Global Scientific JOURNALS

GSJ: Volume 9, Issue 9, September 2021, Online: ISSN 2320-9186
www.globalscientificjournal.com

Isolation and antimicrobial Susceptibility Studies of *Escherichia coli* from Gastrointestinal Contents Sample From Cattle in Maiduguri Abattoir

Ahmed Idriss Jajere[1, *], Saleh Dawud[2] ,Ahmad Abdullahi Garba[3], Dagona Maryam Bulama[4]

[1]Department of Biological Sciences, Federal University Gashua, Gashua, Nigeria
[2]Department of Veterinary Microbiology, University of Maiduguri, Maiduguri, Nigeria
[3]Department of Biological Sciences, Federal University Gashua, Gashua, Nigeria
[4]Department of Biological Sciences, Federal University Gashua, Gashua, Nigeria
Email address: Idrissjajere@gmail.com (A. I. Jajere)
[*]Corresponding author

To cite this article:
Ahmed Idriss Jajere, Saleh Dawud, Ahmad Abdullahi Garba and Dagona Maryam Bulama. Isolation and Antimicrobial Susceptibility studies of Escherichia coli from Gastrointestinal contents sample from cattle in maiduguri abattoir. Global scientific Journal. Vol. 9, No. 9, 2021, pp. 107.

Abstract:

Ruminants are the most important reservoir of zoonotic *E. coli* which is transmitted to humans through the ingestion of food or water contaminated with animal feces or through contact with infected animals or their environment. This study was carried out to isolates and and carried out antimicrobial susceptibility studies of *Escherichia coli* from gastro intestinal content sample from cattle in Maiduguri abattoir, Nigeria. A periodic visits were made to the Maiduguri abattoir where animals (cattle) slaughtered were randomly sampled. A total of eighty (80) rectal swab samples were collected. All the samples were collected aseptically by sterile swab stick and transported in an ice-pack to the Veterinary Microbiology Research Laboratory of the University of Maiduguri immediately and been inoculated into the peptone water and incubated for 24 hours at 37° C for bacteriological assay. The result show that 13 (68%) *E. coli* were isolated from young male cattle, 15 (83%) from young female cattle, 19 (86%) from adult male cattle, and 16 (76%) from adult female cattles. The result showed that the isolates from young male cattle were susceptible to Ciprofloxacin (38.7%), Ofloxacin (23%), Gentamicin (15.4%), and Nitrofurantion (7.7%), whereas, intermediate to Ceftazidime (15.4%) and Cefuroxime (7.7%) but were resistant to Cefixime (7.7%) and Augmentin (7.7%). Ciprofloxacin in which Ofloxacin having the widest zone of inhibition (23mm). Isolates from young female cattle showed Susceptibility towards Ciprofloxacin (26.7%), Ofloxacin (20%), Gentamicin (40%), Nitrofurantion (20%), Ceftazidime (6.7%) and were intermediate to Augmentin (6.7%) but were resistance to Cefixime (13.3%) and Cefuroxime (6.7%). Gentamicin had the widest zone of inhibition of (17mm). Isolates from adult male cattle were susceptible to Ciprofloxacin (31.6%), Ofloxacin (21.0%), Gentamicin (26.3%), and Nitrofurantion (10.5%) but were intermediate to Cefuroxime (5.3%) and Cefixime (5.3%),

and were resistant to Ceftazidime (10.5%). Ciprofloxacin had the widest zone of inhibition of (21mm). Equally, isolates from adult female cattle were susceptible to Ciprofloxacin (25%), Ofloxacin (18.8%), Gentamicin (12.5%), Nitrofurantion (6.3%), and those isolates were Intermediate to Ceftazidime (6.3%) and Cefuroxime (12.5%) but were resistant to Cefixime (6.3%) with Ciprofloxacin having the widest zone of inhibition of (21mm). it can be concluded that there is a high distribution of the bacteria (*E. coli)* among cattle slaughtered for meat in Maiduguri abattoir which may serve as a reservoirs of *E. coli* for transmission through their meat and by-products especially when slaughter conditions are not hygienic enough.

Keywords: E. coli, Antimicrobial, Abattoir

INTRODUCTION

Cattle belong to the family *Bovidae*. These are the biological family of cloven hoofed, ruminant mammals that includes bison, African buffalo, water buffalo, antelopes, wildebeest, impala, gazelles, sheep, goats, muskoxen and domestic cattle (Raji *et al.,* 2003). The family *Bovidae* is scientifically classified as follows;

Kingdom: *Animalia.*

Phylum: *Chordata.*

Class: *Mammalia.*

Order: *Artiodactyla.*

Infra order: *Pecora*

Family: *Bovidae.*

The greatest diversities of bovids occur in Africa. The maximum concentration of species is in the savannas of Eastern Africa. Other bovid species also occur in Europe, Asia and Northern America. *Bovidae* includes three of the five domesticated mammals whose use has spread outside their original ranges namely; cattle, sheep, and goat (Guerrant, 1990). Greater percentage of these animals are found in the Northern part of Nigeria and some serve as a major source[s1][s2] of income to the farmers, protein to the teaming population of Nigeria and their hides are exported to earn foreign exchange. Cattle and other ruminants are major reservoirs of

Escherichia coli (Rhasmussen *et al.,* 1993) and play a significant role in the epidemiology of human infections.

Escherichia coli are a common inhabitant of ruminant and human gut and are considered as an indicator of fecal contamination in food (Olowe *et al.,* 2008). It is one of the organism most frequently isolated from different clinical cases of diarrhea (Tobih *et al.,* 2004) and varieties of diseases such as arthritis, septicemia, omphalitis and complicated air sacculitis in birds (Raji et *al.,* 2003). The very low dose of the organism is required to illicit infection. The fact that ingestion of very few as dose as low as 10 organisms, coupled with the short incubation period of 3 to 4 days in most clinical disease requires prominent attention to be paid in field outbreaks all over the world (Mailafia *et. al.,* 2017).

The organism are commensal that inhabit the gastrointestinal tract of healthy animals and man. This bacterium belongs to the family Enterobacteriaceae (Mailafia *et al.,* 2017).the organism is a gram negative, rod shaped with 2.0um long and 0.25-1.0um diameter can survive on a variety of substrates. It can utilize mixed acid fermentation in anaerobic condition, producing lactate, succinate, ethanol, acetate and carbon dioxide. As well the organisms are straight, facultative anaerobic bacilli, majority are motile, non-spore forming and are facultative inhabitant of the large intestine (Holland *et al.,* 1999). They are triple sugar ion, indole and methyl red positive, catalase and urease negative and reduce nitrates to nitrites. They are lactose fermenters and also ferment other carbohydrates like glucose. They grow readily at 37^{o}C on simple nutrient agar but differentiation can be done on MacConkey agar as lactose fermenters and selectively grows on Eosin Methylene Blue (EMB) agar giving the characteristic greenish metallic sheen. The detection of E. coli 0157:H7 is an indicator of faecal contamination and implies presence of other dangerous pathogens which can compromises the wellbeing of consumers. *E. coli* is the most important organism causing secondary bacterial infection in poultry and may also be primary

pathogens (Grill and Hamer, 2001). Collibacillosis is the most frequent reported diseases in survey of poultry diseases or condemnation at processing (Saif *et al.*, 2008). Collibacillosis is characterized in its acute form by septicemia, resulting in death and in its sub-acute form by pericarditis, air sacculitis (CRD), colisepticemia, coligranuloma (Hjares disease), cellulitis (inflammatory process), swollen head syndrome, salphingitis, osteomyelitis/ synovitis, panophthalmitis, omphalitis or York infection and peri-hepatitis.

Some five million *E. coli* bacteria normally inhabit the human and animal intestinal tracts and are vital to processing vitamins in the diet. However, a number of strains are pathogenic and cause gastroenteritis (Ahmed and Ahmed 2020). These potentially harmful *E. coli* are classified into categories based on the production of virulence factors and on the clinical manifestations that they causes (Fairbrother and Nadeau, 2006) such as enteropathogenic *E.* coli (EPEC), enterotoxigenic *E. coli (*ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and enterohaemorrhagic *E. coli* (EHEC). Most laboratories in developing countries do not have the capacity to detect as well distinguish these strains from other non-pathogenic organisms that possesses similar characteristic (Moses *et al.,* 2006). The organism occurs worldwide and has been isolated from water, soil and food.

Transmission of *E. coli* is through the fecal-oral route. Ruminants are the most important reservoir of zoonotic *E. coli* which is transmitted to humans through the ingestion of food or water contaminated with animal feces or through contact with infected animals or their environment (David, 2015).

Diagnosis of infection caused by *E. coli* is based on isolation and identification of the organism. Serotyping of *E. coli* isolates is important in the epidemiology of *E. coli* infection. In the developed countries, advanced techniques such as Enzyme Linked ImmunoSorbent Assay

(ELISA), Polymerase Chain Reaction (PCR), DNA probes test, etc. have been used to accurately diagnose enterovirulent *E. coli* strains (Saif *et al.*, 2008).

In the European Union (EU) there exist efforts to establish an antibiotic resistance monitoring programmed for the most important microorganisms derived from animals. On the other hand, a great variety of studies has been performed to investigate the antibiotic resistance of the most relevant bacterial species of veterinary and human importance. *E. coli* has been tested very intensely from many different sources, animals and food. Nevertheless, investigation are currently needed in order to gain information on the development of antibiotic resistance in these potentially pathogenic agents entering the food chain and generate data to be used in future risk assessment of antimicrobial on the local, continental and global scale.

Contamination of meat carcasses has pointed to many sources including abattoir workers. In instances where, abattoir workers remuneration is linked to the number of head of cattle slaughtered a day, workers tend to increase the slaughter line speed. A line speed that is too high implies inadequate time for slaughter operatives to carry out their functions, which may lead to increase chances of spillage during evisceration and inadequate sterilization of slaughter equipment leading to increased risk of contamination of carcasses. The generation of abattoir specific microbiological data can be used for training purposes and illustrations so that abattoir workers understand the link between their practices and meat safety (Carter, 1982).

Although little is known about consumer perception and awareness of food safety in Nigeria, generally freshness of meat is often cited as one of the most influential variables impacting on consumers decisions to purchase fresh meat (Inabo, 2014).

E. coli is one of the most frequent causes of many common bacterial infections including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveller’s diarrhea and other clinical infections such as neonatal meningitis and pneumonia (Tarun, 2007). *E. coli* has

been implicated in a variety of diseases condition in poultry such as colisepticemia, coligranuloma, air sacculitis, peritonitis, pericarditis and omphalitis accounting for about 5-50% mortality in poultry flock (Roy *et al.,* 2006). This disease colisepticemia is most common losses in aviculture in many part of the world (Karch *et al.,* 2005). Several reports are available on the involvement of the serotype of *E. coli* in poultry disease (Roy *et al.,* 2003).

An abattoir industry is one of the industries that contribute to the problems of possible foodborne diseases such as bloating, diarrhea, gagging, indigestion, nausea, vomiting, stomach cramps, dehydration and loss of appetite, fatigue and fever. Also potential health hazard which are categorized into three classes; Biological hazards include harmful bacteria, viruses or parasites (e.g. *Salmonella*, hepatitis A and *Trichinella*). Chemical hazards include compound that can cause illness or injury due to immediate or long term exposure and physical hazards include foreign objects in food that can cause harm when eaten, such as glass (Ahmed and Ahmed, 2020) associated with food especially meat, by improper handling of condemned material.

METHODOLOGY

Study Area

The study was conducted between February to May, 2019 in Maiduguri Metropolitan Council, Borno State, which lies within the semi-arid zone of North-Eastern part of Nigeria. Borno State was created from the former North-Eastern State in 1976. Borno is situated between Latitude 11·5 to 12·0North, longitude 13.05 to 14.0 East and altitude of 354 meters above the sea level. Borno state has an area of 6,943633sq km and is the largest state in the federation in terms of land mass. The state occupies the greatest part of Lake Chad Basin and shares borders with three west African countries vi; the Republic of Chad to the Northeast, Niger Republic to the North and Cameroun Republic to the East. Within the country, it neighbors with Adamawa state to the south, Yobe state to the west and Gombe state to the south-west. Borno state is covered by

savannah type of vegetation occupied by tall grasses and stout scattered trees, which are the product of a tropical forest and subsequently free from tsetse-flies as such sheep, cattle, and other livestock are kept.

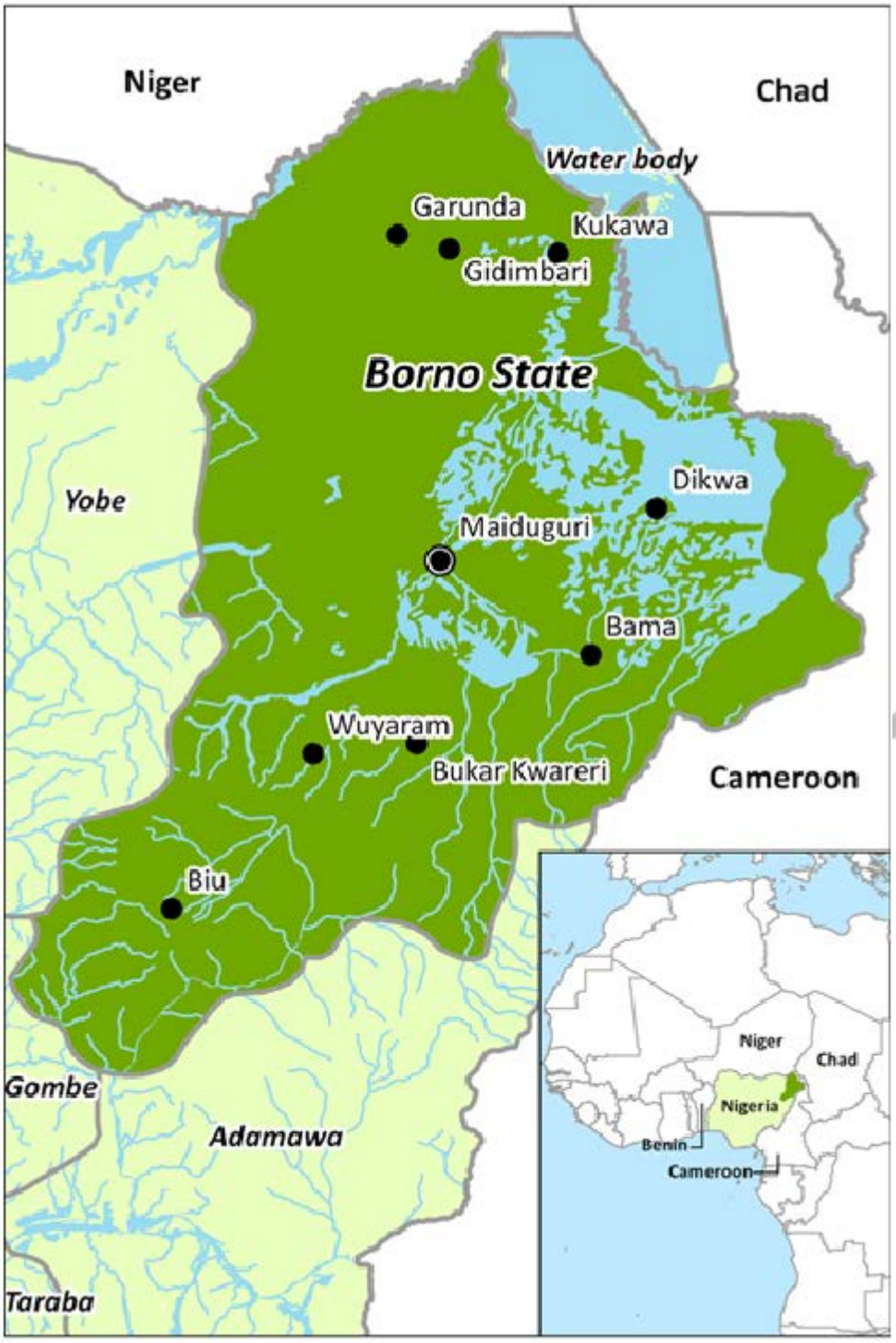

Fig. 1: Showing the study area, Borno State (Maiduguri) which lies within the semi-arid zone of North-Eastern part of Nigeria.

Sampling

A periodic visits were made to the Maiduguri abattoir where animals (cattle) slaughtered were randomly sampled. A total of eighty (80) rectal swab samples were collected. All the samples were collected aseptically by sterile swab stick and transported in an ice-pack to the Veterinary Microbiology Research Laboratory of the University of Maiduguri immediately and been inoculated into the peptone water and incubated for 24 hours at 37° C for bacteriological assay (Jajere *et al.*, 2020)

Preparation of Culture Media

The media used in this study include; peptone water, MacConkey agar, eosin methylene blue agar, nutrient broth medium, methyl-red medium and solution, triple sugar ion agar, Simmon's citrate agar, and urea agar. They were all prepared according to manufacturer's instructions and the prepared media were then stored at 4° C for use during the cultivation (Ahmed and Ahmed, 2020).

Peptone water (Oxoid ltd, England)

12g of peptone reagent was weighed and dissolved in 800ml of distilled water (15g in 1000ml of distilled water), it was swirled to mix before autoclaving at 121° C for 15 minutes, allowed to cool to 47° C and then 10mls each was poured into a sterile Bijou bottles and refrigerated at 4° C

MacConkey agar (Antec Diagnostic Products™, UK)

47g of the MacConkey powder was dissolved in 1 liter of distilled water by gentle mixing until evenly dispersed, it was then boiled to dissolve completely before autoclaving at 121° C for 15

minutes. After autoclaving, it was allowed to cool to about 47° C and then agitated gently to ensure uniform distribution of precipitate. Petri dishes were laid out on a level surface and 16-18mls of medium poured into each dish to give a depth of about 5mm. The surface of the medium was rapidly flamed to remove any air bubbles. It was then covered and allowed to solidify and stored at 4-8° C (Ahmed and Ahmed, 2020).

Eosin Methylene Blue (EMB) agar (OXOID, ENGLAND)

37.5g of the eosin methylene blue powder was weighed and dissolved in 1 liter of distilled water. It was mixed gently until evenly dispersed and then boiled to dissolve completely before autoclaving at 121° C for 15 minutes. After autoclaving, it was allowed to cool to about 47° C before been poured into each petri dish to give a depth of about 5mm. The surface of the medium was rapidly flamed to remove any air bubbles. It was then covered and allowed to solidify before been stored at 4-8° C.

Nutrient agar slopes (LIFESAVE BIOTECH, USA)

28g of nutrient agar powder was dissolved in 1 liter of distilled water, it was swirled to mix and boiled to dissolve, and 5-6mls of agar was poured into Bijou bottles and autoclaved at 121° C for 15 minutes; after autoclaving the bottles were placed in a slanty position until it gelled before storing at room temperature until used.

Triple sugar ion (TSI) agar slant

65g of triple sugar ion powder was weighed and dissolved in 1000mls of distilled water and gentle heat to dissolve the medium completely. It was distributed into test tubes and sterilized by autoclaving at 121° C for 15 minutes, it was then placed in slanty position until it gelled before storing at 4° C until used.

Simmons citrate agar (ACUMEDIA™, USA) Slant

24.2g of Simmons citrate powder was weighed and dissolved in 1000mls of distilled water and gentle heat to dissolve the medium completely. It was distributed into Bijou bottles and sterilized by autoclaving at 121° C for 15 minutes, it was then placed in a slanty position until it gelled before storing at 4° C until used.

Methyl Red Medium and Indicator Solution

10g of methyl red powder was weighed and dissolves in 1 liter of distilled water, it was filtered and 10mls volume was dispersed in each Bijou bottle and been sterilized by autoclaving at 121° C for 15 minutes and 0.25ml of 10% sterile glucose solution was added to each bottle aseptically before used.

Methyl red indicator solution; 0.1g of methyl red indicator powder was weighed and 300mls of absolute ethanol was added, also 200mls of distilled water was added too and swirled to mix and sterilized by autoclaving before been stored at room temperature until used.

Urea Agar Slant

26.4g of urea agar powder was weighed and dissolved in 1000mls of distilled water and boiled to dissolve the medium completely. It was sterilized by autoclaving at 121^{o}C for 15minutes and one ampoule of sterile urea solution was added, it was mixed properly and 10mls was distributed into each bottle and allowed to set in a slope position until used.

Culture and Isolation of *E. coli*

The processing table was first disinfected with alcohol (Ethanol) and antiseptics. Each sample was immediately inoculated unto peptone water on arrival to the laboratory in other to propagate. This was sub cultured unto prepared MacConkey medium using a straight streaking method by a sterile wire loop and each media was labeled accordingly and thereafter incubated at 37^{o}C for 24hours to allow the bacterial growth. The presumptive pink colonies (Lactose fermenting colonies) were picked after 24 hours of growth and then sub cultured unto Eosin methylene blue medium to get pure colony and incubated at 37^{o}C for 24 hours. Colonies that were lactose fermenters on MacConkey which are smooth, glossy and pinkish and appeared greenish metallic sheen with blackish center on Eosin methylene blue medium were considered *E. coli.* All isolates were streaked on nutrient agar slant and stored for future use.

The characteristics isolates were isolated aseptically and characterized using established microbiological methods which includes; colonial morphology, indole test, catalase test, (Cheesbrough, 2000). Isolates that were gram negative were then further tested with various biochemical reagents such as; indole, triple sugar ion, methyl red test, urease test, citrate utilization test using a standard biochemical test method (Cheesbrough, 2000; Ahmed and Ahmed, 2020).

Gram Staining

Gram staining helps to differentiate bacteria into two main groups, thus gram positive (blue/purple) and gram negative (pink/red). Small colony from fresh *Escherichia coli* colonies was picked up from Eosin methylene blue plates with bacteriological wire loop and smeared on separate glass slide and it was been allowed to air dry, then heat fixed by gentle passing it through the flame 3 times. Crystal violet was applied on each smear to stain for 1 minute and then washed with tap water. The smear was flooded with a ligand (Lugol’s iodine) for 1 minute

and again rinsed with tap water, 95% alcohol which serves as a decolorizer was added for few seconds and rinsed with tap water. Safranine was added as a counter stain for 30 seconds and then rinsed with tap water and it was then allowed to air dry, blot examination was done under microscope with high power objective (X100) using oil immersion (Jajere *et al.*, 2020).

Biochemical Characterization of the Presumptive Isolates

The presumptive isolates stored on nutrient agar slants were subjected to biochemical tests which were conventionally carried out on the isolates.

Indole test

Two to three colonies of *E. coli* isolates were inoculated in Bijou bottles containing peptone water using a wire loop and incubated for 24 hours. After 24 hours of incubation two to three drops of Kovac's reagent were added to the medium and shaken. Formation of a pink layer was recorded as a positive result (Cheesbrough, 2000;Ahmed *et al.*, 2020).

Citrate utilization test

Using a straight sterile wire a colony of the test isolates was picked and streaked on Simmon's citrate agar slope and stabbed to the butt before incubating at 37^{o}C for 24 hours. Positive citrate test was indicated with a bright blue colour in the medium. No colour change indicated negative test (Cheesbrough, 2000; Jajere *et al.*, 2020).

Methyl-red (MR) test

Two to three colonies of isolates were inoculated into methyl-red Voges proskauer (MR-VP) broth and incubated for 48 hours. Five to seven drops of methyl red solution were added to the broth and shaken. It was recorded positive since there was formation of a pink-red product within 5-15 minutes (Jajere *et al.*, 2020).

Catalase test

Two to three drops Hydrogen peroxide was added on a glass slide, and a sterile wooden spatula was used to pick a colony of organism (*E. coli*) and immersed it in the H2O2 solution on the glass slide. Immediate gas bubbles indicates positive test and absence of gas bubbles indicates negative test (Jajere *et al.,* 2020).

Urease test

Using a sterile wire loop, colonies of the isolates was picked and streaked on the urease slope and incubated at 37^{o}C for 24 hours. A red pink colour change indicated positive test (Ahmed *et al.,* 2020).

Triple sugar ion test

Using a straight wire, colonies of the test isolates were picked and stabbed to the butt before been streaked on the entire surface (Slope) of the slant, then it was incubated at 37^{o}C for 24 hours. Production of the gas at the butt and the yellow colouration at the surface of slant (acid production) indicated positive test, no gas and acid production indicated negative test (Jajere *et al.,* 2020).

Antimicrobial Susceptibility Testing

The antibiotic susceptibility test was performed on E. coli isolates using the disc diffusion technique which was carried out using Mueller Hinton agar (Bauer *et al.,* 1995). Result of the susceptibility test were compared with the clinical laboratory standards institute (CLSI, 2011) for susceptibility or resistance profiles of the isolates. The isolates were inoculated into 5ml of Mueller Hinton broth and incubated overnight at 37^{O}C. The overnight culture was then standardized with 0.5 MacFarland turbidity standards. Mueller Hinton agar plates were prepared according to the manufacturers guide. The standardized overnight culture (containing

approximately 10^6 CFU/ml) was used to flood the surface of Mueller Hinton agar plate and excess was drained off and allowed to dry while the petri dish lid was in place. The antibiotic sensitivity disc where then aseptically placed on the inoculated Mueller Hilton plates. The antibiotics used in this study includes; Ciprofloxacin (5ug), Ceftazidime (30ug), Oflaxacin (5ug), Cefixime (5ug), Gentamicin (10ug), Augmentin (30ug), Cefuroxime (30ug) and Nitrofurantion (300ug) (Abtek Biologicals Ltd). After the incubation, the test plates were examined. The diameter of each zone of inhibition was measured in millimeter using a ruler on the underside of the plate. The interpretation of the measurement as sensitivity, intermediate and resistance was made according to clinical laboratory standards institute (CLSI, 2011) manual.

RESULTS

4.1 Distribution

A total of 80 faecal samples (19 from young male, 18 from young female, 22 from adult male, and 21 from adult female) were randomly sampled for the isolation of *E. coli*. The result show that 13 (68%) *E. coli* was isolated from young male cattle, 15 (83%) *E. coli* was isolated from young female cattle, 19 (86%) *E. coli* was isolated from adult male cattle, and 16 (76%) *E. coli* was isolated from adult female cattle. The overall isolation rate was 63% .

Table 4.1: Distribution of *E. coli* isolated from cattle slaughtered in Maiduguri abattoir based on age and sex.

Animal species	No. sampled	No. isolated	% of +ve isolates

Young Male	19	13	68.42
Young Female	18	15	83.33
Adult Male	22	19	86.36
Adult Female	21	16	76.19
Total	**80**	**63**	**78.58**

not Significant P>0.05

4.2 Biochemical Tests

For indole test, all the 63 positive isolates shows the formation of a pink layer at the surface of the broth when 2-3 drops of Kovac's reagent was added to the medium and it indicates a positive result .For the citrate utilization test, the 63 positive isolates gives a negative result due to the absence of a bright blue colour within the medium. The methyl red test, 63 positive isolates shows the formation of a pink-red product within 5-15 minutes after 5-7 drops of methyl-red solution were added to the broth and this indicates a positive result. For catalase test, all the 63 positive isolates shows negative for catalase since there were no production of a gas bubbles. For urease test, all the 63 positive isolates were urease negative due to red-pink colour change within the media. For triple sugar ion test, all 63 positive isolates produced a gas at the butt of the test tubes and yellow colouration at the surface of the slant, these indicates a positive result.

Table 4.2. **Biochemical reactions of presumptive *E. coli* isolates from slaughtered cattle.**

Biochemical tests	**Number sampled**	**No. of +ve**	**No. of − ve**	**% of +ve**

Catalase	80	17	63	21.3%
Indole	80	63	17	78.8%
Urease	80	17	63	21.3%
Methyl-red	80	63	17	78.8%
Citrate utilization	80	17	63	21.3%
Triple sugar ion	80	63	17	78.8%

4.3 Antimicrobial Susceptibility Testing of the *E. coli* Isolates

The isolated *E. coli* from cattle (young male, young female, adult male and adult female) were subjected to in vitro antimicrobial susceptibility testing by the modified Bauer-Kirby method.

The result showed that the isolates from young male cattle were susceptible to Ciprofloxacin (38.7%), Ofloxacin (23%), Gentamicin (15.4%), and Nitrofurantion (7.7%), also they are intermediate to Ceftazidime (15.4%) and Cefuroxime (7.7%) but were resistant to Cefixime (7.7%) and Augmentin (7.7%). Ciprofloxacin and Ofloxacin had the widest zone of inhibition (23mm). Isolates from young female cattle were susceptible to Ciprofloxacin (26.7%), Ofloxacin (20%), Gentamicin (40%), Nitrofurantion (20%), and Ceftazidime (6.7%) and were intermediate to Augmentin (6.7%) but were resistance to Cefixime (13.3%) and Cefuroxime (6.7%). Gentamicin had the widest zone of inhibition of (17mm). Isolates from adult male cattle were susceptible to Ciprofloxacin (31.6%), Ofloxacin (21.0%), Gentamicin (26.3%), and Nitrofurantion (10.5%) but were intermediate to Cefuroxime (5.3%) and Cefixime (5.3%), and were resistant to Ceftazidime (10.5%). Ciprofloxacin had the widest zone of inhibition of (21mm).

Equally, isolates from adult female cattle were susceptible to Ciprofloxacin (25%), Ofloxacin (18.8%), Gentamicin (12.5%), Nitrofurantion (6.3%), and those isolates were Intermediate to Ceftazidime (6.3%) and Cefuroxime (12.5%) but were resistant to Cefixime (6.3%). But Ciprofloxacin had the widest zone of inhibition of (21mm).

Table 4.3: Antimicrobial Susceptibility Studies of E. coli Isolates from Cattle to Commonly Used Antimicrobial Agents.

Antimicrobials	Drug Conc.(ug)	No. of Susceptible %	No. of Intermediate %	No. of Resistance %
CAZ	30	0 (0.0)	3 (12.5)	2 (8.3)
CRX	30	0 (0.0)	4 (16.7)	1 (4.2)
GEN	10	15 (62.5)	0 (0.0)	0 (0.0)
CXM	5	0 (0.0)	1 (4.2)	5 (20.8)
OFL	5	13 (54.2)	0 (0.0)	0 (0.0)
AUG	30	0 (0.0)	3 (12.5)	1 (4.2)
NIT	300	7 (29.2)	0 (0.0)	0 (0.0)
CRP	5	19 (79.2)	0 (0.0)	0 (0.0)

CAZ- Ceftazidime, CRX- Cefuroxime, GEN- Gentamicin, CXM- Cefixime, OFL-Ofloxacin, AUG- Augmentin, NIT- Nitrofurantion, CPR- Ciprofloxacin.

Among all the sex and age of cattle tested for antimicrobial sensitivity, adult male showed the highest resistance level of (77.2%) to commonly used antibiotics and as well 19 (79.2%) of the positive isolates were susceptible to Ciprofloxacin, 7 (29.2%) were susceptible to Nitrofurantion, 13 (54.2%) were susceptible to Ofloxacin, 15 (62.5%) were susceptible to Gentamicin, while 3 (12.5%) were intermediate to Ceftazidime, 4 (16.7%) were intermediate to Cefuroxime, 1 (4.2%)

were intermediate to Cefixime, 3 (12.5%) were intermediate to Augmentin, and 2 (8.3%) were resistance to Ceftazidime, 1 (4.2%) were resistance to Cefuroxime, 5 (20.8%) were resistance to Cefixime and 1 (4.2%) were resistance to Augmentin.

Discussion

The study was carried out to isolates and carried out antimicrobial susceptibility studies from gastro intestinal content sample from cattle in Maiduguri abattoir, Nigeria. A total of 80 rectal swab samples were collected from cattle based on age and sex and 63 were positive for *E. coli* organisms. The isolation rate of *E. coli* in this study area was 78.58%. *Escherichia coli* occur aberrantly in most tissues or organs of the body (e.g. gastro intestinal tract, respiratory tract, urogenital tract) in man and animals (Adetosoye, 1980). *Escherichia coli* has been isolated in large numbers from faeces of ruminants and even humans (Moses, 2005, Ameh *et al.*, 2003). The organism is implicated in causing several diseases in animals and man, with ruminant playing an important aspect in the epidemiology of the infections. Zoonotic *E. coli* (0157:H7) has been implicated in human outbreaks of haemorrhagic colitis, neonatal diarrhea and haemorrhagic uraemic syndrome (Griffin and Tauxe, 1991). Meat and meat product from animals can be contaminated by *E. coli* and transmitted to man via consumption of undercooked meat and poor hygienic practice.

Over the past 60 years, antimicrobial have been used in a variety of setting including human medicine, veterinary medicine, plant agriculture and even cosmetics and antibacterial household products (DeVincent and Viola, 2006). All of these uses of antimicrobials potentially contribute to the emergence and spread of antimicrobial resistance but antimicrobial use in animals, particularly in food animals has recently come under particular scrutiny. In large part, concern stems from evidence for direct transfer of resistant pathogens from animals to humans through the food supply (Spika *et al.*, 1987).

The result of the in vitro antimicrobial susceptibility study show that the *Escherichia coli* from cattle were susceptible to Ciprofloxacin, Ofloxacin and Gentamicin but were intermediate to Nitrofurantion and as well resistance to Augmentin, Cefixime, Ceftazidime and Cefuroxime. Generally, the organism were susceptible to the flouroquinolones and this is in agreement with the report by Umolu *et al.,* (2006), Moses (2005), (Ahmed and Ahmed (2020) and Ameh *et al.,* (2003). Flouroquinolones are newer drugs with mode of action central on inhibition of DNA replication which stops the multiplication of the bacterial cells and are relatively expensive and therefore, are less available for abuse. The flouroquinolones (Ciprofloxacin, Ofloxacin and Gentamicin) had the widest zone of inhibition and are inversely related to minimum inhibitory concentration (MIC) values and this is in agreement with earlier report by Ameh *et al.,* (2003). Resistance to Augmentin, Cefixime, Ceftazidime and Cefuroxime is in agreement with those of other workers (Ameh *et al.,* 2003, Adetosoye, 1980 and Ahmed & Ahmed 2020). The high incidence of drug resistance of *Escherichia coli* isolates as observed in this study may suggest that there was a wide spread transfer of resistance plasmid in the past (Adekele *et al.,* 2011) because resistance transfer in *E. coli* and other enterobacteriacae is often embolic and plasmid mediated (Ahmed and Ahmed 2020).

To generate baseline data to be used in future risk assessment of antimicrobial resistance, a number of surveillance systems on the local, continental and global scale is needed. Among the species proposed for surveillance of *E. coli*. The distribution of resistance in commensal *E. coli* is a good indicator for the selective pressure by antibiotic use and resistance problems to be expected in pathogenic bacteria. In food animals, a low distribution and degree of antibiotic resistance in the intestinal flora should be considered as a distinguishing quality and safety mark (Ahmed and Ahmed 2020).

Conclusion

From the result of this study, it can be concluded that there is a high distribution of the bacteria (*E. coli)* among cattle slaughtered for meat in Maiduguri abattoir which may serve as a reservoirs of *E. coli* for transmission through their meat and by-products especially when slaughter conditions are not hygienic enough. This study has also shown the presence of antibiotic-resistant bacteria in cattle slaughtered in Maiduguri abattoir which could be as a result of acquisition of these antibiotic resistant bacteria by contact with carriers or ingestion of food (meat) and water contaminated by faecal droppings of other animals with previous expose to antibiotic. The study has established the presence of antibiotic-resistant bacteria. The level of antimicrobial drug resistance is high which can enter into the food chain. From the study, Ciprofloxacin, Ofloxacin, and Gentamicin had well activity against *Escherichia coli* and may be drugs of choice for the treatment of infections caused by *E. coli* but high resistance were observed to Cefixime, Cefuroxime, Ceftazidime and Augmentin.

REFERENCE

Adetoseye, A.I. (1980). Infective drug resistance among *E. coli* isolated from clinically healthy domestic livestock. Vet. Microbiology, 5: 333-342.

Adekele, E.O. and Omafuvbe, B.O. (2011). Antibiotic resistance of aerobic mesophilic bacteria isolated from poultry faeces, Research Journal of Microbiology, 6(4): 356-365.

Ahmed, Idriss Jajere & Ahmed, Ibrahim Jajere (2020);Phenotypic Identification and Antimicrobial Susceptibility of Escherichia Coli Isolates from Chickens Presented For Slaughter at Fune Local Government Area Yobe, Nigeria. Int Aca. J App Biomed Sci. 1(1)43-51.

Ameh, J.A.; Egwu, G.O.; Onyeyili, P.A (2003). Disc antibiotic sensitivity and minimum inhibitory concentration studies on strains of *E. coli* from diarrheic kids.

Bauer, A.W.P., Kirby, W.M.M., Sherric, J.C., and Turck, M. (1966). Antibiotic Susceptibility testing by a standardized single disc method. Am. J. Clin. Pathology. 45: 493-496.

Carter, G, R., (1982). Essentials of Veterinary Bacteriology and Mycology. Virginia Maryland Regional College of Veterinary Medicine. Virginia Tech. Blacksburg Virginia 24661, Michigan State University Press, East Lancing 1982. Pp. 189-202.

Chessebrought, M. (2000). District Laboratory Practice in Tropical Countries, Low Price Edition, Cambridge University Press, Cambridge pp. 66-68, 140.

Clinical Laboratory and Standards Institute Manual (2011). Performance standards for antibiotic susceptibility testing; MI00-S21. 21st Informational supplement.

David, C., Charles C. D., Michael, W. S., Jan, M. S., Nagaraja, T. J., Richard, D. O., Roberts, A. S., and Griffin, D.D., (2015). Prevalence of *Escherichia coli* 0157 in Cattle Feeds in Midwestern Feedlots. Applied and Environmental Microbiology, American Society for Microbiology. EM 69.9: 5243-5247.

De Vincent, S. J., and Viola, C. (2006), Introduction to animal antimicrobial use data collection in the United State: Methodological options. Prev. Vet. Med. 73: 105-109.

Gill, C.J., and Hamer, D.H., (2001). Food Borne Illness. Curr. Treatment Opt. Gastromenterol. 4:23-38.

Griffin, P.M., and Tauxe, R.V., (1991). The epidemiology of Infections caused by *E. coli 0157:H7,* other enterohemorrhagic *E. coli* and the associated hemolytic Uremic syndrome. Epidemiology Review. 13:60-98.

Guerrant, R.L., (1990). Principles and syndromes of enteric infections, pp. 837-851. In G.L. Mandell, R.G. Douglas., and J.E. Bennett (Ed). Principles and Practice of infectious diseases. Churchill Livingstone, New York.

Holland, R.E., Willson, R.A., Holland, M.S., Yusbasiyan-Gurkan, Y., Mullaney, T.P., White, D.G., (1999). Characterization of *eae E. coli* isolated from healthy and diarrheic calves Vet. Microbiology. 66:251-263.

Inabo, H., (2014). Comparative analysis of Bacterial Biobueden and the antibiotic susceptibility of selected Bacteria from faecal dropping in commensal area, Samaru, Zaria.

Jajere AI, Samuel M, Mustapha AB and Raji M.A. (2020) Phenotypic characterization of Salmonella enterica from chickens in some selected local governments of Yobe State. International Journal of Research and Review. 2020; 7(10): 76-94.

Karch, H., Tarr, P., and Belaszewski, M., (2005). Enterohemorrhagic *E. coli* in Human Medicine. Int. J. Med. Microbiol. 295 (6-7):405-418. PMID 16238016.

Madubulke, S. A., Mailafia, S., Ode, O.J., and Okpo, N. (2018). Phytochemical Screening and Antibacterial Susceptibility of *Escherichia coli* 0157:H7 Isolates on *Acacia ataxacantha* leaves. A Journal of Microbiology and Antimicrobials. Vol. 10(1). pp. 1-8.

Mailafia, S., Desalini, N., and Pwaveno, H. B., (2017). Phenotypic and Molecular Characterization of *E. coli* isolates from Japanese quails in Kelantan, Malaysia. ISOI Journal of Microbiology, Biotechnology and Food Science. 1(3): 01-08.

Mailafia, S., Madubulke, S. A., Raji, M. A., Suleiman. M. M., Olabode. H. O. K., Echioda-Egbole, M. and Okoh, G. P. R., (2017). Phenotypic identification of *Escherichia coli*

0157:H7 isolates from cattle at Suleja Abattoir, Nigeria. African Journal of Microbiology Research. VOL. 11(21), pp. 845-850.

Markku, K. (2001). Shiga-Toxin producing and other diarrheagenic *E. coli* in Finland. Pheno and Genotypic epidemiology. Academic desertification Nat. Public Health Institute, Lab. Of Enteric pathogens; Mannerheiminitie 166, FN-00300 Helsinki, Finland.

Moses, A.E., (2005). Epidemiological of some enteropathogenic *E. coli* serotypes in Borno and Adamawa States of Nigeria. A PhD thesis submitted to the Department of Veterinary Microbiology and Pharmacology University of Maiduguri.

Moses, A.E., Egwu, G.O., and Ameh, J.A., (2006). Phenotypic and Genotypic characterization of *E. coli 0157:H7* and non-0157:H7 serogroups isolated from humans, animals, dairy products and water in Borno and Adamawa States of Nigeria. Research Journal of Medicine and Medical Science, 1(3):96-103.

Olowe, O.A., Okanlawan, B.M., Olowe, R.A., and Olayewi, A.B., (2008). Antimicrobial Resistant Pattern of E. coli from Human Clinical Samples in Osogbo, South-Western Nigeria. Africa J. Microbiology Res. 2:8-11.

Raji, M.A., Jiwa, S.F., Minge, M.U., and Gwakisa, P.S., (2003). *E. coli 0157:H7* reservoir, transmission, diagnosis, and the African Situation; a review, East Africa Med. J. 80(5): 271-276.

Rhasssmusen, M.A., Gray, W.C., Cassey, T.A., and Whip, S.C., (1993). Rumen Content as a Reservoir of Enterohemorrhagic *E. coli* FEMS Microbiology. LEH. 114:77-84.

Roy, I., and Remis, R.S., (2003). Hemorrhagic Colitis associated with rare *E. coli* serotype. N. Engl. J. Med. 308: 681-685.

Spika, H., Blaschke, B., Franke, S., Russmann, H., Schwarzkopf, A., Heesemann, J., and Karch, H. (1987). Differentiation in Virulence pattern of *E. coli* possessing eae genes Med. Microbial. Immunol. 183; 23-31.

Saif, R. and Kuhn, K. (2008). Prevalence of Verotoxin-producing *E. coli* (VTEC) in Bovine coli mastitis and their antibiotic resistance pattern. J. Vet. Med. B. 46: 423-427. Stud. Res. Vet. Med. (Bucharest). 9: 69-76.

Tarun, J., Young, W., MacNawara, A.M., Custer, C., Boesel, B., Lambert-fair, M.A., Murkowski, J., Vugia, D., Werner, S. B., Hollingsworth, J and Morris, J.G., (2007). A new Route of Transmission for *E. coli* infected from dry fermented salami, AM. J. Health 86:1142-1145.

Tobih, J.E., Taiwo, S.S., Olowe, O.A., Olaosun, O.A., and Adejumo, S, O., (2004). Microbiological profiles of discharging ears in Osogbo, Nigeria, Trop. Doc. 36(3): 165-166.