pm 0.15^a$	
Moore	8	$5.91 \pm 0.26^b$	$6.91 \pm 0.26^a$	

**Table 4:** Standard Plate Count of non-EHEC O157:H7 and EHEC O157:H7 in Meat samples from Ife Central LGA.

Location	No of Samples	SPC of non-EHEC O157:H7	SPC of EHEC O157:H7
Opa	16	7.19 ± 0.19 <sup>a</sup>	7.39 ± 0.21 <sup>b</sup>
OIF	20	9.43 ± 0.05 <sup>a</sup>	9.43 ± 0.15 <sup>a</sup>
TMNMI	8	4.74 ± 0.19 <sup>b</sup>	4.64 ± 0.13 <sup>a</sup>
TMNM2	8	4.80 ± 0.15 <sup>b</sup>	4.70 ± 0.18 <sup>b</sup>
ADI	8	6.36 ± 0.08 <sup>a</sup>	6.26 ± 0.28 <sup>a</sup>
OSG	16	6.12 ± 0.15 <sup>a</sup>	6.02 ± 0.23 <sup>a</sup>
OSD	8	6.39 ± 0.12 <sup>a</sup>	6.29 ± 0.23 <sup>a</sup>
ILG	8	6.89 ± 0.12 <sup>b</sup>	5.89 ± 0.14 <sup>a</sup>
Sabo	20	9.43 ± 0.05 <sup>a</sup>	6.43 ± 0.08 <sup>b</sup>
IFMI	14	5.91 ± 0.26 <sup>a</sup>	6.44 ± 0.16 <sup>b</sup>
IFM2	14	6.00 ± 0.17 <sup>a</sup>	6.10 ± 0.27 <sup>a</sup>
ELE	8	6.49 ± 0.12 <sup>a</sup>	6.39 ± 0.22 <sup>b</sup>

Values are the mean ( ± ) standard deviation where n=148. Mean with different superscript along the row (each location) are significantly different at p<0.05.

For that of Ife north LGA, the highest SPC colony count of EHEC O157:H7 was found in meat samples from oduduwa university roundabout with mean value of 8.56 ± 0.08 while the lowest count was found in meat samples from moro with 4.94 ± 0.28 as mean value (Table 5). Overall, highest SPC colony count of EHEC O157:H7 was found in meat samples from Ife market area 1 (Ife central) with mean value of 6.41 ± 0.26 (Table 4) and the lowest count was found in meat samples from Ita-Osa (Ife east) with 4.34 ± 0.21 (Table 3).

**Table 5:** Standard Plate Count of non-EHEC O157:H7 and EHEC O157:H7 in Meat samples from Ife North LGA.

Location	No of Samples	SPC of non-EHEC O157:H7	SPC of EHEC O157:H7
AKM1	20	6.12 ± 0.15 <sup>b</sup>	5.32 ± 0.35 <sup>a</sup>
AKM2	16	6.43 ± 0.05 <sup>a</sup>	7.86 ± 0.65 <sup>b</sup>
OUIR	14	7.44 ± 0.08 <sup>b</sup>	8.56 ± 0.58 <sup>a</sup>
IPE 1	8	7.36 ± 0.08 <sup>b</sup>	6.56 ± 0.68 <sup>b</sup>
MOR	8	5.91 ± 0.26 <sup>a</sup>	4.94 ± 0.28 <sup>b</sup>
IPE 2	8	6.00 ± 0.17 <sup>a</sup>	5.02 ± 0.56 <sup>a</sup>

Values are the mean ( ± ) standard deviation where n=74. Mean with different superscript along the row (each location) are significantly different at p<0.05.

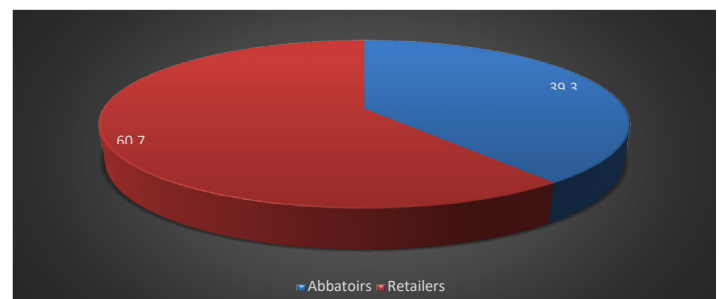
**KEYS:** AKM1: Akinola Market 1, AKM2: Akinola Market 2, OUIR: Oduduwa University Roundabout, IPE1: Ipetumodu 1, MOR: Moro, IPE2: Ipetumodu 2

The average percentage occurrence of EHEC O157:H7 in the abattoirs for both seasons was 39% while that of retailers for both seasons was 60%. This shows that the retail outlets had a higher incidence rate in the LGAs than the abattoirs (Figure 3).

### Results of Antibiotics Susceptibility Pattern of EHEC O157:H7 Isolates

When compared with CLSI 2019, most isolates showed different resistant patterns against the antibiotics used. The detailed antibiotics susceptibility pattern of the isolates summarized in

Table 6 and details about the percentage-wise antibiotics profile of isolates from the different samples showed that most of the isolates were susceptible to all the antibiotics used in the test. EHEC O157:H7 isolates showed 90% sensitivity to meropenem followed by vancomycin at 88.8% and cefuroxime at 86.40%. In the present study 90.45% of isolates showed resistance to ampicillin and 90.45% ofloxacin, followed by 86.57% of isolates showing resistance to chloramphenicol and 84.40% showing resistance to cotrimazole. It was also observed that 80.30%, 65.80% and 62.10% of isolates were resistant to gentamicin, tetracycline and ciprofloxacin respectively. 28.60% were resistant to ceftazidime and 24.44% to augmentin. Findings also revealed that 21.77% of isolates were resistant to nitrofurantoin, 21.40% resistant to ceftriaxone. 14.29% resistant to cefixime, 13.60% resistant to cefuroxime, 11.20% resistant to vancomycin and 9.20% resistant to meropenem (Figure 4).

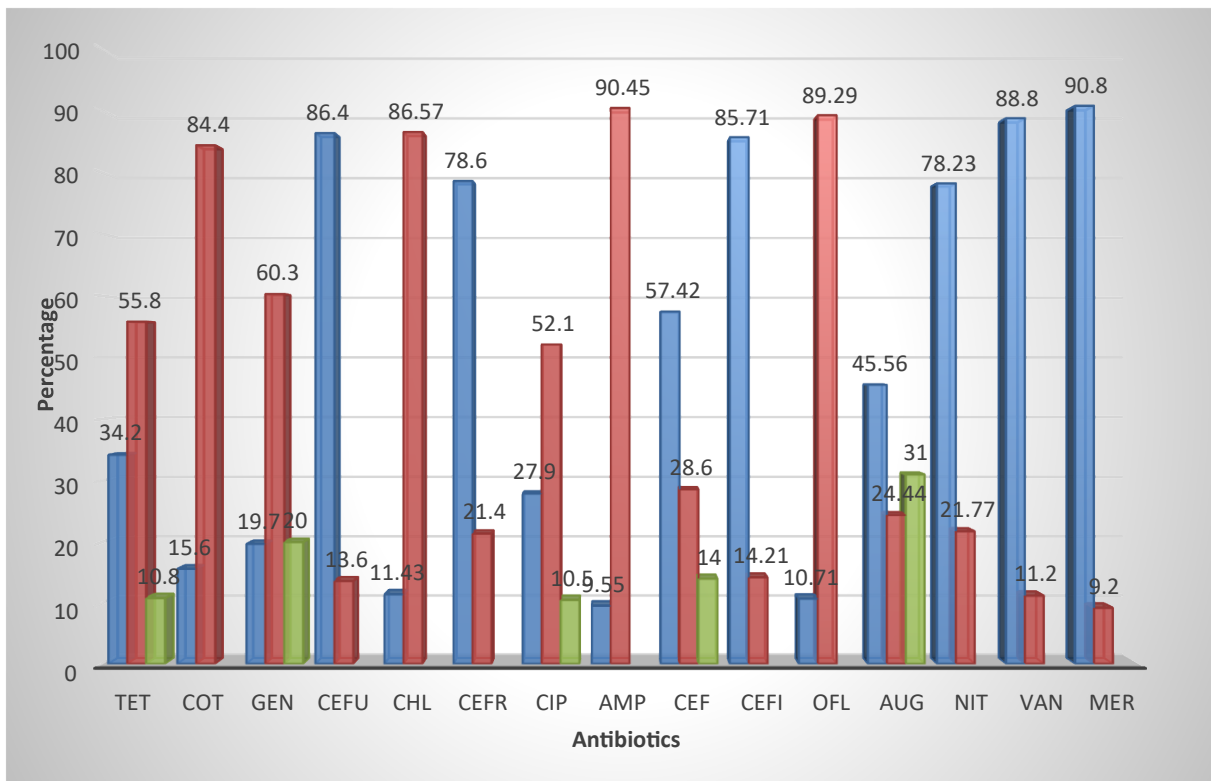


**Figure 3:** A Pie chart showing the Percentage variations between Abattoirs and Retail outlets for EHEC O157:H7 in all LGAs.

EHEC O157:H7 isolates were found to be most susceptible to meropenem (90.80%) followed by vancomycin (88.80%), cefuroxime (86.40%), cefixime (85.71%). High resistance was found to be against ampicillin (90.45%) followed by chloramphenicol (86.57%) (Figure 4). The resistance pattern of EHEC O157:H7 isolates to antibiotics are shown in Figure 4. The resistance pattern varies between susceptibility, resistance and intermediate pattern according to CLSI, 2019.

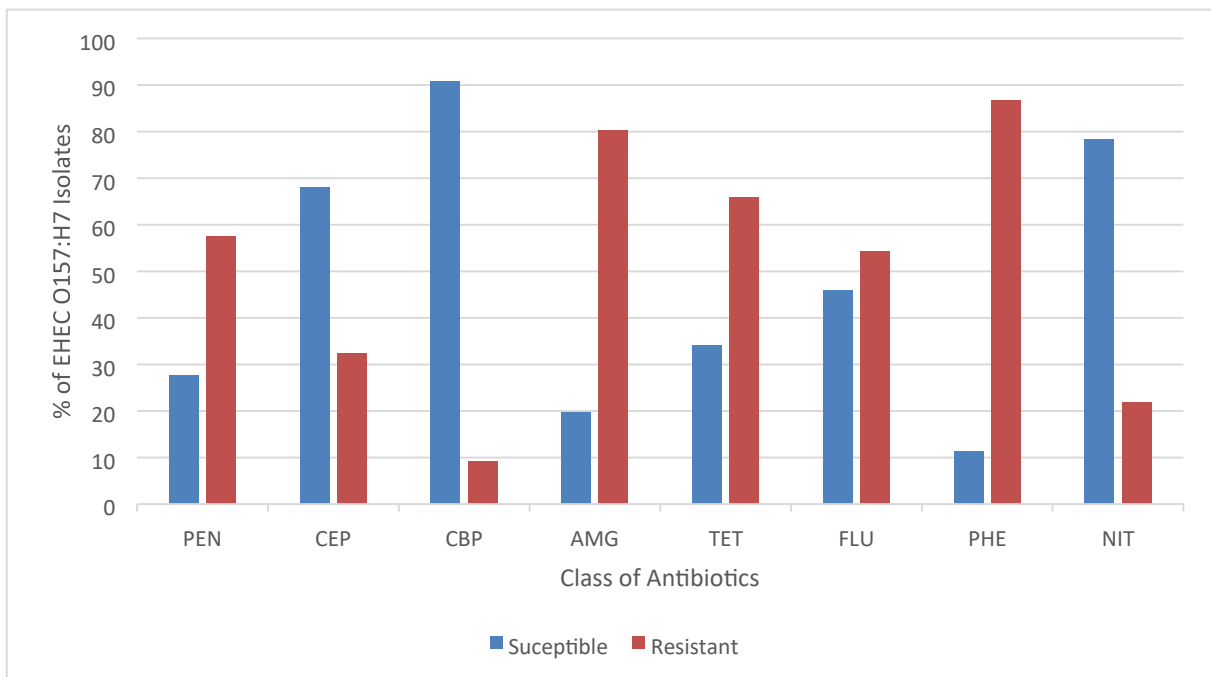
**Table 6:** Percentage Resistance Pattern of EHEC O157:H7 Isolates to Antibiotics used.

Antibiotics	Suceptible (%)	Resistant (%)	Intermediate (%)
Tetracycline (10ug)	34.20	55.80	10.8
Cotrimozaxole (25ug)	15.60	84.40	-
Gentamicin (10ug)	19.70	60.30	20
Cefuroxime (30ug)	86.40	13.60	-
Chloramphenicol (10ug)	11.43	86.57	-
Ceftriaxone (30ug)	78.60	21.40	-
Ciprofloxacin (5ug)	27.90	52.10	10.5
Ampicilin (30ug)	9.55	90.45	-
Ceftazidime (30ug)	71.42	28.60	14
Cefixime (5ug)	85.71	14.21	-
Ofloxacin (5ug)	10.71	89.29	-
Augmentin (30ug)	45.56	24.44	31
Nitrofurantoin (30ug)	78.23	21.77	-
Vancomycin (30ug)	88.80	11.20	-
Meropenem (30ug)	90.80	9.20	-



■ Suceptible    ■ Resistant    ■ Intermediate

**Figure 4:** Antibiotics resistance pattern of EHEC O157:H7 Isolates.



■ Suceptible    ■ Resistant

**Figure 5:** Pattern of Antibiotics Resistance of EHEC O157:H7 isolates in relation to classes of Antibiotics.

**KEYS:** PEN: Penicillin, CEP: Cephems, CBP: Carbapenem, AMG: Aminoglycosides, TET: Tetracycline, FLU: Fluoroquinolones, PHE: Phenicol, NIT: Nitrofurantoin

## Intermediate

The class of antibiotics that was most effective for the EHEC O157:H7 isolates were carbapenems with a rate of 90% while the class of phenicols were least effective with a rate of 10% (Figure 5). About 46% of the isolates had the highest multiple antibiotics resistance index of  $>0.80$  and 20 isolates having the lowest MAR index of  $<0.20$ . About 85% isolates were within the range of 0.20-0.79 (Table 6).

**Table 7:** Multidrug resistant profile of EHEC O157:H7

MAR index/ Range	Number of Isolates
$< 0.20$	20
0.20 - 0.39	24
0.40 - 0.59	25
0.60 - 0.79	36
$> 0.80$	46

MAR: Multiple Antibiotics Resistance

## Statistical Analysis

Chi-Square was used for the Statistical Analysis. Prevalence was expressed as the percent positive samples from the total samples tested. Differences in the prevalence of EHEC O157:H7 in abattoirs and retail outlets was assessed. The significance level of the presence of EHEC O157:H7 and non-EHEC O157:H7 was also analyzed, and the significance level was also set at  $p < 0.05$  which was considered statistically significant.

## Discussion

Ile-Ife city have municipal abattoirs which render slaughter services to their respective dwellers. Butcher shops, restaurants and other meat vendors or retailers get their beef from cattle slaughtered in the abattoirs of their respective locations. Nevertheless, backyard, illegal and unhygienic slaughtering of animals is a common practice and pattern. These abattoirs are scarcely equipped with necessary facilities for proper slaughtering practices. Shortage of clean water is one of the serious problems most abattoirs are facing in Ile-Ife. In addition to this, the sanitary condition and environment of most Abattoir and meat retailers are generally poor; Generally, food providing places e.g Abattoirs are loosely monitored and regulated by the appropriate health and food regulating Bodies. In Ile-Ife, fresh beef cuts are commonly purchased from Abattoirs and meat vendors and are consumed at home either being cooked through boiling, smoking or frying. In Abattoirs, a beef carcass is kept in open-air at environmental temperature (in Ile-Ife.  $7^{\circ}\text{C}$  to  $25^{\circ}\text{C}$  daily temperature). Food borne infections are major health concerns in developing countries including Nigeria. The contamination rate of EHEC O157:H7 within the beef samples were quantified. The CFUs ranged from  $5.70 \pm 0.13$  to  $6.41 \pm 0.26$  ( $\log_{10}$  cfu/g). These results demonstrated that a high number of cattle were with EHEC O157:H7. An isolation rate of 49% EHEC O157:H7 was obtained from the raw beef sampled. This result is similar to the findings of Fashina [13], Aboh's [14] and Frederick [15] who reported higher prevalence from different sources of water. However, this finding is in contrast with the findings of Yakubu in 2020 [16] who reported that 0.52% was obtained

from water sourced from households where small ruminants were reared. Based on the antibiotic's susceptibility pattern of EHEC O157:H7 in this study, EHEC O157:H7 isolates revealed highest resistance to ampicillin which is 90.85%, followed by 86.93% to chloramphenicol, 85.63% to cefixime, followed by others. Lowest resistance of the isolates was shown against meropenem (8.50%), vancomycin (9.20%) and ceftazidime (11.20%). The results of this study agreed with the findings of Amézquita-López et al. [17] who observed 100% resistance of EHEC O157:H7 to ampicillin isolated from domestic farm animals in rural communities in northwestern Mexico to ampicillin. It is also in line with the report of Mashak [18] who also reported 100% resistance of EHEC O157:H7 to more than two antibiotic agents. It was reported in the study that the highest levels of resistance against ampicillin (100%), tetracycline (83.33%), gentamicin (83.33%), amikacin (58.33%) and sulfamethoxazole (58.33%) which is similar to the resistance against ampicillin.

This same pattern is observed in the study of Beyi in 2017 [19] who observed 100% susceptibility of EHEC O157:H7 to five of the ten antibiotics used. Similarly, Deji Agboola and his group [20] in their study of molecular identification of EHEC O157:H7, from retail meat in Ibadan, Southwest Nigeria reported that out of the 130 isolates, 72 (55.4%) were resistant to at least one or more of the antibiotics tested including quinolone/fluoroquinolones. This also confirms the rise in antimicrobial resistant pattern. In a recent study conducted by Shinde, all the EHEC O157:H7 isolates obtained were resistant to at least one or more antimicrobial agents used in this study [21]. Resistance toward penicillin G, piperacillin, tetracycline, cotrimoxazole, ampicillin, and nitrofurantoin was observed. He observed that High occurrence of antimicrobial resistance in EHEC O157:H7 isolates from cattle may confer a selective advantage toward intestinal colonization [21].

In respect to high resistance of isolates towards ampicillin, Obaidat [22] in a study of EHEC O157:H7 in dairy cattle in Jordan, they reported that a high percentage of the isolates demonstrated resistance to amoxicillin-clavulanic acid (75.0%), ampicillin (62.5%), and kanamycin (58.3%). However, in this study Meropenem, Vancomycin and Ceftazidime were found to be the most effective antibiotics against the EHEC O157:H7 isolates. Furthermore, Bedasa buttressed that resistance of 90%, 80% and 77.5% was developed to Vancomycin, Ampicillin and Streptomycin respectively [23]. These varying antimicrobials resistant pattern may be because of repeated exposure of the organisms to these antibiotics thereby causing the organism to build up resistance against the antibiotics. The EHEC O157:H7 isolates in this study were (56%) multidrug resistant, that is, they were resistant to three or more tested class of antibiotics which is in similarity with the earlier study conducted by Ayalew [24]. Ayalew reported 93.2% multidrug resistant EHEC O157:H7, this slight variation may be due to change in resistant genes of EHEC O157:H7, natural resistance in which the pathogen possesses characteristics that inhibit the action of the antibiotic or acquired resistance in which there is a change in the genetic characteristics of the pathogen or horizontal

---

gene transfer in which genetic characteristics are transferred by members of the same generation. The antibiotics susceptibility pattern revealed in this study is in conformity with the work of other authors Adefarakan [25], Urumova [26], BukarKolo [26] who reported similar percentage of resistance to various antibiotics. The variation in the percentage of resistance to the antimicrobials tested in this study compared to other work may be attributed to different rates at which antimicrobials were used in different study areas and the distinction in the type of samples from which EHEC O157:H7 was isolated [27]. Due to resistance pattern observed in the isolates in this study, the isolates were found to be resistant to most of the antibiotics used which is of serious public health implication especially for those that consume the meat contaminated with such isolates without cooking properly. Hygiene level should be on the increase in cattle farms to reduce the occurrence of food borne pathogens and also the controlled use of antibiotics in the feeds of animals should be encouraged as the resistance of EHEC O157:H7 isolates against above mentioned antibiotics agent may be due to indiscriminate and irrational use in the fields of grazing [28]. The use of antibiotics in agriculture is contributing to the problem of antibiotic resistance amongst pathogenic bacteria. These drugs are widely used in animal production in Nigeria and are readily available over the counter [29]. This study correlates with a study carried out by Amosun [29] which showed high resistance of over 70% to amoxicillin, ampicillin and streptomycin among EHEC O157:H7 isolates [30]. Over the years, antimicrobial resistance has increased and the normal intestinal microbial flora of humans have become a reservoir for resistant genes. The use of antimicrobial agents in animal production has been identified as an important factor which select for antimicrobial resistant bacterial strains [31]. This may occur as a result of inevitable genetic response to the selective pressure which is strong and imposed by antimicrobial chemotherapy and plays a vital role in the evolution of antibiotic resistance among bacteria. Globally, antimicrobial resistant bacteria resident in the gut of carrier animals contribute significantly to environmental contamination and the spread of antimicrobial resistant bacterial strains, hence the need to continuously monitor antimicrobial resistance in zoonotic and commensal bacteria of animal origin for the protection of public health [32].

It is worth considering that antibiotic resistance in foodborne pathogens have increased virulence and can increase the burden on human health by increasing the risk of contracting an infection in immunocompromised persons and reducing the treatment options for illnesses. In this study, most isolates displayed a high resistance profile to antibiotics, indicating the potential for higher resistance to occur in the future. Attention must be given to antibiotics with high potency of isolates being resistant to them. As a result of this, measures should be put in place to ensure hygienic practices during slaughtering and during post-process handling of beef to reduce the risk of transmission of multi drug resistant EHEC O157:H7 to humans. Also proper legislation is required to regulate access to and use of antimicrobial agents in animal production in order to prevent the increasing incidence of resistance.

## Conclusion

This Findings suggests that cross-contamination of meat samples especially with EHEC O157:H7 occurs at Abattoirs and in other retail meat shops in Ile-Ife proving that cattle act as reservoir host for EHEC O157:H7 resulting in higher food contamination. In the beef retail shops samples, beef carcasses were not hoisted but kept on the floor for dressing. During the various handling of the carcass the intestinal contents were handled at same place with the whole meat. This has resulted in the cross contamination of the other meat parts during slaughter process. This clearly indicates the need for proper handling and processing of meat before selling before consumption. It is also important to note that during slaughter and in the kitchen where ever meat is prepared, care should be taken to avoid cross contamination. Antibiotics resistance is a serious threat to global health and has been established in this study with the large number of isolates that are resistant to the antibiotics used. Resistance of antibiotics of EHEC O157:H7 in cattle identified in one country do not respect international borders. Resistance of antibiotics cuts across borders. A robust evidence base that accurately describes the global burden of antimicrobial resistance will be essential for mitigating this challenge globally and this study has provided. The establishment of an integrated surveillance system for human and animal foodborne pathogens across the food chain is recommended. In addition, educational programs about the adverse health consequences of improperly cooked meat is essential in Ile-Ife. Further recommendations include educational programs for farmers, enacting regulations to restrict access to clinically important antibiotics, developing guidelines for veterinarians for the diet of cattles, and research into the potential drivers and mitigation strategies needed to reduce antibiotics resistance in Ile-Ife, Osun state.

## References

1. Mayer CL, Leibowitz CS, Kurosawa S, et al. Shiga toxins and the pathophysiology of hemolytic uremic syndrome in humans and animals. *Toxins Basel*. 2012; 4: 1261-1287.
2. Sethulekshmi C, Latha C, Sunil B. Occurrence of enterohaemorrhagic *Escherichia coli* in raw meat samples in Kerala. *International Journal of Advanced Research in Biological Sciences*. 2016; 3: 220-222.
3. Stella AE, Maluta RP, Rigobelo EC, et al. Virulence genes in isolates of *Escherichia coli* from samples of milk and feces from dairy cattle. *Journal of Food Protection*, 2012; 75: 1698-1700.
4. Parvej M, Ashraful MD, Mio S, et al. Prevalence of virulence genes of Diarrheagenic *Escherichia coli* in fecal samples obtained from cattle, poultry and Diarrheic patients in Bangladesh. *Japanese Journal of infectious diseases*. 2020; 73: 76-82.
5. Salvadori M, Elisabetta Bertoni. Update on hemolytic uremic syndrome: Diagnostic and therapeutic recommendations. *World Journal of Nephrology*. 2013; 2: 56-76.
6. Isibor JO, Afe OE, Regina EO, et al. *Escherichia coli* O157:H7-prevalence and risk factors of infection in Edo state, Nigeria. *American Journal of research communication*. 2013; 1: 35-50.
7. Abdissa R, Woyshet H, Akafete TF, et al. Prevalence of



- Escherichia coli* O157:H7 in beef cattle at slaughter and beef carcasses at retail shops in Ethiopia. Biomedical Clinical Infectious Diseases. 2017; 17: 277.
8. Saeedi P, Yazdanparast M, Behzadi E, et al. A review on strategies for decreasing *Escherichia coli* O157:H7 risk in animals. Microbial Pathogenesis. 2017; 103: 186-195.
  9. Samad A, Abbas F, Ahmad Z, et al. Multiplex polymerase chain reaction detection of shiga toxin genes and antibiotic sensitivity of *Escherichia coli* O157:H7 isolated from beef meat in Quetta, Pakistan. Journal of Food Safety. 2018; 23: 57-67.
  10. Dubreuil JD. EAST 1 toxin: An enigmatic molecule associated with Sporadic episodes of diarrhea in humans and animals. Journal of Microbiology. 2019; 57: 541-549.
  11. Perry JD. A decade of development of chromogenic culture media for clinical microbiology in an era of molecular Diagnostics. Clinical Microbiology Reviews. 2017; 3: 449-479.
  12. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 2019.
  13. Fashina CD, Babalola GO, Osunde MO. Prevalence and Molecular Characterization of *E. coli* O157:H7 Isolated from Water Bodies in Ile-Ife and Environs. Journal of Bacteriology Parasitology. 2018; 9: 340-342.
  14. Aboh AE, Giwa JF, Giwa A. Microbiological assessment of well waters In Samaru, Zaria, Kaduna, State, Nigeria. Annual African Medicine. 2015; 14: 32-36.
  15. Frederick Adzitey. Antibiotic Resistance of *Escherichia coli* Isolated from Beef and its Related Samples in Techiman Municipality of Ghana. Asian J Anim Sci. 2015; 9: 233-240.
  16. Yakubu RO, Lawan MK, Kwaga JKP, et al. Isolation, Molecular Detection and Antimicrobial Susceptibility Profile of *Escherichia coli* O157:H7 in Household - reared Small Ruminants in Zaria Metropolis, Kaduna State, Nigeria. Sahel Journal of Veterinary Science. 2020; 17: 16-23.
  17. Amézquita-López BA, Quiñones B, Soto-Beltrán M, et al. Antimicrobial resistance profiles of Shiga toxin-producing *Escherichia coli* O157 and Non-O157 recovered from domestic farm animals in rural communities in Northwestern Mexico. Antimicrob Resist Infect Control. 2016; 5: 1.
  18. Mashak Z. Prevalence and Antibiotic Resistance of *Escherichia coli* O157:H7 Isolated from Raw Meat Samples of Ruminants and Poultry. Journal of Food and Nutrition Research. 2018; 6: 96-102.
  19. Beyi A, Akafete F, Teklu F, et al. Prevalence and antimicrobial susceptibility of *Escherichia coli* O157:H7 in beef at butcher shops and restaurants in central Ethiopia. BMC Microbiology. 2017; 17: 233-236.
  20. Deji-Agboola, Anotu M, Ayodele OB, et al. Molecular Identification of *E. Coli* O157:H7, Virulence Genes and Quinolone /Fluoroquinolone Resistant Genes Among *E. Coli* Isolates From Retailed Meat In Ibadan, Southwest Nigeria. African Journal of Science and Nature. 2019; 9: 15-23.
  21. Shinde DB, Singhvi S, Koratkar SS, et al. Isolation and characterization of *Escherichia coli* serotype O157:H7 and other verotoxin-producing *Escherichia coli* in healthy Indian cattle. Veterinary World. 2020; 13: 2269-2274.
  22. Shinde DB, Singhvi S, Koratkar SS, et al. Isolation and characterization of *Escherichia coli* serotype O157:H7 and other verotoxin-producing *Escherichia coli* in healthy Indian cattle. Veterinary World. 2020; 13: 2269-2274.
  23. Bedasa S, Daniel S, Ashebr, et al. Incidence of Haemorrhagic shiga toxin *Escherichia coli*. International Journal of Food Contamination. 2018; 5: 24.
  24. Ayalew Assefa A. Prevalence of *Escherichia coli* O157:H7 in foods of animal origin in Ethiopia: A meta- analysis. Cogent Food and Agriculture. 2019; 5: 164-298.
  25. Adefarakan TA, Oluduro AO, David OM, et al. Prevalence of Antibiotic Resistance and Molecular Characterization of *Escherichia coli* from Feces of Apparently Healthy Rams and Goats in Ile-Ife, Southwest, Nigeria. International Journal of Science. 2014; 16: 447-460.
  26. Urumova V, Lyutskanov M, Petrov V. Investigations on Prevalence and Antimicrobial Resistance of Enterohaemorrhagic *Escherichia coli* (EHEC) Among Dairy Farms in the north part of the Republic of Bulgaria. Mac Vet Rev. 2015; 38: 21-29.
  27. Bukar-kolo YM, Peter ID, Bukar MM, et al. Prevalence and Antibiotic Susceptibility of Aerobic Bacterial Flora of the Skin, Wound and Anterior Vagina of Sheep, Goats and Dogs in Maiduguri, Nigeria. Alex. Journal of Veterinary Science. 2016; 49: 6-12.
  28. Adesokan HK, Akanbi IO, Akanbi IB, et al. Pattern of antimicrobial usage in livestock animals in south-western Nigeria: the need for alternative plans. Onderstepoort Journal of Veterinary Research. 2015; 82: 1-6.
  29. Abreham S, Teklu A, Cox E, et al. *Escherichia coli* O157:H7: Distribution, molecular characterization, antimicrobial resistance patterns and source. Biomedicine Microbiology. 2019; 19: 34-45.
  30. Amosun EA, Ojo OE, Alao IK, et al. Antimicrobial resistance among commensal *Escherichia coli* from cattle faeces and beef in Ibadan, Nigeria. African Journal of Biotechnology. 2012; 11: 12240-12245.
  31. WHO. Fact sheets, Antimicrobial resistance. 2021.
  32. Onifade AK, Oladoja MA, Fadipe DO. Antibiotics Sensitivity Pattern of *Escherichia coli* isolated from Children of School Age in Ondo State, Nigeria. Elsevier Journal. 2015; 45: 31-33.