DETECTION AND MOLECULAR CHARACTERIZATION OF VIRULENT AND ANTIMICROBIAL RESISTANT *Escherichia coli* FROM CATTLE FAECES AND MANURE IN BAUCHI STATE, NIGERIA

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DECLARATION

I hereby declare that the work described in the thesis entitled "**Detection and Molecular characterization of virulent and antimicrobial resistant *Escherichia coli* from cattle faeces and manure in Bauchi state, Nigeria"** was carried out by me in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria under the supervision of Professor Junaidu Kabir, Professor Mohammed Bello and Dr. S. N. A. Sa'idu.The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project report was previously presented for another degree or diploma at this or any other university.

Shehu AbdulQadir ZAILANI _____ _____

Name of Student Signature Date

CERTIFICATION

This thesis titled "**Detection and Molecular characterization of virulent and antimicrobial resistant *Escherichia coli* from cattle faeces and manure in Bauchi state, Nigeria**" meets the regulations governing the award of the degree of Doctor of Philosophy in Veterinary Public Health and Preventive Medicine of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Professor J. Kabir Chairman, Supervisory Committee	_____ Signature	_____ Date
Professor M. Bello Member, Supervisory Committee	_____ Signature	_____ Date
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Professor S. A. Abdullahi Dean School of Postgraduate Studies	_____ Signature	_____ Date

DEDICATION

This thesis is dedicated to Almighty Allah (SWT), His beloved prophet Muhammad (PBUH) and his companions and those who follow his teachings, my late parents Malam Zailani Abdullahi and Hajiya A'ishatu Abubakar, may Jannatul Firdausi be their final abode and to my family especially my wife Rashida Abubakar Ila and her sister Rabiatu and the children, Mustapha Yakubu Lame, Hafsat Usman, Abdulrahim Usman, Sa'adatu Usman (Raheema), Khadija Usman (Fadeela), AbdulQadir Usman (Abeed) and Abubakar Sadiq Usman (Akhram).

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ABSTRACT

This research assessed the practice of manure use, its role in dissemination of virulent and antimicrobial resistant *Escherichia coli* from cattle in Bauchi State, Nigeria. The objectives were to isolate and identify shiga toxin producing *E. coli* (STEC) in cattle faeces and manure from abattoirs, cattle farms (CF) and livestock markets (LM); to determine the anti-microbial susceptibility of the isolates against some antimicrobial agents and multiple antibiotic resistance (MAR) indices, to determine the presence and distribution of antibiotic resistance (*tetA, B, C, D, K*) and virulence genes (*eaeA, stx1, stx2*) in the isolates using PCR, and to the evaluate practice of cattle manure use and socio-economic factors promoting it on farm lands through administration of questionnaires. A purposive sampling study involving cattle population in the abattoirs, CF and LM and farmers using cattle manure on their farms was carried out. One local government area (LGA) was selected from each of the three agro-climatic zones of the State based on large cattle population and largest slaughter facility selected with Bauchi (southern), Darazo (central) and Katagum (northern) LGAs being selected. Abattoir with highest slaughter figures in each of the LGAs was selected; similar criteria were used in the selection of the CF and LM from the selected LGAs. Three (3) cattle farms with more than fifty (50) animals were considered; one farm in each of three (3) districts of the selected LGAs was considered. One major slaughter facility and one cattle market were selected from each of the LGAs based on the number of animals slaughtered and the number of animals available at the markets respectively. Standard laboratory procedures for the isolation and identification of *E.*

coli were followed and biochemical characterization of the isolates followed by serological identification, antibiotic susceptibility tests and multiplex polymerase chain reaction (mPCR) for detection of virulence and resistance genes. Also three hundred (300) structured questionnaires were administered to respondents across the three (3) LGAs, where fifty (50) were administered to individuals keeping animals (manure producers) and fifty (50) to crop farmers using manures on their farm lands (manure consumers) in each of the selected LGAs. Three hundred and fourteen (314) faecal samples and seven (7) manure samples out of nine hundred and ninety (990) faecal and one hundred and sixty-five (165) manure samples respectively were presumptive positive for *E. coli*, among which 51(16.2%) positive isolates were from the abattoirs, 107(34.1%) from CF and 156(49.6%) were from the LM. The result also indicated highest number of positives 135(42.9%) from Bauchi, followed by 130(41.4) from Katagum and then 49(15.9) from Darazo. On the basis of sex males had more positives 219(69.7%) than females with 95(30.3) out of three hundred and fourteen (314) presumptive positives for *E. coli*. Biochemical test results showed 75(24%) isolates from faecal samples to be positive, while 7(4.24%) isolates from manure samples were positive for *E. coli*. Out of the isolates 30 were *E. coli* O157:H7, while 20 were non-O157. Antibiotic susceptibility results showed high susceptibility of tested isolates to chloramphenicol - 40(80%), followed by Gentamicin and Imipenem - 35(70%). Highest frequency of resistance was recorded for cefazoline - 44(88%) and closely followed by sulfamethoxazole and Enrofloxacin - 42(84%) and 40(80%) respectively. Virulence and antimicrobial resistance genes were detected from the

isolates confirmed to be *E. coli* O157:H7 and non-O157. The study showed that majority 99(63.8) of respondents that used manure on their farm lands have acquired basic education. Most respondents 125 (80.6%) indicated manure being cheaper, accessible and available as their reasons for using it. Most farmers - 142 (91.6%) that used manure on their farms used cattle manure and the majority 141(90.9%) of them planted ≥ 2 crops types on their farms. It was observed that farmers were aware of consumer preferences for crops grown with manure. In conclusion *Escherichia coli* were found to be more in male cattle 219(22.1%) than in female cattle 95(9.6%) and more in adult cattle 271(27.4%) than in young ones 43(4.34%). All isolates showed multiple drug resistance (MDR) with some isolates showing resistance to up to 8 antimicrobial agents belonging to 7 groups of antimicrobial agents. The MAR index of 0.2 was observed in most isolates where 98% of the. Virulence and antimicrobial resistance genes were detected from the *E. coli* isolates of cattle faeces in the study area from the abattoirs, CF and LM. Respondents levels of education to biosecurity was statistically significant ($p<0.05$), with economic consideration as respondents' reasons for manure use.

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LIST OF ABBREVIATIONS

Abbreviations	Meaning
Ab	Abattoir
AAAF	Azare Abattoir Adult Female
AAAM	Azare Abattoir Adult Male
AAM	Azare Abattoir Manure
ACAF	Azare Cattle Adult Female
ACAM	Azare Cattle Adult Male
ACYM	Azare Cattle Young Male
ALM	Azare Livestock Manure
AMC	Amoxicillin clavulanate
AMR	Antimicrobial Resistance
ARGs	Antimicrobial Resistant Genes
BCAF	Bauchi Cattle Adult Female
BCAM	Bauchi Cattle Adult Male
BLAF	Bauchi Livestock Adult Female
BLAM	Bauchi Livestock Adult Male
BSADP	Bauchi State Agricultural Development Project
CDCP	Centers for Disease Control and Prevention
CF	Cattle Farms
CLSI	Clinical and Laboratory Standards Institute

Abbreviations	Meaning
COMPASS	Community Participation for Action in the Social Sector
DAAM	Darazo Abattoir Adult Male
DAEC	Diffusely Adherent Escherichia coli
DAYM	Darazo Abattoir Young Male
DCAF	Darazo Cattle Adult Female
DCAM	Darazo Cattle Adult Male
DCYF	Darazo Cattle Young Female
DCYM	Darazo Cattle Young Male
DLAM	Darazo Livestock Adult Male
DNA	Deoxyribonucleic Acid
eaeA	Intimin coding gene
EAEC	Enteroaggregative *Escherichia coli*
EAF	EPEC Adherence factor
ECDPC	European Center for Disease Control and Prevention
EDTA	Ethylene Diamine Tetra Acetic ACID
EFSA	European Food Safety Authority
EHEC	Enterohemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
ENR	Enrofloxacin
EPEC	Enteropathogenic *Escherichia coli*
ESBLs	Extended Spectrum beta lactamases

Abbreviations	Meaning
ETEC	Enterotoxigenic *Escherichia coli*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HUS	Haemolytic Uremic syndrome
ISO	International Standards Organization
LGA	Local Government Area
LM	Livestock Market
MAR	Multiple Antibiotic Resistance
MARNR	Ministry of Animal Resources and Nomadic Resettlement
MDR	Multiple Drug Resistance
MIC	Minimum Inhibitory Concentration
mPCR	Multiplex Polymerase Chain Reaction
NCE	National Certificate of Education
ND	National Diploma
PCR	Polymerase Chain Reaction
STEC	Shiga toxin producing *Escherichia coli*
Stx	Shiga toxin gene
TBE	Tris / Borate/ EDTA
Tet	tetracycline genes
TIM	Ticarcillin clavulanate
USEPA	United State Environmental Protection Agency
UTI	Urinary Tract Infection

Abbreviations	**Meaning**
VTEC	Verocytotoxic *Escherichia coli*
WHO	World Health Organization
Z I	Zone of Inhibition

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Escherichia coli (*E. coli*)is a type of bacteria that normally live in the intestine of humans and animals (Schulman, 2017). Some *E. coli* strains can cause a wide variety of intestinal and extra-intestinal diseases, such as urinary tract infections and neonatal meningitis (EFSA, 2011). *Escherichia coli* can easily and frequently exchange genetic information through horizontal gene transfer (EFSA, 2011). The organism can undergo conjugation and transformation or transduction with other related bacteria, such as other *E. coli* strains, *Salmonella* and *Shigella* (EFSA, 2011). Therefore, *E. coli* strains may exhibit characteristics that have been acquired from a wide variety of sources (EFSA, 2011).

Diarrheagenic strains of *E. coli* strains are among the most common etiologic agents of diarrhea and based on their specific virulence factors and phenotypic traits are divided into enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), Vero toxin-producing/Shiga toxin-producing *E. coli* (VTEC/STEC) which include its well-known subgroup eneterohaemorrhagic*E. coli* (EHEC), enteroinvasive*E. coli* (EIEC), enteroaggregative*E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (Jafari*et al*., 2012). Some assays for the detection of diarrheagenic *E. coli* available include an auto-microfluidic thin-film chip (AMTC) instrument integrating one-step multiplex PCR (mPCR) with reverse dot blot hybridization (RDBH) was developed for high-throughput detection of DEC (Yun, *et al*., 2018). Among the molecular detection

methods, Polymerase Chain Reaction (PCR), is a commonly used method that gives rapid, reliable results with a high sensitivity and specificity (Stacy-Phipps *et al*., 1995; Bellin *et al*., 2001).

Some *E. coli* strains have been categorized into pathogenicity groups based on their virulence properties(Caprioli *etal*., 2005). One of these groups is characterized by its production of potent cytotoxins that inhibit protein synthesis within eukaryotic cells(Caprioli *et al*., 2005). These toxins are either termed verocytotoxins (VT), because of their activity on Vero cells, or Shiga toxins (*stx*), because of their similarity with the toxin produced by *Shigella dysenteriae* (Caprioli *et al*., 2005). *Escherichia coli* O157:H7 in humans causes food-borne infections with symptoms that range from mild diarrhea to haemorrhagic colitis (Caprioli *et al*., 2005). Most patients recover from the infection within the first 10 days, but in a small percentage of patients (up to 10%), particularly young children and the elderly, the infection may lead to haemolytic uraemic syndrome (HUS) (WHO,2005).

Enteropathogenic *Escherichiacoli* are present in the faeces of humans, wildlife and domestic livestock (Wang *et al.,* 2013). Although, theymay be found in faeces, water and soil, only a small proportion (< 1%) of the bacteria are harmful (Wang *et al.,* 2013). Most strains of *Escherichia coli* commonly found in th e large intestine of of humans and other warm-blooded animals are commensals, existing in a symbiotic state providing resistance against pathogenic organisms (Katouli, 2010). There are several pathogenic strains of *Escherichia coli*, with one of the best known and of zoonotic concern is *E. coli* O157:H7. This strain (*E. coli* O157:H7) and some other

pathogenic ones produce toxins that can cause serious human illness. All *E. coli* are classified on the basis of the presence or absence of surface antigens (somatic (O), flagellar (H) and capsular polysaccharide antigens (K)) and a numerical system that distinguishes them based on harmless and harmful bacteria (Riemann and Cliver, 1998).

Humans are considered to be incidental hosts of *E. coli* O157:H7, although infection may also spread among patients due to the low infectious dose (Kaper *et al.*, 2004). Cattle and sheep have been most often linked to human disease due to *E. coli*O157:H7 and those cattle in particular, are considered the primary reservoir host for the organism (Caprioli *et al.*, 2005; Pennington, 2010). Early outbreaks of *E. coli* O157:H7 were traced to ground beef, fruit, vegetable, and undercooked food, on December 24, 2009, the United states department of Agriculture Food Safety and Inspection Services (FSIS) issued a recall of 248,000 pounds of beef and poultry products that have been contaminated with *E. coli* O157:H7 (CDC, 2016).

Livestock manure represents a valuable resource in the agriculture sector. As fertilizer it is a source of nitrogen, phosphorous and organic matter, all crucial for the sustainability of farming. But manure can also have negative impacts on the environment, depending on how it is used. Manure can contaminate surface and groundwater, for example, or cause air pollution in the form of malodorous emissions and greenhouse gases (Cordis, 2013). However, unless animal manure is managed carefully to minimize odour, nutrient losses and emissions, it becomes a source of environmental pollution, a threat to surface water sources (Van Boheemen, 1987) and

can also be a direct threat to human and livestock health. Application of livestock manure to agricultural farm land is regarded as beneficial to soil due to its high nutrient content and therefore serves as an organic fertilizer (Environment Agency, 2005; Merrington*et al.*, 2002; Burton and Turner, 2003). Prior to the 20th Century, livestock manure offered the only method of enriching the soil (Burton and Turner, 2003). However, in addition to spreading pathogenic bacteria, manure used is important in the dissemination of antimicrobial resistance genes in the environment (Thanner*et al*., 2016). The spreading of manure clearly increased the abundance of resistance genes in the soil (Christian and Stephan, 2006).

1.2 Statement of Research Problem

Many pathogens of livestock as well as humans can be found in manure of bothlivestock and poultry; these pathogens include bacteria, protozoans and viruses (Kirk, 2016). It has also been observed that many farmers fertilize their farms with fresh or composted cattle manure but do not realize the hidden dangers thatthe manure may harbour disease causing organisms such as *E. coli*, *Listeria*, *Salmonella*, *Cryptosporidium*,etc. These organisms are faecal to oral in transmission and may therefore be passed to humans if vegetables from gardens are not properly washed (Bolen, 2017).

A comprehensive approach to land application of manure is necessary to maximize agricultural value and minimize environmental and human health effects (Shober and Magruire, 2018).Thus special precautions should be followed in handling such

manure, through protocols such as wearing of protective equipment, avoiding hand contact with mouth, eyes and nose and washing of hands after handling livestock manureand can be of help and protective. Activities of small children within the vicinity of livestock and manure should be carefully monitored and farmers should also be aware that farm companion animals can serve a means of transmitting*E. coli* O157:H7 to humans (Davis, 2000). Compost is rich in nutrients, humic matter, and microorganisms; it may be added to agricultural soil as a fertilizer to improve soil fertility and promote the growth of crops and microorganisms, and as a soil amendment to relieve heavy metal pollution (Huang *et al.,* 2016). It has also been mentioned by Mingou*et al*. (2018) that composting eliminates antibiotic residues and pathogens in livestock manure before its application as an organic fertilizer in agro-ecosystems, even though the impacts of composting on antibiotic resistance genes (ARGs) in livestock manure and their temporal succession following the application of compost to land are not well understood.

According to Aderibigbe (2014), the shedding of *E. coli* O157:H7 by cattle and the use of organic manure may be responsible for the contamination of crops by this organism in Zaria and environs. He also indicated that the organism is capable of long term survival outside its mammalian host in the natural environment and can therefore be transmitted to humans and animals through contamination of feeds and human animal based products. Also, a study by Lawan *et al*. (2015) in Zaria showed that pathogenic *E. coli* can contaminate and persist in the environment as a result of the discharge of untreated abattoir waste. Cattle manure is a well-documented source of

zoonotic pathogens, such as *E. coli* O157:H7, *Salmonella* spp., and *Cryptosporidium parvum*(Rhoades *et al*., 2009). Animals may carry these pathogens without showing signs and may also have sporadic faecal shedding (Rhoades *et al*., 2009; William *et al.,* 2015) that contributes to the difficulty in discerning their presence in the animal's gastrointestinal tract and hence faecal matter.

The increasing habit of people eating salad along with fruits, especially in local restaurants, is capable of exposing such individuals to enterotoxigenic *E. coli.* Furthermore, the peoples' practice of washing fruits and vegetables with contaminated water could also lead to serious disease outbreaks due to *E. coli* even though such fruits might not have been initially contaminated.Some studies indicated increasing frequency of*E. coli* O157:H7 isolation from fresh produce, including bean sprouts, cantaloupes, apples, and lettuce leaf (Ackers *et al.,* 1998; Hilborn *et al*., 1999).

The mechanisms by which the pathogen is introduced into the lettuce plant are not fully understood. However, it is speculated that the plant becomes contaminated when grown in fields fertilized with improperly treated manure (Beuchat, 1999). There are limited studies on the role of manure use in Nigeria and the problems of manure contaminating surface and ground waters with pathogenic bacteria such as *E. coli* and the spread of antibiotic resistance genes by such organisms. The existing federal and state government agricultural programs do not incorporate impact assessment of manure use in agricultural development projects. There is also paucity of information on the frequency and occurrence of human health problems amongst farmers due to the prolonged use of manure in Bauchi state.

1.3 Justification

Animal manures are indeed a valuable source of nutrients for crops and grasslands. However, global intensification of animal production concentrates manure within smaller areas, creating nutrient hot spots. In contrast, nutrient deficits often develop in areas with limited animal production that could benefit from manure applications (Shober and Maguire, 2018). A comprehensive approach to manure application is necessary to maximize agricultural values and minimize environmental and human health effects,regulations and incentive programs that promote beneficial use can enhance productive use of manures and prevent environmental problems, and inmanure transportation programs and infrastructure are needed to relocate manures from areas of nutrient surplus to areas of deficit (Shober and Maguire, 2014). Peter and Owino (2019) also mentioned that most farmers in sub-saharan Africa do not apply recommended manure management practices and also that a vast majority of them used manure produced by their own animals.

Also, in anticipation that Nigeria's economy may soon be driven by Agriculture, manure output from livestock production may likely increase due to growth in commercial production systems (Oyinbo and Rekwot, 2014). Manure utilization in crop cultivation is expected to be on the increase due to cost of inorganic fertilizer. Manure obtained from cattle and poultry will predominate even though there are limited studies on the practice and its environmental and public health implications. Farmers often pay herdsmen for their animals to stay for specified periods on their

farms in an attempt to improve the farmlands fertility from the animal droppings. The effect of this practice on the spread of pathogenic and virulent bacterial strains has not been investigated. There is increasing prevalence of antibiotic resistance among bacteria worldwide due to spread of pathogenic organisms on the environment and indiscriminate use of antibiotics, this problem is considered as one of the most intractable challenges in 21st century public health (Wichman *et al*., 2014).

Consumption of fresh fruits and vegetables is increasingly becoming popular among individuals in their bid for healthier living (Opara *et al*., 2007). During the last few decades, pre-prepared minimally processed fruits and vegetables have become popular worldwide among consumers (European Commission, 2002). In several countries, pre-processing of manure intended for use in agriculture is reported to reduce the negative impact of manure use on the environment and food safety (Burton and Turner, 2003; Jen, 2014). The prevailing manure use practice in Nigeria and the lack of regulatory control of biological pollution raises concern for the public health risks associated with manure use. There is dearth of information on the risk-benefits analysis on the increasing trend of the use of manure by Nigerian farmers due to high cost and non-availability of inorganic fertilizers.Therefore, carrying out this research will help to generate information on the dangers of frequent use of cattle manure and faeces by farmers.

1.4 Aim and Objectives

1.4.1 Aim of the study

The study was designedto assess the practice of cattle faeces and manure use and its role in dissemination of virulent and antimicrobial resistant *Escherichia coli* from cattle to humans and environment in Bauchi State, Nigeria.

1.4.2 Objectives of the study

i. To isolate and identify shiga toxin producing *E. coli* (STEC) from cattle faeces and manure obtainedfrom abattoirs, cattle farms (CF) and livestock markets (LM).

ii. To determine the antimicrobial susceptibilities of the *E. coli*isolates against common antimicrobial agents and Multipleantibiotic resistance (MAR) indices

iii. To determine the presence and distribution of antibiotic resistance genes (*tetA,B,C,D,K*) in the VTECisolates ofcattle faeces and manure from abattoirs, CF and LM using multiplex PCR.

iv. To determine the presence and distribution of virulence genes (*eaeA*, *stx1*, *stx2*) in the VTECisolates incattle faeces and manure from abattoirs, CF and LM using multiplex PCR.

v. To evaluate the practice and pattern of cattle faeces and manure usage and the socio-economic factors that promotes it on farm lands in Bauchi state,through administration of structured questionnaire(s).

1.4.3 Research questions

i. Are there *E. coli* (STEC) in cattle faeces and manure from the abattoirs, cattle farms and livestock markets in Bauchi state?

ii. Are the *E. coli* isolates from cattle faeces and manure samples susceptible to common antimicrobial agents harbouring antibiotic resistance genes?

iii. Are there antibiotic resistance genes (*tetA,B,C,D* and *K*) in the VTEC isolates from cattle faeces and manure samples in abattoirs, cattle farms and livestock markets in Bauchi state?

iv. Are there virulence genes (*eaeA, stx1* and *stx2*) in the VTEC isolates from cattle faeces and manure samples in abattoirs, cattle farms and livestock markets in Bauchi state?

v. What are the significant socio-economic factors encouraging the use of cattle faeces and manure on crop farm lands by farmers in Bauchi state?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background of the Study

Escherichia coli are gram-negative bacterium with an outer membrane containing lipopolysaccharides, allowing them to go under proteobacteria (Phylum). *E. coli* are anaerobic gram-negative bacterium that allows them to be categorized under Gamma Proteobacteria (Class). *E. coli* are rod-shaped anaerobic gram-negative bacterium, allowing them to be categorized under enterobacterials (Order). They are classified in the family *Enterobacteriaceae*(Orskov, 1984). Its taxonomic features include Gram-negative, straight rods (1.1-1.5 μm wide and 2.0-6.0 μm long) that may be motile by peritrichous flagella or non-motile and do not form spores.

Colonies on nutrient agar may be smooth (S), low-convex, moist, gray, with a shiny surface and entire edge and easily dispersible in saline, or they may be rough (R), dry and difficult to disperse well in saline. S forms, which usually grow as glistening colonies on ordinary agar media and show turbid growth in fluid media, have developed polysaccharide side chains as part of their lipopolysaccharide (LPS) outer membrane, whereas R forms, which usually will show dry and wrinkled colony forms on agar and which will agglutinate spontaneously in fluid media and have lost their polysaccharide side chains by mutation.

Escherichia coli is a non-spore-forming, Gram-negative bacterium, usually motile by peritrichous flagella. *Escherichia* coli is the most common cause of acute urinary tract infections as well as urinary tract sepsis. It has also been known to cause neonatal meningitis and sepsis and also abscesses in a number of organ systems. *Escherichia coli* may also cause acute enteritis in humans as well as animals and is a general cause of 'traveller'sdiarrhoea', a dysentery-like disease affecting humans, and haemorrhagic colitis often referred to as 'bloody diarrhoea' (Percival, and Williams, 2014).

E. coli can grow both aerobically and anaerobically and are capable of utilizing simple carbon and nitrogen sources for all their metabolic and energy needs(Orskov, 1984).Glucose and other carbohydrates are fermented with the production of pyruvate, which is further converted into lactic, acetic and formic acids, and part of the formic acid is split by a complex hydrogenlyase system into equal amounts of CO_2 and H_2.Some strains are however anaerogenic, lactose is fermented by most strains, but fermentation may be delayed or absent. *E. coli* usually can also use acetate as a sole source of carbon, but citrate cannot be used (Antonia, 1996).

Escherichia coli are normal members of the gastro-intestinal micro flora of humans and animals; however, enterohaemorrhagic *E. coli* strain O157:H7 can cause severe hemorrhagic disease and death in humans (Buchanan and Doyle, 1997). Since the first outbreak of *E. coli* O157:H7 infections in 1982, there have been many other outbreaks in the United States of America (U.S.A.), with estimates of approximately 73,000 *E. coli* O157:H7 infections and 61 associated deaths occurring annually (CDC, 2001). Theorganism is a gram-negative bacterium and the main aerobic commensal bacterial

species. (Von Baum and Marre 2005; Alhaj *et al.*,2007). The native habitat of *E. coli* is the enteric tract of humans and other warm-blooded animals.

Therefore, the organismis widely disseminated in the environment through the faeces of humans and other animals and its presence in water is generally considered to indicate faecal contamination and the possible presence of enteric pathogens (Alhaj *et al.,* 2007). Germany officially announced that they tentatively linked bean sprouts grown in northern part of the country to a massive *Escherichia coli* outbreak, where the European center for infectious Disease Prevention and Control (ECDC) said it received reports of 1,605 enterohaemorrhagic *E. coli* O104:H4 infections of 658 cases of hemolytic uremic syndrome (HUS), a potentially fatal kidney condition that stands out in the outbreak due to higher proportion of cases that is typically seen in *E. coli* outbreaks (Schnirring, 2011).

Unlike other microorganism, *E. coli* is able to acquire resistance easily, thereforebecoming a good bio-indicator model for surveillance studies of virulence and antimicrobial resistance (Von Baum and Marre 2005; Alhaj *et al.,* 2007). A pathogenic strain of this bacterium is also important cause of bacterial infections. Outbreaks of enterohemorrhagic*E. coli* O157:H7 infections have been associated with lettuce and other leaf crops which occurred with increasing frequency in recent years (Mahbub, 2004). Contaminated manure and polluted irrigation water are other probable vehicles for the pathogen in many outbreaks. It has also been observed by Alonso *et al*. (2017) that rapid increase in the rate of antimicrobial resistance (AMR)

reinforced by some opposite tendency in development of new active drugs may no longer be a health threats as recognized by World Health organization (WHO).

In the last decade, increases in *E. coli* O157:H7 outbreaks were associated with environmental contamination in water and through fresh produce such as green leaves or vegetables. Both intrinsic (genetic adaptation) and extrinsic factors may contribute and help E. coli O157:H7 to survive in adverse environments (Chekabab*et al*., 2013).Several outbreaks of *E. coli* O157:H7 infections have been associated with contaminated water. The organism can survive for many weeks in deionized water and lake water, depending on temperature of the water (Chalmers, 2000).

2.2 Characterization of Virulent (verocytotoxigenic) *E. coli*

Escherichia coli is a predominant species of facultative anaerobes in the human gut and usually harmless to the host; however, some groups of pathogenic *E. coli* has emerged that causes diarrheal disease in humans. Referred to as Diarrheagenic *E. coli* (Itoh *et al*., 1998) or commonly as pathogenic *E. coli*, the groups are classified based on their unique virulence factors and can only be identified by their traits. Hence, analysis for pathogenic *E. coli* often requires that the isolates be first identified as *E. coli* before testing for virulence markers. The pathogenic groups and perhaps others that are not yet well characterized (Feng, 1993, Itoh *et al*., 1998). Of the pathogenic group, only 4 groups have been implicated in food or water borne illness.

Enterotoxigenic *E. coli* (ETEC) is recognized as the causative agent of travelers' diarrhea and the illness is characterized by watery diarrhea with little or no fever.

ETEC infections occur commonly in under-developed countries but, in the U.S.A., it has been implicated in sporadic waterborne outbreaks as well as due to the consumption of soft cheeses, Mexican-style foods and raw vegetables. Pathogenesis of ETEC is due to the production of any of several enterotoxins. ETEC may also produce a heat stable toxin (ST) that is of low molecular size (4 kDa) and resistant to boiling for 30 min (Itoh *et al*., 1998).

Enteropathogenic *E. coli* (EPEC) causes a profuse diarrhea and it is a leading cause of infantile diarrhea in developing countries. EPEC outbreaks have been linked to the consumption of contaminated drinking water as well as some meat products. Pathogenesis of EPEC involves intimin protein (encoded by *eaeA* gene) that causes attachment and effacing lesions (CDC, 1996); but it also involves a plasmid-encoded protein referred to as EPEC adherence factor (EAF) that enables localized adherence of bacteria to intestinal cells (Mehlman, 1984). Production of EAF can be demonstrated in Hep-2 cells and the presence of *eae* gene can be tested by PCR assays (Itoh *et al*., 1998).

Enterohemorrhagic *E. coli* (EHEC) is recognized as the primary cause of hemorrhagic colitis (HC) or bloody diarrhea, which can progress to the potentially fatal hemolytic uremic syndrome (HUS). EHEC are typified by the production of verotoxin or Shiga toxins (*stx*). Although *stx1* and *stx*2 are most often implicated in human illness, several variants of *Stx*1 and *Stx*2 exist. There are many serotypes of *stx*-producing *E. coli* (STEC), but only those that have been clinically associated with HC are

designated as EHEC. Of these, O157:H7 is the prototypic EHEC and most often implicated in illness worldwide (Itoh *et al*., 1998 and CDC, 2007).

The infectious dose for O157:H7 is estimated to be 10 - 100 cells; but no information is available for other EHEC serotypes. EHEC infections are mostly food or water borne and have implicated undercooked ground beef (CDC, 2007), raw milk (Karmali, 1989), unpasteurized apple juice and sprouts and vegetables (Doyle and Schoeni, 1987). EHEC O157:H7 are phenotypically distinct from *E. coli* in that they exhibit slow or no fermentation of sorbitol and do not have glucuronidase activity; hence, these traits are often used to isolate this pathogen from foods.

Enteroinvasive *E. coli* (EIEC) closely resemble *Shigella* and cause an invasive, dysenteric form of diarrhea in humans. Like *Shigella*, there are no known animal reservoirs; hence the primary source for EIEC appears to be infected humans. Unlike typical *E. coli*, EIEC are non-motile, do not decarboxylate lysine and do not ferment lactose, so they are anaerogenic. Pathogenicity of EIEC is primarily due its ability to invade and destroy colonic tissue. The invasion phenotype, encoded by a high molecular weight plasmid, can be detected by invasion assays (Griffin and Tauxe, 1991) or by PCR.

2.3 Factors Affecting Survival Rates of Pathogens in Cattle Manure

The survival rate or duration of persistence of each bacterial pathogen in manure gives an indication of its threat to cause infections and diseases. The survival rate varies from a few days to several months, depending on the particular bacterial species being

monitored and its ability to respond to the hostile environment of the cattle manure into which they are excreted (Watcharasukarn *et. al*., 2009). Literature have shown some controversies in the recorded survival rates or die off rates of microbes (Haruta, 2015)this is because laboratory-based studies executed under controlled conditions generated results that differed from those in field-based seasonal studies that were exposed to external influences from the environment.

In a study conducted by Kudva *et al*. (1998), the findings highlighted that the survival period of pathogenic bacteria was lower in laboratory studies as opposed to observations in field studies which were influenced by fluctuations in ambient temperature. Consequently, the emphasis has pointed to the fact that laboratory results are difficult to apply in field conditions (Soupir*et al*., 2008). Notwithstanding, for the subsequent use of manure either in recycling, land application or disposal, destruction of the aforementioned pathogens is very pivotal (Watcharasukarn *et al*., 2009). Several authors have reported the multiplicity of factors that affected the inactivation of pathogens in manure and these included temperature, pH, moisture content, nutrient availability/organic content, biological interactions, time and the density of the organisms in manure (Pachepsky *et al*., 2006).

Contaminated manure and manure slurry maypose a risk when used as a fertilizer for crops. Several conditions may influence the survival of pathogens in manure or slurry. They include temperature,pH, solids content, microbial content, oxidation-reductionpotential (ORP) and time. However, few reportsattempting to quantify the impact of thesefactors on survival of *Salmonella* or *E. coli*O157:H7 in manure were

found. The objective ofthis study was to measure the impact of a few selectedfactors on the growth/survival of *E. coli*O157:H7 and *Salmonella* Typhimurium in cow manureand manure slurry (Himathongkham*et al*., 1999)

Laboratory studies have shown that *E. coli* cansurvive for up to several months inexperimentally inoculated animal feces (Kudva*et al.,* 1998; Bolton *et al.,* 1999; Jiang *et al*.,2002). The principal manner of disinfectionduring composting is based on time-temperaturerelationships that destroy pathogens, althoughantagonistic microorganisms may also play a rolein the process (Himathongkham and Riemann,1999; Larney*et al.,* 2003). The temperatureevolution pattern in the composting system canbe divided into four different phases: mesophilic,thermophilic, cooling and maturation. Aftermixing of the compost material, the temperatureraises rapidly reaching a maximum within ninedays, after which it gradually declines to theambient level over about 30 days (Ishii *et al.*,2000).Cross-contamination of manure produce orimproperly composted manure used for soil improvement may be a source of pathogen contamination during preharvesting. Summer is a period of high prevalence of *E. coli*(David and Page, 2016), and also as stated in the work of Hancock *et al.* (2001) who mentioned that animals may shed increasing loads of *E. coli* O157 from stress due to transportation to abattoirs for slaughter regardless of the season.

Competition with soil microorganisms and adverse environmental conditions can influence pathogen survival, but little information regarding the degree to which Shiga toxigenic *E. coli* non-O157 cells can survive in manure-amended soils is available. A

study, with the objective to determine the fate of STEC non-O157 cells in cattle manure in two different composting systems showed that STEC is widespread in animal feces; therefore, application of untreated manure to vegetable cultures may pose a risk for the transmission of STEC to food (Goncalves andMarin 2007). There was a report that large quantities of livestock waste are spread over agricultural soil in Brazil which serves as a means of environmental contamination with pathogens capable of spreading antimicrobial resistance genes. It was also observed that STEC strains of *E. coli*non-O157 carrying the *stx*2 gene can survive in a composting system for four to 30 days, depending on the temperature reached during composting. Therefore other good alternative systems should be adapted to minimize the risk of pathogens spreadon agricultural soil (Goncalves andMarin, 2007)

2.4 Epidemiology of *Escherichia coli*

Shiga toxin-producing *Escherichia coli* (STEC) have emerged as important foodborne zoonoses associated with outbreaks and sporadic cases of diarrhea, haemorrhagic colitis and haemolytic uraemic syndrome in humans (Griffin *et al*., 1998). The association of *E. coli* O157 with outbreaks of haemorrhagic colitis in humans was observed for the first time in the United States of America in 1982 (Riley *et al*., 2003). Since then outbreaks of disease associated with *E. coli* O157 have been reported from all over the world (Gyles, 2007). In New Zealand, the first case of *E. coli* O157 was reported in 1993 in an 11- month-old boy (Wright *et al*., 1993). Subsequently many cases of *E. coli* O157 have been recorded in New Zealand: in 2009 137 cases (3.6 per 100,000 population) of *E. coli* O157 were recorded in humans (Anonymous, 2010). It

is likely that infection is under reported as many faecal samples from asymptomatic or mild cases may not be assessed for the presence of *E. coli* O157 (Baker *et al*., 1999). Cattle and sheep are the main reservoirs of *E. coli* O157 (Synge *et al*., 2003). Faecal excretion of *E. coli* O157 may cause infection in humans due to direct contact with these animals or contact with food or water contaminated with their faeces during slaughter or grazing, respectively (Armstrong *et al*., 1996). Therefore, cattle play an important role in the epidemiology of *E. coli* O157 with cattle and sheep believed to be the major reservoir of *E. coli* O157 in New Zealand (Cookson *et al.*, 2006). However, very little information is available about the epidemiology of carriage of STEC, particularly *E. coli* O157, in New Zealand. In New Zealand, approximately 1.4 million bobby calves are slaughtered annually for local and export markets (Anonymous, 2010). A better understanding of the epidemiology of *E. coli* O157 is likely to assist in reducing the increasing number of human cases and will also help in designing interventions to avoid possible losses to red meat and dairy export industries that are economically important in many countries (Cavanagh, 2003).

2.5 Diseases caused by *Escherichia coli* O157:H7 in humans and animals

E. coli O157:H7 is a toxin producing bacteria that causes intestinal disease in people which lasts about one week. Diarrhea with blood is typical. Severe cases can lead to kidney problems which can be life threatening, especially in children or the elderly (Fast Facts, 2016). According to Center for Food Security and Public Health(CFSPH, 2016) there are many types of *E. coli* bacteria responsible for diseases in humans and the designation O157:H7 refers to the way scientists classify various *E. coli* bacteria.

Some types of *E. coli* are normally found in the intestines of people and animals but do not cause illness. *E. coli* O157:H7 does not cause illness in animals, but the animals can serve as carriers of the bacteria. These bacteria can be found in cattle, sheep, pigs, deer, dogs and poultry.

Infected animals, especially young ones, can shed the bacteria in their feces. Cattle are the main carriers. Animals, particularly livestock, get *E. coli* O157:H7 by ingesting the bacteria in the feed contaminated with the feces of infected animals, or by exposure to items contaminated with feces (e.g. food or water) or non-living objects (CFSPH, 2016). Human disease from *E. coli* O157: H7 is most often associated with eating improperly cooked meat, particularly ground beef, or from products such as unpasteurized milk and some processed meats. Exposure in animals can be minimized by preventing fecal contamination of feed and water. This can reduce the chance for ingestion of the bacteria (Fast facts, 2016).

Because consumers have developed a better understanding of the health benefits associated with eating fresh produce, salads are increasingly being served in the home, in restaurants and asready-to-eat pre-packaged foods. The associated increase in food-borne diseases must be seen inview of these benefits. Bacterial pathogens can survive on growing plants over prolonged periodsof time under field conditions (Ackers *et al.*, 1998; Hilborn *et al*., 1999). In addition, the ability of human pathogens to infiltrate plant tissues has been recognized recently. Prepackaged, precut lettuce (i.e. damaged plant tissue) may pose a risk as an emerging source of food-borne illness. Surprisingly,

there are no clear differences in pathogen contamination reported between organically grown produce and conventionally grown produce (Allerberger and Sessitsch, 2009).

Vegetables are often eaten raw, and hence are potential vehicles to transmit human pathogens. Vegetables can become contaminated throughout the food chain from production to consumption, via, for example, treatment with water for fertilization, irrigation or pest control, contact with animal or human excreta, food handlers or by cross-contamination in domestic environments (Allerberger and Sessitsch, 2009).The obesity epidemic in Europe and in North America has prompted consumers to eat healthier diets that include more fresh vegetables. Salads constitute one of the most popular diet foods. The rise in food-borne illness associated with salad vegetables can be attributed partly to this higher consumption level (Sivapalasingam *et al.,* 2004; WHO, 2004), and with the increase in availability of ready-to-eat vegetables (Warriner *et al.,* 2003)

2.5.1 Treatment prevention and control

The CDC recommends thorough washing of hands after contact with animals or their products, thorough cooking of meat, avoid raw milk or unpasteurized dairy products and preventing cross contamination during food preparation as preventive measures. Where they mentioned that healthy adults require no treatment for E. coli O157:H7 because most of the infections are self-limiting, moreover for acute diarrheal cases, antibiotic have not proven useful, in fact some studies have shown antibiotics may increase the chance of developing HUS (Patrick, 2019).

2.6 Antibiotic resistance genes (*tet*)

The development of antimicrobial resistance can be seen as a global problem in microbial ecology and is the best-known example of a rapid adaptation of bacteria to a new ecosystem (Carattoli, 2009). Micro-organisms are ubiquitous in nature and there is a continuous exchange of genetic information between bacteria inhabiting diverse ecological niches including, humans,animals and the broader environment. Widespread use of antimicrobial agents led to the emergence of antibiotic resistance in many important pathogens (Levy, 2002). This resistance contributes to higher rates of morbidity and, in the case of severe bacterial infections, therapeutic failure (Masterton *et al*., 2006). Degrees of antimicrobial susceptibility/resistance in clinical isolates are often defined in terms of the Minimum Inhibitory Concentration (MIC) of an antimicrobial compound required to prevent bacterial growth. Bacteria can be defined as being resistant to an antimicrobial compound, when its MIC is higher than its wild-type counterpart (EUCAST, 2000; Acar and Rostel, 2001). For this reason, the 'epidemiological cut-off values' will vary among different species and geographical regions.

Antibiotic resistance has been recognized as an emerging worldwide problem in both human and animals. The use of antibiotics is considered to be the most important factor for the emergence, selection and dissemination of resistant bacteria, which intrinsically acquire the resistance gene from the environment. Antimicrobial agents are important in the treatment of bacterial infections. The global increase in antibiotic-resistant bacteria is of major concern and thus, antibiotic use for medical and

agricultural applications is a major risk factor for the increased occurrence of resistant organisms. (Neu, 1992; Witte, 1998; Alhaj *et al.,*2007). Besides medical use in humans, there is the troubling issue of their use in agriculture, specifically in livestock production where antibiotics have long been regularly used not only for the treatment of infections, but also as a means of getting animals to market faster through growth promotion by adding feed-based antibiotics constantly (Witte, 1998). Antibiotic resistance (ABR) is recognized as one health challenge because of rapid emergence and dissemination of resistant bacteria and genes among humans, animals and environment on a global scale Roushame*t al*. (2018).

Resistant bacteria can be shed in faeces, where they can share extra chromosomal antibiotic resistance plasmids (R-plasmids) with native bacteria and may also be disseminated to other animals. Antibiotics accumulate in the tissues of animals and hence, can be ingested by consumers whose own resident micro flora may become resistant (Timothy *et al.,* 1997). Hence, this is an important means of dissemination of resistance in humans through the food chain (Barton, 1998). Beta–lactamases target the peptidases of bacterial cell-wall biosynthetic process andextended-spectrum beta-lactamases (ESBLs) have evolved through a series of substitutions of amino acids and provide resistance to third generation cephalosporins. ESBLs are produced by gram-negative bacteria and have been reported from many species (Ittoo *et al*., 2010).

Antibiotic resistance in pathogenic bacteria has been an increasing medical problem for decades (Kastner*et al.,* 2005). Microbes may develop resistance to antibiotics under selective pressure, or they may acquire antibiotic resistance determinants

without direct exposure to an antibiotic. Furthermore, acquired resistance determinants are spread among different species and even genera which include potential and obligate pathogens (Kastner*et al.*,2005). This is especially favored in settings that allow the close association of densely packed microorganisms such as the intestine of humans and animals (Salyers*et al.*, 2004). Consequently, antimicrobial-resistant bacteria are selected for; thereby posing a serious public health threat in that antimicrobial treatment effectiveness may be reduced (Schroeder, 2002). Even in humans antibiotic resistance have been a serious problem as stated by Adenipekun *et. al.* (2016) who showed the emergence of Multiple Drug Resistance (MDR) of two (2) or more antimicrobials encountered in the treatment of infected patient with urinary tract infection (UTI) demonstrate circulating MDR *E. coli* in Nigerian community.

Certain management practices such intensive management have shown evidence that antimicrobial drug residues in livestock effluent can contribute to elevated antimicrobial resistance (AMR) levels, hence it is important to improve our understanding of how current management systems may impact AMR transmission to the public. Culture-independent methods can contribute to this understanding, as the genetic dynamics of AMR are complex and pan-microbial. For instance, antimicrobial resistance genes ARGs often move together in a networked fashion, and can be exchanged between distantly-related bacteria (Forsberg, 2012; Avijit *et al*., 2016). Transfer rates among bacteria increase under conditions that induce stress, including antibiotic exposure (Guerin, 2009). Pan-microbial approaches enable access to this microbial resistance ecology and provide information on how livestock production

practices influence the density and composition of ARGs (i.e., the resistome (Noyes*et al.,* 2016). With this insight, practical mitigation strategies can be proposed to minimize the flow of ARGs into aquatic, terrestrial and atmospheric ecosystems.

However, very little is currently known about the cattle production resistome. The few published studies are descriptive in nature, utilizing samples taken from a small number of non-commercial animals (Durso *et al.,* 2011; Wichmann*et al.,* 2014). A lack of basic study design information is a major impediment to execution of larger, inferential studies. For instance, the sample size needed to detect resistome differences between conventional and organic operations is currently not estimable because of lack of baseline of normal resistome variability among different breeds of cattle. In addition, many livestock production factors may compound the relationship between antimicrobial use and the resistome, e.g., stocking density or ration formulation.

2.7 Virulence genes of *Escherichia coli*

Shiga toxin-producing *Escherichia coli* (STEC) was recognized as a cause of bloody diarrhea and hemolytic uremic syndrome (HUS) for the first time in two independent studies in 1982 (Karmali*et al.,* 1983; Riley *et al.,* 1983) and later found to be the main cause of diarrhea associated HUS with a high number of cases worldwide. Non-sorbitol-fermenting STEC (NSF) O157:H7 was the first STEC serotype that was isolated in association with HUS and has been the most frequently reported cause of diarrhea-associated HUS (Gyles, 2007). However, STEC strains of other serogroups like O26, O103, O111, O121, and O145 have also been recognized to cause severe disease and outbreaks (ECDPC, 2013; Gould*et al.*, 2013).

Shiga toxins 1 and 2 (*stx1*and *stx2*) are essential virulence factors of STEC. The term STEC is used to describe any *E. coli* producing Shiga toxin, whereas the term enterohaemorrhagic *Escherichia coli* (EHEC) is often used to describe the subset of STEC strains responsible for causing hemorrhagic colitis and HUS (Gyles, 2007). In addition to *stx*1, *stx*2, and *eae*, the presence and absence of various other genes have been investigated as potential virulence markers for HUS and outbreaks (Gyles, 2007).

The ability of *E. coli* to colonize different anatomical sites is due in part to genome plasticity and remodeling by acquisition or loss of genetic material from which it acquired resistance or virulence factors. Therefore, horizontal transfer is an important factor in the evolution and adaptation of *E. coli* to different niches (Ahmed *et al.*, 2010). The interaction between bacteria and epithelial cells is a multifactorial and complex phenomenon which involves several adhesins produced according to the stage of infection, while adherence to epithelial cells is essential for successful colonization and establishment; the expression of other genes encoding toxins, siderophores, lipopolysaccharide (LPS), capsule, and invasins determines the disease severity and the strain's virulence (Hilbert *et al.*, 2008). Certain strains can cause acute infections and recurrent infections that do not respond to common antimicrobial treatments.

Urinary tract infections treatment generally includes β-lactam antibiotics, fluoroquinolones, or trimethoprim/sulfamethoxazole (Chulain *et al.*, 2005) but may vary according to patient age, sex, pathogen involved, course of disease, and the urinary tract anatomic area involved (Johnson and Stell, 2000). The increased

resistance may be related to changes in the bacterial genome by mutation or acquisition by horizontal transfer of an extra chromosomal or chromosomal material (Moura *et al.,* 2009).

2.8 *Escherichia coli* Serotyping

According to O'Sullivan *et al.,* (2007) serotyping of *E. coli* is based on the use of specific antisera and the detection of somatic O- and H- antigens expressed by these bacteria. Currently, a total number of 181 O-antigens and 53 H-antigens are available for the organism. Different approaches can be applied for agglutination of these specific antisera, including screening of all types of antigens simultaneously in a microplate format, or using several pools of antisera which are tested separately. Kits for O and H serotyping for VTEC are commercially available. Certain isolates may be non-motile, requiring supplementary motility tests in specific motility media. However, a minority of strains do not serotype satisfactorily. Serotyping can also be done by DNA-based methods. Full serotyping is usually carried out in national reference laboratories (O'Sullivan*et al.,* 2007).

Classical antibody serotyping of *E. coli* is an important method of diagnostic microbiology for epidemiological purposes, as well as for a rough virulence assessment. However, serotyping is so tedious that its use is restricted to a few reference laboratories. To improve this situation a method was developed and validated a genetic approach for serotyping based on the microarray technology (Karin *et al.,* 2007). The gene encoding the O-antigen flippase (*wzx*) were selected as target

sequences for the O-antigen polymerase (*wzy*) were selected as target sequences for the O-antigen, where *fliC* and related genes, which code for the flagellar monomer, were chosen as representative for the H phenotype. Starting with detailed bioinformatic analysis and oligonucleotide design, an ArrayTube-based assay was coupled with a site-specific, linear multiplex labeling procedure and hybridization analysis of the biotinylated amplicons (Karin *et al.*, 2007).

E. coli has been the most important bacterial pathogen involved in neonatal diarrhea or collibacillosis and has important public health implication due to the involvement of serotype O157. There is the need for continuous research to determine the presence of virulent factors of the various serotypes of *E. coli*. Serotyping of O antigen of *E. coli* was carried out usingavailable O antigen containing *E. coli* antisera(O'Sullivan *et al.*, 2007). The procedure involved the use of 2ml ofcommercially prepared polyclonal *E. coli* antisera ofserotypes O26, O118, O111, O157 and O1O3 sourcedfrom any reputable research institute or University.Each antiserum would be diluted based on the manufacturer'srecommendation. Slide agglutination tests would then be carried out to identify each serotype and the positive result recorded when small minute granules appeared(O'Sullivan *et al.*, 2007).

E. coli plays an important role as a member of the gut microbiota; the nonpathogenic ***E. coli***that makes up the component of the gut microflora of humans and animals. They are commensals, when they do not acquire virulence factors; however, pathogenic strains also exist, including various diarrheagenic *E. coli* pathotypes and extra intestinal pathogenic *E. coli* that cause illness outside of the GI-tract. *E. coli* have

also been traditionally serotyped using antisera against the ca. 186 O-antigens and 53 H-flagellar antigens. Phenotypic methods, including bacteriophage typing and O- and H- serotyping for differentiating and characterizing *E. coli* have been used for many years; however, these methods are generally time consuming and not always accurate. Advances in next generation sequencing technologies have also been made it possible to develop genetic-based subtyping and molecular serotyping methods for *E. coli,* which are more discriminatory compared to phenotypic typing methods(Karin *et al.,* 2007). Furthermore, whole genome sequencing (WGS) of *E. coli* is replacing established subtyping methods such as pulsed-field gel electrophoresis, providing a major advancement in the ability to investigate food-borne disease outbreaks and for trace-back to sources. Whole – genome sequencing (WGS) is rapidly becoming the method of choice for outbreak investigations and public health surveillance of microbial pathogens (Holmes*et al.,* 2018).

2.9 Molecular assessment of *Escherichia coli*

Several studies have identified genetic elements associated with particular seropathotypes that appear to play a role in influencing bacterial virulence (Coombes *et al*., 2008; Bugarel *et al.,* 2012**)**. Bacterial virulence traits of STEC strains are governed by the dynamic exchange and loss of mobile genetic elements such as plasmids, transposons, insertion sequences, integrons, bacteriophages, and genomic islands **(**Kelly *et al*., 2009). These genetic loci characterize the pathogenicity of bacterial species by determining what toxins are produced and how the bacterium

attaches to and invades host cells, modulates the host cell cycle and immune responses, survives in stressful environments, and produces biofilms. Many virulence loci have been characterized for STEC, including a number of pathogenicity islands (e.g., the locus of enterocyte effacement [LEE], the locus of proteolysis activity [LPA], the high-pathogenicity island [HPI], the *E. coli* type III secretion apparatus [ETT2], the urease gene cluster, the long polar fimbrial operon, O-island 36 [OI-36], OI-57, OI-71, OI-122, OI-141, and OI-154), two key virulence plasmids (pO157 and pO113), and chromosomal lambdoid bacteriophage insertions that carry genes for two types of Shiga toxin (Fratamico *et al*., 2002 and Coombes *et al*., 2008**).** Variants of the genes encoding the Shiga toxins have been reported, and certain variants, including stx_2 and stx_{2c}, are more likely to be associated with HC and HUS **(**Friedrich, 2002; Beutin *et al*., 2008**).**

Polymerase chain reaction (PCR) is one of the molecular tests that have been designed for the detection of many virulence genes and are often the most sensitive methods for detecting them; however, using these techniques for the screening of more than one gene is labour intensive and costly. There are a number of nucleic acid based methods reported for the detection and characterization of VTEC (O'Sullivan, 2007). The most commonly reported methods are based on the use of the polymerase chain reaction (PCR) to amplify a specific gene target in VTEC.

PCR remains a powerful molecular biology technique that was introduced to facilitate the detection of these virulence factors (Nataro and Kaper, 1998); however, its direct application to fecal specimens is impeded by the presence of inhibitors in such crude

materials (Lou *et al*., 1997). For this reason, most studies using this technique worked with isolated colonies and/or extraction and partial DNA purification (Stacy-Phipps*et al.,* 1995; Pass*et al.,* 2000).The primers used in the PCR may detect a characteristic virulence or antibiotic resistance factor in VTEC, i.e. *stx*genes (s*tx1*, s*tx2* or s*tx3* subtypes) as well as others such as *eae* (intimin) gene sequences. There are a number of primer pairs available which will detect genes in a single assay and others which discriminate between the variants. This can give additional characterization of the strains and information regarding their virulence potential with some variants more associated with severe clinical illness (O'Sullivan, 2007).

Conventional PCR relies on amplification of the target gene(s) in a thermocycler, separation of PCR products by gel electrophoresis, followed by visualization and analysis of the resultant electrophoretic patterns, a process that can take a number of hours. The development of real-time PCR which uses fluorescence to detect the presence/absence of a particular gene in real time has greatly increased the sensitivity and speed of PCR-based detection methods (O'Sullivan, 2007). Real-time PCR assays have been developed for the detection of VTEC carrying the major associated virulence genes i.e. *eae, stx1* and *stx2* and also for serogroups specific gene targets for O157, O26, O111, O103 and O145. Some of these targets have been combined into multiplex assays (O'Sullivan, 2007).

Paton *et al.* (1993) described a PCR for the amplification of *stx1* and *stx2* sequences in primary human fecal cultures. However, oligonucleotide hybridization probes were required to distinguish between the two toxins in a separate test. The presence of *stx*-

positive fecal cultures in asymptomatic individuals (Brian *et al.*, 1992; Paton *et al.*, 1993) suggested that other virulence factors besides *stx*are required to cause serious disease in humans. Fratamico *et al.,* 1995) described a multiplex PCR capable of detecting *stx1*, *stx2*, *eaeA*, and EHEC *hlyA*sequences. However, this PCR was not tested with fecal samples; primers for each target gene sequence showed differential sensitivities, and *stx*primers were unable to distinguish *stx1* from *stx2* by agarose gel electrophoresis. Ideally, PCR-based detection methods should be rapid and sensitive without requiring extensive sample preparation. More recently Paton and Paton (1998) developed a multiplex PCR utilizing four PCR primer pairs for the detection of *stx1*, *stx2*, *eaeA*, and EHEC *hlyA*in human feces and foodstuffs.However, the relatively lengthy PCR template preparation protocol used was considered inappropriate for testing large numbers of samples.

2.9.1 Biochemical tests for *Escherichia coli*

2.9.1.1 Conventional biochemical test for Escherichia coli

Some of the tests used in the conventional biochemical tests for the identification of *E. coli* are:Simmon's Citrate Agar which is a defined medium used to determine if an organism can use citrate as its sole carbon source. It is often used to differentiate between members of *Enterobacteriaceae*. In organisms capable of utilizing citrate as a carbon source, the enzyme citrase hydrolyzes citrate into oxaloacetic acid and acetic acid. The oxaloacetic acid is then hydrolyzed into pyruvic acid and CO_2. The CO_2 produced reacts with components of the medium to produce an alkaline

compound (e.g. Na_2CO_3) andthe resultant alkaline pH turns the pH indicator (bromthymol blue) from green to bluewhich is a positive result. *Klebsiella pneumoniae* and *Proteusbmirabilis* arehexampleshofmcitratempositive organisms. *Escherichia coli* and *Shigella dysenteria*e are citrate negative(Balows *et al*., 1991).

Methyl Red / Voges-Proskauer (MR/VP) test is used to determine which fermentation pathway is used to utilize glucose. In the mixed acid fermentation pathway, glucose is fermented and produces several organic acids (lactic, acetic, succinic, and formic acids). The stable production of enough acid to overcome the phosphate buffer will result in a pH of below 4.4. If the pH indicator (methyl red) is added to an aliquot of the culture broth and the pH is below 4.4, a red color will appear. If the MR turns yellow, the pH is above 6.0 and the mixed acid fermentation pathway has not been utilized. The 2,3 butanediol fermentation pathway will ferment glucose and produce a 2,3 butanediol end product instead of organic acids. In order to test this pathway, an aliquot of the MR/VP culture is removed and a-naphthol and KOH are added. They are shaken together vigorously and set aside for about one hour until the results can be read. The Voges-Proskauer test detects the presence of acetoin, a precursor of 2,3 butanediol. If the culture is positive for acetoin, it will turn “brownish-red to pink”. If the culture is negative for acetoin, it will turn “brownish-green to yellow”. Note: A culture will usually only be positive for onepathway: either MR+ or VP+. *Escherichia coli* is MR+ and VP-. In contrast, *Enterobacter aerogenes* and *Klebsiella*

pneumoniae are MR- and VP+. *Pseudomonas aeruginosa* is a glucose non-fermenter and is thus MR- and VP-General and Medical Microbiology (G.M.M., 2018).

Urease test is a test used to identify bacteria capable of hydrolyzing urea using the enzyme urease. It is commonly used to distinguish the genus *Proteus* from other enteric bacteria. The hydrolysis of urea forms the weak base, ammonia, as one of its products. This weak base raises the pH of the media above 8.4 and the pH indicator, phenol red, turns from yellow to pink. *Proteus mirabilis* is a rapid hydrolyzer of urea (center tube pictured here). The tube on the far right was inoculated with a urease negative organism and the tube on the far left was un-inoculated(G.M.M., 2018).

Motility agar is a differential medium used to determine whether an organism is equipped with flagella and thus capable of swimming away from a stab mark. The results of motility agar are often difficult to interpret. Generally, if the entire tube is turbid, this indicates that the bacteria have moved away from the stab mark (are motile). The organisms in the two tubes pictured on the right are motile. If, however, the stab mark is clearly visible and the rest of the tube is not turbid, the organism is likely non-motile. G.M.M (2018).

2.9.1.2Microbact system biochemical test

The Microbact™ Gram-negative system is used for the identification of aerobic and facultative anaerobic Gram-negative bacteria (Enterobacteriaceae and miscellaneous Gram- negative bacteria). The Microbact™ Gram-negative system is a standardized

micro-substrate system designed to simulate conventional biochemical substrates used for the identification of Enterobacteriaceae and common miscellaneous Gram-negative bacilli (MGNB). Organism identification is based on pH change and substrate utilizations as established by published reference methodologies. The Microbact™ Gram-negative product consists of two separate substrate strips, 12A and 12B. Each strip consists of 12 different biochemical substrates. The 12A strip may be used alone for identification of oxidase-negative, nitrate-positive glucose fermenters comprising 15 genera and may be useful for screening pathogenic *Enterobacteriaceae* from enteric and urine specimens or identification of other common isolates. The 12B strip can be used in conjunction with the 12A strip for the identification of oxidase-positive, nitrate-negative, and glucose-non-fermenters (MGNB) as well as the *Enterobacteriaceae*.

It is important to note that the 12 substrates contained in the 12A strips are available in a solid microplate format, referred to as 12E. The 12B strips can be used alongside the 12E, but in a separate tray. The 24E solid microplate format contains the 24 substrates contained in the combination of both the 12A and 12B strips as described in theMicrobact Identification Kits, manual (2018) Oxoid limited (Balows *et al*., 1991).

Isolation of organisms is carried out on an18-24 hour pure culture of the organism to be identified. Appropriate agar media, for example MacConkey (CM0007), Eosin Methylene Blue (CM0069), Blood or Chocolate (CM0331 and SR0050), may be used to grow organisms. Qualified Personnel should collect specimens according to standards routinely used, perform an oxidase test on the organism to be identified.

Oxidase positive organisms cannot be identified using the Microbact™ 12A (12E) alone and should be examined with the Microbact™.

The inoculum is prepared by picking 1-3 isolated colonies from an 18-24 hour culture and emulsified in 2.5ml of sterile saline solution if 12A/E alone is being used or 5.0 ml of sterile saline if 24E (12A/E and 12B) are being used,then mixed thoroughly to prepare a homogeneous suspension. If the organism has been grown on a selective medium and the colony is small or inhibited, it may be necessary to emulsify the colony in 5.0 ml of peptone water and incubate at 35° ± 2°C for four hours. Using a sterile Pasteur pipette, transfer one drop of the peptone water culture into the appropriate volume of sterile saline solution (0.85%) (Balows *et al*., 1991).

The inoculation is carried out by setting the wells of individual substrate sets can be exposed by cutting the end tag of the sealing strip and slowly peeling it back. Place the strip or plate in the holding tray and using a sterile Pasteur pipette add 4 drops (approximately 100 µl) of the bacterial suspension, or half fill each well in the set. Using a sterile pipette or dropper bottle, overlay the substrates underlined on the holding tray with sterile mineral oil, i.e. wells 1, 2 and 3 for 12A (12E) or 24E and wells 8 and 12 for 12B or wells 20 and 24 for 24E. (Well 8 for 12B and 20 for 24E is not overlaid with oil for oxidase- positive, miscellaneous Gram-negative bacilli) as described inMicrobact Identification Kits (M.I.K),(2018).

Incubation is carried out after resealing the inoculated rows with the adhesive seal and write the specimen identification number on the end tag with a marker pen. Incubate at

35° ± 2°C for 18-24 hours. When *Pseudomonasfluorescens* appears as the organism of choice, repeat the test at an incubation temperature of 25° ± 2°C.To determine the purity of the inoculum, it is advisable to inoculate a solid non-selective medium with the test suspension to act as a culture purity check.Reading the test strip is carried out sequentially as follows; where the 12A (12E) strip should be read at 18-24 hours. The 12B/24E strip is read at 24 hours when identifying Enterobacteriaceae. All systems should be read after 48 hours for the identification of Miscellaneous Gram-negative bacilli. Then followed by Removing the strips or tray from the incubator, peel back the sealing tape. Record all positive results. The reactions are evaluated as positive or negative by comparing them with the colour chart. Record the results under the appropriate heading on the report formM.I.K (2018).

2.9.2 Cattle faeces and manure

Cattle manure is basically made up of digested grass and grain. Cow dung is high in organic materials and rich in nutrients. It contains about 3 percent nitrogen, 2 percent phosphorus, and 1 percent potassium (3-2-1 NPK). In addition, cow manure contains high levels of ammonia and potentially dangerous pathogens. For this reason, it's usually recommended that it be aged or composted prior to its use as cow manure fertilizer.The use of cattle manure, or cow dung, in the garden is a popular practice in many rural areas. This type of manure is not as rich in nitrogen as many other types; however, the high ammonia levels can burn plants when the fresh manure is directly applied. Composted cow manure, on the other hand, can provide numerous benefits to the garden when compared to the chemical fertilizer especially in terms of cost of the

product and availability to farmers and its effect on the soil fertility Tilley (2019).

2.10 *Escherichia coli* in cattle faeces and manure

Animal manure may contain numerous pathogenic bacteria including *Escherichia coli* O157:H7 and Salmonella (Pell, 1997) which are food borne pathogens. Grazing cattle that are infected with food borne pathogens shed these pathogens during defaecation (Looper*et al*. 2006); consequently, infected faeces may inoculate forages that are subsequently grazed leading to the spreadof the pathogenic bacteria to additional animals. *Escherichia coli* O157:H7 from the faeces of naturally-infected animals can survive for 162 days (Avery *et al*. 2004), and salmonella is reported to survive in soil fertilized with cattle faeces for 203–231 days (Islam *et al*. 2004). Previous studies indicate that feedlot pen soil (Berry and Miller 2005), livestock water tanks (LeJeune*et al*. 2001), and the area surrounding livestock water tanks (Looper*et al.*, 2006) may also serve as reservoirs for pathogenic bacteria. A faecal sample refers to faeces collected directly from the rectum of the animals in sterile polythene or freshly voided non-contaminated faeces.

2.10.1 Factors promoting use of cattlemanure

Livestock production effluent has been implicated in the transmission of antimicrobial resistant bacteria into aquatic, terrestrial and atmospheric ecosystems (McEachran2015;Sura,2015). Regulation of livestock manure and wastewater is currently designed to limit levels of organic nutrients and coliform bacteria within

ground and surface waters, and manured soils. Such regulations may not adequately control transfer of (ARGs) from livestock facilities into the surrounding environment, particularly when antimicrobial drug residues are present in effluents (Zhang, 2013 and Zhu 2013). However, significant increases in soil ARG levels have been observed after application of antimicrobial-free soil (Kyselkova, 2013;Udikovic-Kolic, *et. al*., 2014), or when manure application rates are high or manure is improperly stored (Ghosh and LaPara, 2007)).

Animals generate waste, wheremany traditional crop and livestock operations use manure as a fertilizer, letting animals roam on land after harvest to build up organic matter (Honeyman, 1996). It is evident that antimicrobial drug residues in livestock effluent can contribute to elevated antimicrobial resistance (AMR) levels, hence it is important to improve our understanding of how current management systems may impact AMR transmission to the public. Culture-independent methods can contribute to this understanding, as the genetic dynamics of AMR are complex and pan-microbial. For instance, ARGs often move together in a networked fashion (Gow*et al.*, 2008; Herrick*et al.*, 2014), and can be exchanged between distantly-related bacteria (Doucet-Populaire*et al.*, 1991; Forsberg, 2012).

Transfer rates among bacteria increase under conditions that induce stress, including antibiotic exposure (Beaber*et al.*, 2004 and Guerin, 2009). Pan-microbial approaches enable access to this microbial resistance ecology and provide information on how livestock production practices influence the density and composition of ARGs. With this insight, practical mitigation strategies can be proposed to minimize the flow of

ARGs into aquatic, terrestrial and atmospheric ecosystems.Declining soil fertility coupled with minimal nutrient inputs have contributed to low crop yields in sub-Saharan Africa; a major constraint to food security and economic development in most African countries. The use of cattle manure in Agriculture is increasing as an alternative source of fertilizer and as a means of increasing or preserving soil organic matter (Muhereza, 2012).

The depleted soil nutrients can be replenished with addition of fertilizers; however, most farmers use little mineral fertilizer, due to the high cost/price compared to the price of crop output and/or poor distribution in rural markets. Therefore, the efficient management of nutrients through manure recycling within the crop-livestock system is necessary to support food production. Various animal manures including goat manure, chicken manure, rabbit manure and cattle manure can be used to improve soil fertility, with the latter used widely. Cattle manure, either aged or fresh manure is applied prior to crop establishment and by a few farmers in combination with inorganic fertilizers at planting (Muhereza, 2012). However, Mazza*et al*. (2010) recommend that fresh manure must be used with caution in the garden because it may contain pathogenic bacteria such as *E. coli, Listeria*, and *Salmonella*.

Human population pressure on the limited land resource has led to the evolution of mixed crop-livestock farming systems as an efficient and sustainable means of producing food (McIntyre *et al*., 1992 and Rufino 2008). Crops and livestock are no longer viewed as separate and inevitably competitive enterprises but complementary to each other. Though livestock is not a major driving force of the global economy, it

plays a crucial role in food security and supports the livelihoods of approximately 1.3 billion people (Antwi *et al*., 2010).

2.10.2 *E. coli* in cattle manure

Despite significant advances in our understanding of *E. coli* transmission in industrialized nations, little is still known about the transmission of the bacterium in the developing world. Zoonotic transmission of *E. coli* and similar pathogens may in fact be very common in rural, subsistence-based agricultural economies. In rural Africa, for example, people traditionally keeplivestock in close proximity to the homestead or even inside the domicile (Innocent *et al*., 2008). In such situations, humans and livestock come into exceptionally close contact both directly and through cross contamination of the shared environment. Such contact may increase the risks of pathogen transmission, especially for microbes such as *E. coli* that are transmitted via the fecal-oral route (Innocent *et al*., 2008).

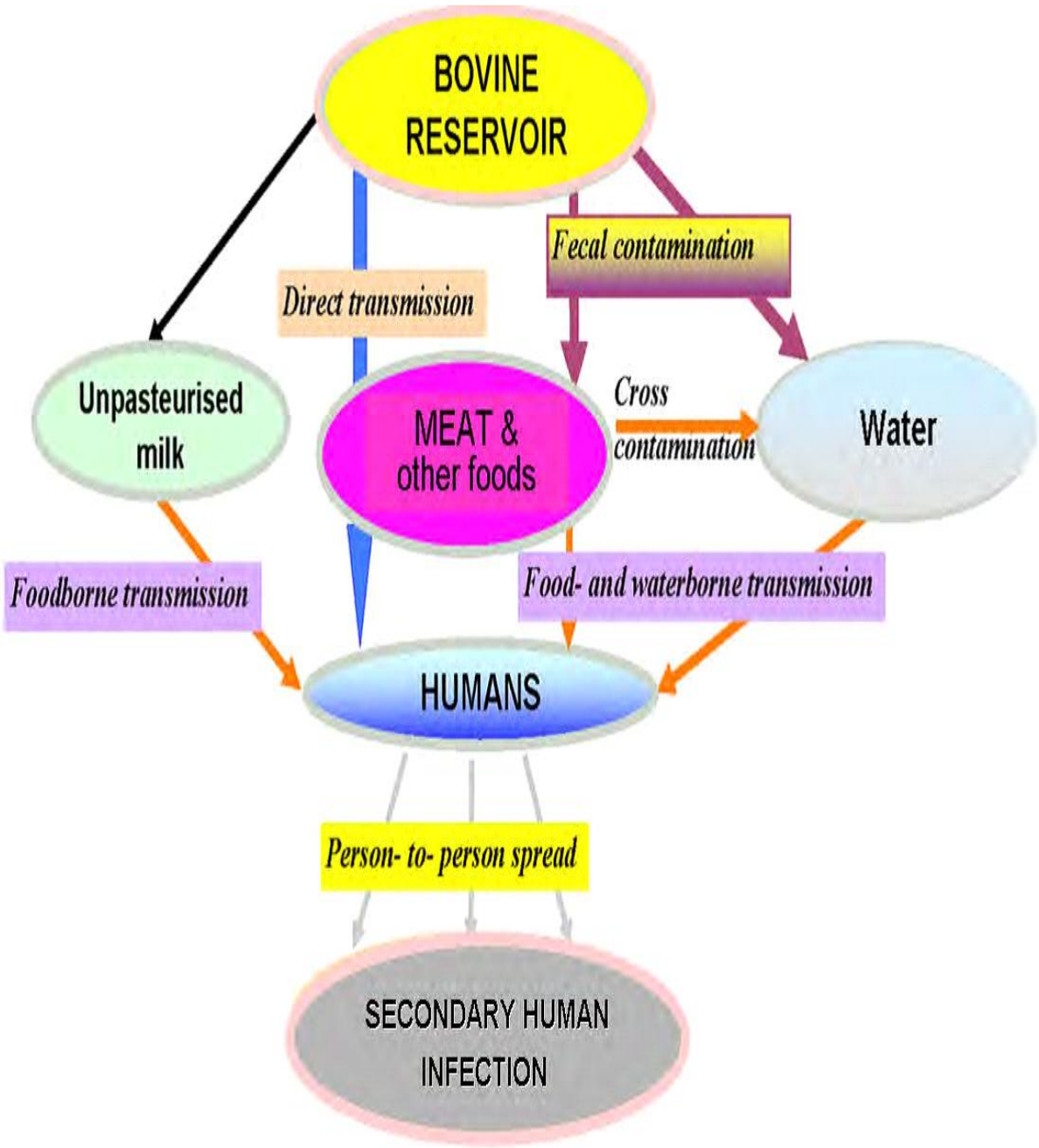


Figure 2.1: Cycle of transmission of *E. coli* between human and animals

Adapted from Armstrong*et al.* (1996)

Livestock and poultry manure can contain a variety of pathogens. Some are host-adapted and, therefore, not a health risk for humans. Others can produce infection in humans and are thus termed zoonotic. The more common zoonotic pathogens in manure include *Escherichia coli* O157:H7, *Campylobacter*, *Salmonella*, *Cryptosporidium parvum*, and *Giardia lamblia*(Christy *et al*., 2016). Viruses can also be associated with manure, although less is known about their survival in manure. (United States Environmental Protection Agency (USEPA), 2013). Survival of microorganisms in manure, soils, and water varies greatly (from days to as much as a year) depending upon the organism and the environmental conditions. Risks from manure-associated pathogens can arise when runoff spills, or infiltration enable microorganisms to reach surface water or groundwater, or when land-applied manure, or irrigation water impacted by manure, comes into contact with food crops. The level of risk to humans depends upon a number of factors that dictate how readily the microorganisms are transported through the environment and how long they remain infectious, as well as the numbers of microbes and their infectious doses (USEPA, 2013).

2.10.3 *Escherichia coli* dissemination through cattle manure

Livestock industries are growing tremendously along with the human population ensuing in the increase rate of generation of organic residues, which pose problems that warrant strategies for disposal and/or management (Karim *et al*., 2005). Agricultural animals, including cattle produce copious quantities of animal manure consisting of animal excreta (feces and urine) along with bedding, microorganisms,

process-generated wastewater, secretions (nose, throat, blood, vagina, mammary glands, skin and placenta), undigested and spilled feed, antibiotics, nutrients and fur (Sakar *et al*., 2009). In addition, animal manure is known to harbor a wide variety of microorganisms which can be pathogenic or non-pathogenic to both animals and humans (Godwin and Moore, 1997). However, (Hutchison *et. al*., 2005) mentioned that the levels and types of pathogens occurring in livestock wastes vary with animal species, dietary sources, health status and age of the animals, physical and chemical characteristics of the manure produced as well as the storage facilities of the manure (Spiehs and Goyal, 2007).

Cattle manure harbors microbial constituents that make it a potential source of pollution in the environment and infections in humans. Knowledge of, and microbial assessment of, manure is crucial in a bid to prevent public health and environmental hazards through the development of better management practices and policies that should govern manure handling (Christy *et al*., 2016). Literally, livestock practices vary from one individual to another and from one geographical location to the other, but eventually, influence the microbial structure of manure released by the animals. Clearly, manure provides different biological and physicochemical environment to microorganisms (Wang *et al*., 2004). Specifically, Pachepsky *et al*. (2006) noted that many manure-based pathogens exist, and the major manure based zoonotic bacteria, including Salmonella spp., *Campylobacter* spp., *Listeriamonocytogenes*, *Yersinia enterocolitica*, *Escherichia coli* and protozoa viz. *Cryptosporidiumparvum* and *Giardia lamblia*, are present; however, with others being less common.

2.10.4 Antibioticresistant *Escherichia coli*

E. coli is a frequent cause of life threatening bloodstream infections and other commoninfections, such as the urinary tract infections (Kennedy *et al.,* 2008). Antibiotic resistance rates in *E. coli* are rapidly rising, especially with regard to flouroquinolones and third – and fourth – generations' cephalosporins. Surprisingly, most of these multidrug-resistant strains are acquired in the community rather than in healthcare settings (Mesa *et al.,* 2006: Laupland*et al.,* 2008).Drug resistant *E. coli* are rapidly acquired via food and water, and there is a major turnover of drug-resistant *E. coli* each day (Corpet, 1988). When people eat sterile food, there is a rapid and substantial fall in the number of drug-resistant *E. coli* these people carry (Corpet, 1988). What remains unclear is where the drug-resistant *E. coli* in our food are coming from. Are they mainly human strains that contaminate our food (and water), or are these strains derived from food animals. A study by Amosun*et al.,* (2012) monitored antimicrobial susceptibility in commensal *Escherichia coli* from the faeces of on-farm and slaughter cattle and at abattoir as well as beef sold to general public in markets at Ibadan, Nigeria.And stated that commensal bacteria contribute to the distribution and persistence of antimicrobial resistance in the environment (Agga*et al.,* 2019).

Global increase in the incidence of antimicrobial resistance in bacterial strains is a major concern to all stakeholders in animal production, veterinary practice and public health (WHO, 2001). Realizing the dangers associated with the emergence and dissemination of resistant bacterial strains, many advanced countries have taken giant

strides in formulating and promoting policies to limit the use of antimicrobial agents in food animals (WHO, 2001, 2002; Obeng*et al.,* 2012). However, in developing nations of Africa, livestock farmers with little knowledge on the use of antimicrobial agents and the attendant consequences, have unrestricted access to these agents and use them without recourse to expert advice or supervision. Commensal *E. coli* is often used as indicator bacteria for monitoring and assessing the level of antimicrobial resistance in the community (Kijima-Tanaka *et al.,* 2003). The ease of transferability of resistance trait among bacteria through mobile genetic elements and the pre-ponderance of *E. coli* in the faeces and environment may facilitate the development and spread of antimicrobial resistance (Kang *et al.*, 2005; Lee *et al.,* 2006).

Antimicrobial resistant bacteria can enter the meat production chain from carrier animals and get transmitted to humans (Hammerum and Heuer, 2009). Over dependence on antimicrobial usage in animal production as well as unhygienic practices during meat production and marketing are important factors responsible for the emergence of antimicrobial resistance in bacteria of animal origin and their eventual transmission to humans (WHO, 2000).

2.10.5 Knowledge,Attitude and Practice(KAP) of farmer's use of manure

Organic agriculture is the most dynamic and rapidly growing sector of the global food industry (Ellis *et al.,* 2006). The yields from organic agriculture are not less than from conventional agriculture (Ramesh, *et al.,* 2005). Badgley*et al.* (2006) showed that organic food can fulfill the demands for food and sustain the environment, for example Thailand is predominantly an agricultural-based country with a favorable

climate and well-developed agricultural processing facilities, being among the top ten agricultural exporters in the world. In Thailand, production of organic crops is undertaken mainly by smallholders, farmers groups, or large agro-enterprises using organized groups of contract farmers (Chouichom and Yamao, 2010).

Despite the importance of manure application as a key outcome in one of the communities in Rwanda's Girinka program, no previous study has been carried out to assess the knowledge, attitudes, and practices of Girinka farmers in Rwanda concerning manure usage and its effect on soil fertility and crop yields. According to Smathers, (1982) as cited in Alonge and Martin (1995), farmers' attitudes and perceptions are considered the most influential factors in successful adoption and diffusion of sustainable agriculture practices.Organic farming is gaining popularity in Malaysia today as it can diversify agricultural production system toward attaining improved productivity, farm income, and food safety and is seen as a sustainable alternative to chemical-based agricultural systems (Stockdale *et al.*, 2001; Biao *et al.*, 2003). International Federation of Organic Agriculture Movements (IFOAM) (2000) has defined organic agriculture as 'a process that develops a viable and sustainable agro ecosystem". Interest in organically produced food is increasing throughout the world in response to concerns about intensive agricultural practices and their potential effect on human health as well as on the environment.

The growth in consumer demand for organically produced food and the standardization of organic farming methods have created a distinguished marketing opportunity for agricultural producers. Organic farming systems differ from

conventional systems in several aspects such as: no artificial pesticides or fertilizers are used on organic farms;organic farms generally have a wider crop rotation scheme, and also have larger areas of non-crop habitats (Seyed*et al.,* 2010). By not using soluble chemical fertilizers and limiting the use of natural biocides in organic farming, it means that it is largely dependent on biological processes for the supply of nutrients and for protection of crops from pests and disease (Gosling *et al.*, 2006).

Organic farming produces safe and nutritious food as it helps prevent soil pollution by stopping risky chemical reactions in the soil and avoiding produce contamination, as well as soil erosion, by wind and rain. Organic farming hurdles the natural biological process of the soil, organic manure use on farms are good for soil health, water quality and the environment and it promotes bio-diversity, biological process and activities of the soil. Hadriman (2004) found that the nutritional value was an important factor that influences consumers' preferences in purchasing chemical free vegetable, followed by desire, freshness, health effect and taste.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

Bauchi State is located on the Northern edge of the Jos Plateau, at an elevation of 616m above sea level (COMPASS, 2009). The State occupies a total land area of 549,260 km² representing about 5.3% of Nigeria's total land mass and is located between latitudes 9° 3' and 12° 3' North of the Equator and longitude 8° 50' and 11° East of the Greenwich meridian (COMPASS, 2009). The State is bordered by seven other states, namely Kano and Jigawa States to the North, Taraba and Plateau States to the South, Gombe and Yobe States to the East and Kaduna State to the West. The State has an estimated population of 4.7 million and is divided into three senatorial zones corresponding to the 3 existing agricultural zones with 20 LGAs (Figure 3.1BSADP Archive, 1986). The State has an estimated cattle population of 1.8 million as at 2015 (MANR, 2015). The cattle population in Bauchi state is predominantly kept under the extensive management system where they roam around to graze for pasture;however, there are few farms where semi intensive management is practiced. Cattle are mostly rearedto provide meat for local consumption and industrial uses

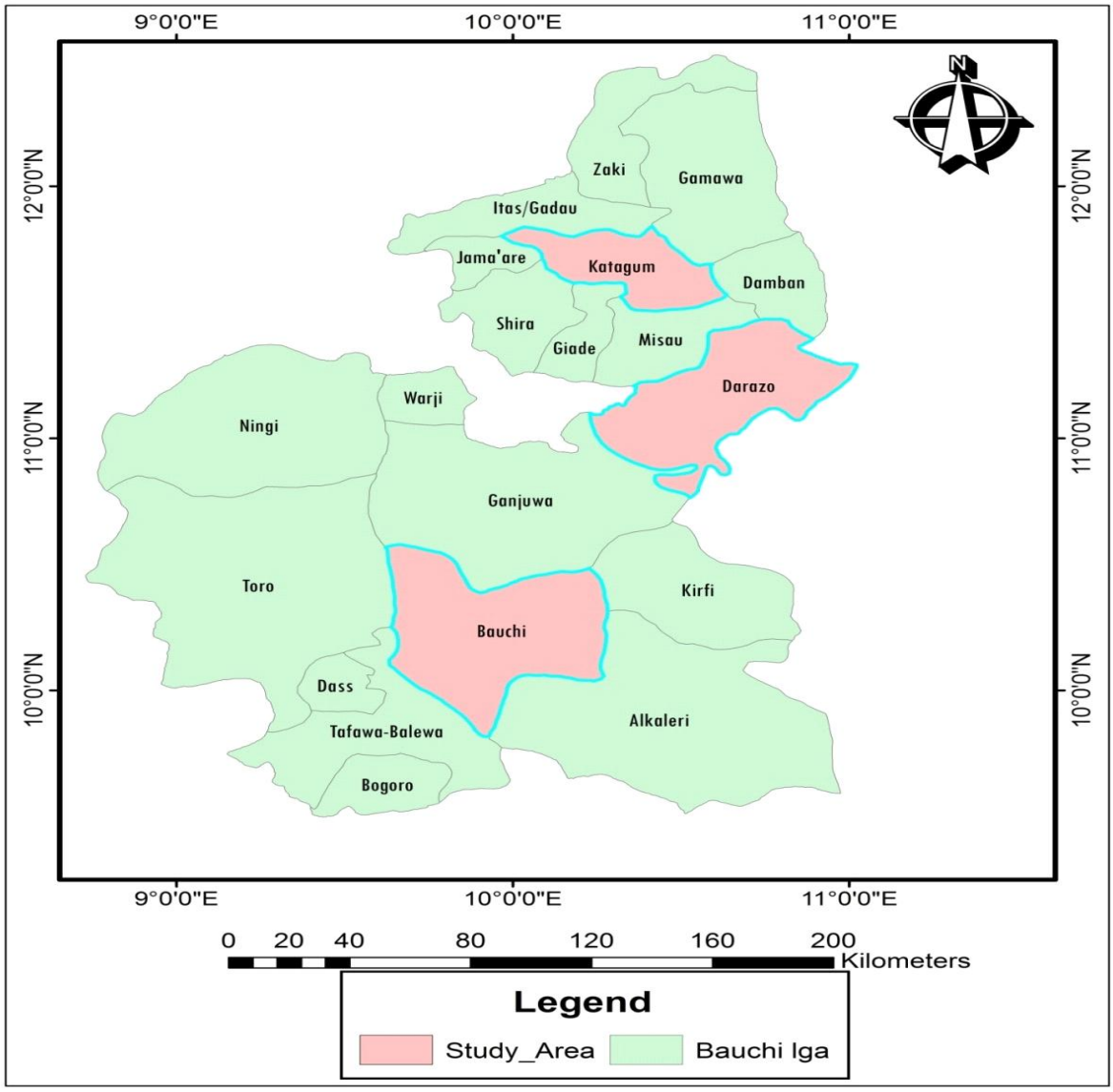


Figure: 3.1 Map of Bauchi State Showing the Study Area.
Source: Modified from Administrative Map of Bauchi state (MANR Archive 2015.)

3.2 Study Design

The study was a descriptive cross-sectional study involving cattle presented to theabattoirs or slaughter slabs for slaughter, cattle population in farms and livestock markets as well as farmers that usedcattle manure on their farm lands in Bauchi state.One LGA was selected from each of the three agro-climaticzones of the State. Being the LGA with the largest cattle population and having the largest slaughter facility was used to represent the zone. Accordingly, Bauchi, Darazo and Katagum LGAs were selected for the study representing the southern, central and northern zones of the state respectively.Three locations were selected for the sampling;an abattoir, cattle farm and livestock market from each of the selected LGA. The abattoir or slaughter slab with highest slaughterfigures in each of the LGA was selected. Similar criteria were used in the selection of the livestock markets and cattle farms from the selected LGAs.

The abattoir or slaughter slab, cattle farms and the livestock markets in each LGA were selected based on number of animals slaughtered or estimated animal population in each of the LGA;one major abattoir/slaughter slab was selected from each of the LGA in accordance with the slaughter figures. Three (3) cattle farms (one farm per district) with more than fifty (50) animals were also consideredin each LGA;and a livestock market was selected from each of the selected LGA based on the number of animals available in the markets. Animals were selected inthe cattle farms and

livestock markets through random sampling without any consideration to age, sex, health and body condition score.

3.3 Determination of number of samples

The sample size was determined using the formula of Thrusfield, (2005)

$$\mathbf{n=(1.96^2 \times P_{exp}(1 - P_{exp})) / d^2;}$$

Where:

n = required sample size;

P_{exp} = expected prevalence

d = desired absolute precision (5%) at a confidence level of 95%.

Thus, for cattle faeces:

Using the above formula and prevalence of 31.2% (Musa et al., 2012) and desired 5% confidence interval of 95%, a total sample size of 330 was obtained for faeces. To increase precision and reduce error, each LGA was assigned 330 sample size to be collected from abattoirs, cattle farms and livestock markets. Thus the sample size was therefore 990 and was shared proportionately in the study area (the abattoirs, cattle farms and livestock markets).

While the sample size (n) for manure was determined through the expected prevalence of *E. coli* (P_{exp}) of 12.2 % from manure samples (Smith *et al*., 2009) and desired absolute precision of 5% at confidence interval of 95%.

The sample size was calculated at 165 which were shared among the sampling areas in the three LGAs (Bauchi (n=63), Darazo (n=42) and Katagum (n=60)).

The sharing of samples was based on the existing cattle population in the selected LGAs.

3.4 The sampling and samples processing

The sampling took place between March and August, 2016 and the period covered isolation and identification of the organisms (*Escherichia coli*) from the samples as well as the phenotypic and genotypic identification of the isolates obtained from the samples. For faecal samples, about two grams (2g) of faecal sample were collected from the rectum of the sampled animals using sterile polythene bags. Cattle of both sexes were sampled and those less than one-year-old or until they are weaned were considered young, while those above one year of age were considered as adults. The collected faecal samples were transported to the laboratory on ice packed cool box soon after collection.

Similarly, samples were collected from heaps of manure at the sampling sites. In doing this, heaps were divided into four parts using a disinfected shovel. Five (5) grams of manure were collected from each of the four heap parts using a sterile spoon and mixed on a sterile tray to form an aggregate sample. Thereafter, two (2) grams of the aggregate sample were then transferred into a labeled sterile plastic screw capped tube, labeled and immediately transported to the laboratory in ice

packed box. Sampling equipment were always cleaned and disinfected in-between sampling so as to prevent microbial transfer between heaps and farms. In addition, a fresh clean pair of latex gloves was worn for each individual sampling. Sample mixing trays were also washed with clean tap water and thereafter rinsed with methylated spirit and allowed to dry immediately before mixing the manure samples.

3.5 Distribution of collected faecal and manure samples

3.5.1 Distribution of cattle faecal samples collected from selected Local government Areas of Bauchi State

A total of 990 faecal samples were collected for the study with equal number of (n=330) samples collected from each of the three Local Government in the study area. However, with regard to locations of sample collection, sampling was such that 247samples were collected from the abattoirs while 412and 331samples were collected from cattle farms and livestock markets respectively (AppendixIIIa). Also the samples were collected proportionately with a variation in the sample size from one sampling site to another due to estimated cattle population in the study area as confirmed via a pilot study previously carried out before the commencement of the study. Manure sample refers to cattle faeces that have been kept for more than 24hr in the abattoirs, cattle farms or livestock markets.

3.5.2 Distribution of cattle manure samples collected from Selected Local Government Areas of Bauchi State

Also a total of 165 cattle manure samples were collected from the three selected LGAs for the study with 63of the samples from Bauchi LGA, 42from Darazo LGA and 60

from Katagum LGA. Out of these samples collected 45were from abattoirs, 55from cattle farms and 65from livestock markets (AppendixIIIb).

3.5.3 Monthly distribution of cattle faecal and manure samples collection

The faecal and manure samples were collected from the study areas from March to August, 2016 and a total of 990 faecal and 165 manure samples were collected from abattoirs, cattle farms and livestock markets (Appendix IV). The period covered isolation and identification of the organisms (*Escherichia coli*) from the samples as well as the phenotypic and genotypic identification of the isolates obtained from the samples.

3.6 Laboratory investigations

3.6.1 Phenotypic identification of*Escherichia coli* (STEC) from faecal and manure samples

3.6.1.1 Isolation of verocytotoxigenic Escherichia coli (STEC)

The procedure was in accordance with the International Standard Organization reference method (ISO,2001) for isolation of *E. coli* and *E. coli* O157 (Giau*et al.*, 2016). Briefly, 10g of the cattle faecal sample was suspended into 90ml of 0.1% of peptone water and then homogenized. Then 10ml of the homogenized sample was inoculated into 90ml of tryptone soy broth and incubated at 37^{o}c for 24hr for enrichment. A loop full of the overnight culture was streaked on Eosin Methylene Blue (Oxoid, U.K) and further incubated at 37^{o}c for 24hrs for the detection of *E. coli.* A greenish metallic sheen colony from the incubated enriched culture suspected to be

E. coli was streaked onsorbitol MacConkey Agar incorporated with cefexime tellurite selective supplement (Oxoid, U.K.) for the detection of *E. coli* O157.A secondary culture was then prepared from the well- separated colonies from the above culture and further inoculated on nutrient agar (Oxoid, U.K.) slants and incubated at 37°C and then stored at 4^{o}C for further identification.

3.6.1.2Biochemical tests

Colonies grown on nutrient agar slants were subjected to biochemical tests that included Indole, Methyl red, VogesProskauer, Citrate (IMViC), Motility and Triple sugar iron (TSI) as described by Konema*et al.* (2008), Cheesbrough, (2000) and Quinn *et. al.*(2002) for identification of *E. coli*. Isolates that yielded test results in these biochemical consistent with those of *E. coli*were further characterized biochemically using Microbact 12E (MB1130A+), according to the manufacturer's (Oxoid, U.K.) instruction.

3.6.1.3 Characteristics of Escherichia coli on conventional and Microbact Biochemical tests

Isolates that were positive for indole, motility and methyl red as well as citrate andVogesProskauernegative were considered as *E. coli* for conventional biochemical test. With regard to the Microbact test the reactions were evaluated as positive or negative by comparing them with a colour chart, for *E. coli*after24 hours incubationyellow indicate negative while green-bluepositive and the results were recorded under the appropriate heading on the report form.An octal coding system was

adopted for Microbact 12E (MB1130A+). Each group of three reactions produces a single digit of the code. Using the results obtained, the indices of the positive reactions are circledwhere the sum of the indices in each group of three reactions forms the code number and the code is entered into the computer package for further interpretation (Balows *et al*., 1991).

3 .6.1.4Serological test on Escherichia coli isolates

Isolates that were presumptively identified by biochemical tests were further tested by slide agglutination using *E. coli* O157:H7 diagnostic kit from Wellcolex (U.K.) to identify the serogroups using test procedure as described by the kits manufacturers (ThermoScientific^{TM} Remel^{TM} R30959501).Each antiserum was diluted using the manufacturer's recommendation. Slide agglutination tests were then used to identify each serotype and positive result was recorded when small minute granules appeared.

3.6.2 Determination of antimicrobial susceptibility of *Escherichia coli* isolates against commonantimicrobial agents and MAR indices

Antibiotic susceptibility tests were carried out on fifty the (50) isolates (positive via serological tests for *E. coli*) to determine their antibiotic resistance profiles using the disk diffusion method developed by Kirby–Bauer (1966) and standardized by the World Health organization as modified by Sozman*et al*. (2011). Twelve (12) commonly used commercially available antimicrobial disks impregnated with Imipenem (10µg), tetracycline (30µg), Sulfamethoxazole (23.75µg), ciprofloxacin (5µg), chloramphenicol (30µg), Amoxicillin / clavulanate (10+20µg), Ticarcillin

clavulanate (75+10µg), Gentamicin (10µg), Erythromycin (15µg), Enrofloxacin (5µg), Ceftriaxone (30µg) and Cefazolin (30µg) obtained from Oxoid (UK)were used.

The antibiotic susceptibility test procedure consisted of inoculating the bacteria in tryptone soy broth and incubated at 37^{o}C for 24hr. The turbidity of the broth was adjusted to 0.5 McFarland standard and a sterile swab stick was dipped into the overnight culture broth and excess moisture was expressed by pressing the swab against the side of the tube.The surfaces of the Mueller Hinton agar were swabbed completely and then turned at 90^{o} and the swabbing process was repeated until the entire circumference of the plate was covered. The media were allowed to dry for about 5 min before placing the antibiotic discs using antibiotic dispenser (Oxoid). Then each disc was lightly touched with a sterile inoculating loop to make sure it was in good contact with the agar surface, and then incubated upside down at 37^{o}C overnight. After incubation the culture with the anti-microbial disks was interpreted by using a transparent plastic metric ruler across the zone of inhibition (Z I), at the widest diameter to measure from edges of the zones in millimeters. A chart was used to report results on a table as sensitive, intermediate or resistant according to CLSI, (2014). Isolates resistant to tetracycline were further screened for the presence of *tet* resistance genes.

3.6.3 Detection of antibiotic resistance genes in *Escherichia coli* O157:H7

Of the fifty (50) positive isolates,twenty five (25) thatwere resistant to tetracycline were further subjected to the multiplex PCR for detection of antibiotic resistance genes.

3.6.3.1DNA Extraction

A loopful of each of the bacterial isolates (*E. coli* O157:H7) was suspended in two hundred microliter (200µl) of DNase free water in a microphage tube. The suspension was heated in a water bath at 96^{o}C for 5min and centrifuged at 15000g for 2min. The pellets were discarded while the supernatant containing the DNA was used for polymerase chain reaction (PCR) (Jothikumar and Griffiths, 2002).

3.6.3.2 Polymerase Chain Reaction for Detection of tetA, tetB, tetC, tetD and tetK Genes

A multiplex polymerase chain reaction (PCR) was carried out for the detection of the antibiotic resistance genes using TopTaq™ Master Mix PCR kit (Qiagen®). A 25µl reaction mixture contained 12.5µl TopTaq Master Mix 2x, 7.5µl DNase-free water, 2.5µl of 200ng DNA template and 0.25µM of primer cocktail (Table 3.1) was used for amplifying the 210bp, 659bp, 418bp, 787bp and 169bp for *tet*A, *tet*B, *tet*C, *tet*D and *tet*Kgenesrespectively as described earlier (Roberts, 1996; Levy *et al*., 1999; Chopra and Roberts 2011). The reaction began with an initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 53°C for 1 min and 72°C for 1 min. The final extension step was performed at 72°C for 7 min. Sterile DNase-free water and a confirmed *E. coli* O157:H7 isolate were used as negative and positive controls respectively.The PCR result is interpreted by comparing the amplified segments on gel

to the various known nucleotide sequences that resemble the "ladder" according to their electrical charge and molecular size termed gel electrophoresis.

Table 3.1 Sequences of oligonucleotide primers used for PCR (Paton and Paton, 1998; Levy *et al*., 1999; Chopra and Roberts (2011))

Primer	Sequence (5' to 3')	Expected Amplicon Size(bp)
*tet*A-F	GCT ACA TCC TGC TTG CCT TC	
*tet*A-R	CAT AGA TCG CCG TGA AGA GG	210
*tet*B-F	CAG TGC TGT TGTTGT CAT TAA	
*tet*B-R	GCT TGG ATT ACT GAG TGT AA	659
*tet*C-F	CTT GAG AGC CTT CAA CCC AG	
*tet*C-R	ATG GTC GTCATC TAC CTG CC	418
*tet*D-F	AAA CCA TTA CGG CAT CCA GC	
*tet*D-R	GAC CGG ATA CAC CAT CCA TC	787
*tet*K-F	TCG ATA GGA ACA GCA GTA	
tetK-R	CAG CAG ATC CTA CTC CTT	169
stx1F	ATA AAT CGC CAT TCG TTG ACT AC	
stx1R	AGA ACG CCC ACT GAG ATC ATC	180
stx2F	GGC ACT GTC TGA AAC TGC TCC	
stx2R	TCG CCA GTT ATC TGA CAT TCT	255

eaeAF	GAC CCG GCA CAA GCA TAA GC	
eaeAR	CCA CCT GCA GCA ACA AGA GG	384

3.6.4 Detection of virulence genes in *Escherichia coli* O157:H7

Of the fifty (50) positive isolates twenty-five (25) were subjected to the multiplex PCR for detection of virulence and resistance genes, the remaining isolates were lost during storage.

3.6.4.1 Polymerase Chain Reaction for the Detection of Virulence Genes (stx1, stx2 and eaeA)

A multiplex polymerase chain reaction (PCR) was carried out using TopTaq™ Master Mix PCR kit (Qiagen®) where a 25µl reaction mixture containing 12.5µl TopTaq Master Mix 2x, 7.5µl RNase-free water, 2.5µl of 200ng DNA template and 0.25µM of six-primer cocktail (Table 3.5) was used for amplifying the 180bp, 255bp and 384bp for *Stx*1, *Stx*2 and *eae*A genesrespectively as described by Paton and Paton, (1998). DNA Samples extracted from the isolates were subjected to 35 PCR cycles, each consisting of 1 min of denaturation at 95°C; 2 min of annealing at 65°C for the first 10

cycles, decrementing to 60°C by cycle 15; and 1.5 min of elongation at 72°C, incrementing to 2.5 min from cycles 25 to 35. Sterile DNase-free water and a confirmed *E. coli* O157:H7 isolate were used as negative and positive controls respectively.

3.6.4.2 Reading and interpretation of PCR results

A 1.5% agarose gel was prepared by suspending 1.5 grams of agarose powder in 100ml of 1X Tris-Borate-EDTA (TBE) buffer and heated on a hot plate until completely dissolved. Ethidium bromide (2.5µl/100ml), was added into the liquid agarose before pouring the contents into a gel caster set and allowed to solidify at room temperature. The caster was rightly placed into an electrophoresis tank and flooded with 1XTBE buffer to the maximum level before carefully removing the comb. The PCR products (5µl each + gel loading dye) were then loaded into the gel wells using a 10µl Eppendorf pipette. Four microliters (4µl) of 100bp ladder (BioLabs Inc. New England) mixed with 2µl Gel Loading Dye Blue 6X (BioLabs Inc. New England) were also loaded into one of the wells before connecting the tank to a power pack and plugged to the mains supply. The products were electrophoresed at 85volts for 50min. Immediately after electrophoresis, the agarose gel was viewed using a Gel Doc™ XR+ (BioRad). The gel image was captured and labeled accordingly.

3.7 Questionnaire administration

For the evaluation of practices and pattern of cattle manure use, three hundred (300) structured questionnaires were used, with one hundred (100) being administered to

respondents in each LGA as earlier stated (cattle owners, abattoir managers and crop farmers using manure on their farm land). The questionnaires (Appendix I) were designed to access information such as type of crops planted on their farm, types of manure used, frequency and mode of application of the manure, means of conveying the manure to their farms and health related problems observed in relation to the use of cattle manure. Other information such as reasons for using cattle manure, composting of manure before application and the benefits of using cattle manure were also determined as well as the management practices for the cattle farms.

The respondents in each of the three (3) LGAs were categorized into two (2) groups as those whose animals or animals under their control produce the manure (producers) and those considered as end users of the manure (who use the manure on their farm lands). Fifty respondents in each category were identified and given the questionnaires making a total of one hundred (100) questionnaires administered in each LGA.

3.8 Data analyses

Data generated were presented as simple descriptive statistics in forms of tables and charts.The results obtained were inserted into Microsoft excel and analysedusing chi-square (χ^2) and Fisher exact tests toshow association between variables such asoccurrence of *E. coli* in cattle faeces and manure at p-value < 0.05.

CHAPTER FOUR

4.0 RESULTS

4.1 Phenotypic identification of *Escherichia coli* from faecal and manure samples

Of the total 990 of cattle faeces and 165 manure samples examined, 314(31.7%) and 7(4.24%) faecal and manure samples were found positive for *E. coli* respectively (Table 4.1). And of the *E. coli* positive samples from faeces by LGAs 135(13.6%) was the highest and obtained from Bauchi LGA followed by Katagum with 130(13.1%) and the least from Darazo 49(4.9%) (Table 4.2)

Also, of the *E. coli* positive manure samples (7); 1(06%) and 6(3.64%) were from Bauchi and Katagum LGAs respectively (Table 4.1)

4.1.2 Distribution of *Escherichia coli*positive from faecal samples by sex

Out of the990 faecal samples collected247were from theabattoirs, 412 from the cattle farms and 331 from the livestock markets in the study area, of which 655(66.1%)were collected from male cattle and 335(33.9%) from females. Also out of the 655 samples from male, 219(22.9%) were positive for *E. coli* of which 34 were from the abattoirs, 60 from cattle farms and 125 from livestock markets (Table 4.2); and of the 334 samples from female cattle, 95(9.6%) were positive and made up of 17 from abattoirs, 47 from cattle farms and 31 from livestock markets (Table 4.2). The distribution of *E. coli* between male and female cattle was found to be highly significant (χ^2; p=0.008, p <0.05).

4.1.3 Distribution of *Escherichia coli* positive from faecal samples by age

on the basis of the age animals, out of the 990 faecal samples collected from the abattoirs, cattle farms and livestock markets, 119 were from young animals, while 871 were from adult animals. Out of the 871 samples from adult cattle; 271 (27.4%) were positive for *E. coli* of which 51 were from abattoirs, and 86 from cattle farms while 134 were from the livestock markets (Table 4.2). Of the 119 samples from young cattle 43(4.43%) were positive for *E. coli*, out of which 21 were from the cattle farms and 22 from livestock markets with no positive from the abattoir. (Table 4.2) The distribution of *E. coli* between young and adult cattle was found to be highly significant (χ^2; p=0.008).

Of the 314 samples positive for *E. coli*; abattoirs had 51(16.2%) cattle farms had 107(34.9%) while livestock markets had 156(49.6%) (Table 4.3).

Table 4.1*E. coli* presumptive isolates from faecal and manure samplesbased on sampling sites

Sampling sites	Faeces		Manure	
	Sample size	No. positive(%)	Sample size	No. positive(%)
Abattoir	247	51(5.2)	45	1(0.6)

Cattle farms	412	107(10.8)	55	0(0.0)
Livestock markets	331	156(15.7)	65	6(3.64)
Total	**990**	**314(31.7)**	**165**	**7(4.24)**

Table 4.2: Occurrence of *E. coli* in faeces of male and female cattle from Bauchi, Darazo and Katagum LGA of Bauchi State, Nigeria

LGA	No of samples collected	Abattoir		Cattle farms		Livestock Markets		Total positive
		Male No (%)	Female No (%)	Male No (%)	Female No (%)	Male No (%)	Female No (%)	
Bauchi	330	25(11.4)	8(8.42)	16(7.3)	26(27.4)	45(20.5)	15(15.8)	135(13.6)
Darazo	330	9(4.1)	8(8.42)	9(4.1)	15(15.8)	6(2.74)	2(2.10)	49(4.95)
Katagum	330	0(0)	1(1.05)	35(15.9)	6(6.32)	74(33.8)	14(14.7)	130(13.1)
Total	**990**	***34(15.5)**	****17(17.9)**	***60(29.4)**	****47(49.5)**	***125(57.1)**	****31(32.6)**	**314(31.7)**

Significant at P<0.05 ***(Male = *219(22.1%); Female = **95(9.6%))***

Table 4.3: Occurrence of *E. coli* in faeces of young and adult cattle from Bauchi, Darazo and Katagum LGA of Bauchi State, Nigeria

LGA	No of samples collected	Abattoir		Cattle farms		Livestock Markets		Total
		Young	Adult	Young	Adult	Young	Adult	
		No positive (%)	No positive (%)	No positive (%)	No positive (%)	No positive (%)	No positive (%)	No positive (%)
Bauchi	330	0(0)	33(3.33)	9(0.9)	33(3.33)	0(0)	60(6.06)	135(13.6)
Darazo	330	0(0)	17(1.72)	6(0.61)	18(1.82)	1(0.1)	7(0.71)	49(4.95)
Katagum	330	0(0)	1(0.1)	6(0.61)	35(3.54)	21(2.12)	67(6.77)	130(13.1)
Total	**990**	***0(0)**	****51(5.15)**	***21(2.12)**	****86(8.69)**	***22(2.22)**	****134(13.5)**	**314(31.7)**

Significant at P≤0.05 ***(Young = *43 (4.43%); Adult = **271 (27.4%))***

4.1.4 Biochemical test of *Escherichia coli* isolates from faecal samples

Out of the 990 faecal samples collected, 314 showed greenish metallic sheen on EMB and were further subjected to conventional biochemical tests (IMViC) where 75(24.0%) were positive for the tests, and were subjected to Microbact 12E biochemical test.

4.1.5 Biochemical tests using the Microbact 12E test kit for faecal samples

The 75*E. coli*isolates positive by biochemical tests, were further tested using Microbact 12E biochemical test kit,where 48(64%)were confirmed as*E. coli*,while 27(36%) were identified as other gram negative microorganisms such as *Klebsiella*2(2.67%), *Citrobacter*15(20%), and *Serratia*10(13.3%).

4.1.6 Results of agglutination test for *Escherichia coli* isolates from faecal samples

Out of the 48 isolates confirmed to be *E. coli* using Microbact 12E test kit, werefurther tested by agglutination using the *E. coli* O157 test kit, 28(58.3%) were shown to be*E. coli* O157while 20(41.7%) were *E. coli*non - O157.

4.1.7 Biochemical test of isolated *Escherichia coli* from manure samples

Out of the 165 manure samples collected, 7(4.24%) showed greenish metallic sheen on EMB and were further subjected to conventional biochemical screening tests (IMViC) in which5(3.03%) were positive for the tests, and were subjected to Microbact 12E biochemical testing.

4.1.8 Biochemical test using the Microbact 12E test kit for cattle manure

The 5(3.03%) isolates identified as *E. coli*based on the biochemical screening tests were tested using the Microbact 12E biochemical test kit which lead to confirmation of 2(1.21%) as *E. coli*, while 3(1.82%) were identified as other gram negative microorganisms (*Klebsiella*, *Citrobacter*, and *Serratia.*)

4.1.9 Serological testing of *Escherichia coli* isolated from cattle manure

The 2 isolates positive for biochemical test(Microbact 12E) were further confirmed as *E. coli* O157 using the *E. coli* O157 agglutination test kit.

4.2 Results of antimicrobial sensitivity testing of *Escherichia coli* isolates

4.2.1 Results of antimicrobial resistance of isolates from cattle faeces and manure

The 50 confirmed*E. coli*isolates which were subjected to antibiotic susceptibility against 12 commercially available(commonly used) antimicrobial agents showed highest frequency of resistance to Cephazolin by 44(88%) of the isolates, followed by Sulfamethoxazole 42(84%) isolates showing resistance and Enrofloxacin showing 40(80%) isolates showing resistance. Highest susceptibility of the isolates to the antimicrobial agents was against chloramphenicol 40(80%) followed by gentamicin and Imipenem 35(70%)and thenCeftriaxone to which 30(60%)of the isolates were susceptible (Table 4.4).

Table 4.4: Antimicrobial susceptibility patterns of *E. coli* isolates from cattle faeces and manure to 12 antibiotics in Bauchi state, Nigeria (N=50)

Antibiotic tested	Antimicrobial sensitivity pattern		
	No. (%) Susceptible	**No. (%) Intermediate**	**No. (%) Resistant**
Amoxicillin clavulanate	16 (32)	13 (26.0)	21 (42)
Cefazolin	0 (0.0)	6 (12.0)	44 (88)
Ceftriaxone	30 (60)	7 (14.0)	13 (26)
Chloramphenicol	40 (80)	6 (12.0)	4 (8)
Ciprofloxacin	23 (46)	6 (12.0)	21 (42)
Enrofloxacin	0 (0.0)	10 (20.0)	40 (80)
Erythromycin	0 (0.0)	15 (30.0)	35 (70)
Gentamicin	35 (70)	5 (10.0)	10 (20)
Imipenem	35 (70)	7 (14.0)	8 (16)
Tetracycline	23 (46)	3 (6.0)	24 (48)
Ticarcillin clavulanate	23 (46)	13 (26.0)	14 (28)
Sulfamethoxazole	0 (0.0)	8 (16.0)	42 (84)

4.2.2 Occurrence multidrug resistance profile and Multiple Antibiotic Resistance(MAR) indices amongst *Escherichia coli* isolates from faeces and manure

The multi-drug resistance profiles of isolates were determined for this study. All the isolates were resistant to three or more antibacterial agents (Tables 4.5). As shown in the table,49 (98%) out of the 50 isolates exhibited multi-drug resistance.The *E. coli* O157:H7 strains showed6% (3/50) resistance to three antimicrobials, as well asa 30% (15/50) resistance to five other antimicrobials,and the isolates [6% (3/50)] also showed a resistance to eight antimicrobials tested.It was also observed that only 6% (3/50) of the isolates were from manure samples, and each of the three were resistant to six, seven and eight antimicrobials respectively, all the 94% (47/50) of the isolates that showed resistance to antibacterial agents tested were from cattle faecal samples.

The proportion of isolates with MAR index greater than 0.2 was 98% (49) while those with MAR index of less than 0.2 was 2%.

Table 4.5: Multidrug resistance profile and Multiple Antibiotic Resistance (MAR) indices of *E. coli* isolates from Faeces and Manure in selected Local Government Areas of Bauchi State, Nigeria

No. of Antibiotics	Antibiotic profile	No. of Isolate(s) (%)	MAR Index
2	ENR, KZ,	1(2)	0.16
3	TE, SXT, KZ SXT, E, CIP SXT, ENR, TIM	3(6)	0.25
4	SXT, E, ENR, TIM SXT, E, ENR, TIM **TE, SXT, ENR, KZ** **TE, SXT, ENR, KZ** **TE, SXT, ENR, KZ** SXT, E, CRO, KZ, SXT, E, KZ, TIM TE, ENR, KZ, AMC SXT, ENR, CRO, KZ IPM, E, CIP, KZ,	10(20)	0.33
5	TE, SXT, E, ENR, KZ SXT, E, CIP, CN, KZ, TE, E, CIP, ENR, KZ, SXT, E, ENR, KZ, AMC TE, SXT, E, ENR, KZ **TE, SXT, E, ENR, KZ** **TE, SXT, E, ENR, KZ** **TE, SXT, E, ENR, KZ** SXT, E, CIP, ENR, KZ E, ENR, CRO, KZ, AMC SXT, E, CRO, KZ, AMC C, SXT, ENR, KZ, AMC SXT, E, CRO, KZ, AMC TE, SXT, E, CN, KZ SXT, E, ENR, CN, KZ	15(30)	0.41

Table 4.5 (continuation)

No. of Antibiotics	Antibiotic profile	No. of Isolate(s) (%)	MAR Index
6	SXT, CIP, ENR, CN, KZ, TIM	11(22)	0.50
	IPM, SXT, ENR, CN, KZ, TIM		
	IPM, E, CRO, KZ, AMC, TIM		
	TE, SXT, ENR, CRO, KZ, TIM		
	TE, SXT, E, ENR, KZ, TIM		
	TE, SXT, E, ENR, KZ, TIM		
	TE, SXT, E, ENR, KZ, AMC		
	IPM, SXT, E, ENR, KZ, AMC		
	TE, SXT, ENR, CN, KZ, AMC		
	TE, SXT, E, ENR, CRO, KZ,		
	SXT, CIP, ENR, KZ, AMC, TIM		
7	TE, E, ENR, CRO, CN, KZ, AMC	7(14)	0.58
	TE, C, E, ENR, CRO, KZ, AMC		
	SXT, E, CIP, ENR, CRO, CN, KZ,		
	TE, SXT, E, ENR, CN, KZ, AMC		
	TE, SXT, E, CIP, CRO, KZ, AMC		
	TE, SXT, CIP, ENR, KZ, AMC, TIM		
	IPM, TE, E, CIP, ENR, CN, AMC		
8	IPM, TE, SXT, E, CIP, ENR, KZ, AMC	3(6)	0.66
	TE, C, SXT, E, CIP, ENR, KZ, AMC		
	IPM, TE, C, SXT, ENR, CRO, KZ, TIM		
	Total	**50(100)**	

4.3 Detection and distributions of the antibiotic resistance genes of *Escherichia coli* isolates

4.3.1 Detection of resistance genes (*tet* genes)

Of the 25 *E. coli* isolates that were subjected to multiplex PCR for the detection of antibiotic resistance genes, nine 9(36.0%) of the isolates were shown to harbour*tet*A (210bp) and *tet*K (169bp) genes (Plate 1).Also, only two 2(8.0%) isolates were positive for*tet*B gene (659bp).

4.3.2 Distribution of resistance genes based on sample location

Nine (9) of the *E. coli* isolates were found to harbour resistance genes, with eight (8) harbouring*tetA* and *tetK*, and two (2) having only *tetB.* No resistance genes were detected from isolates obtained from the abattoirs, while up to four (4) different *tet* genesand five (5) different *tet*resistance genes were detected from livestock markets and cattle farms respectively.

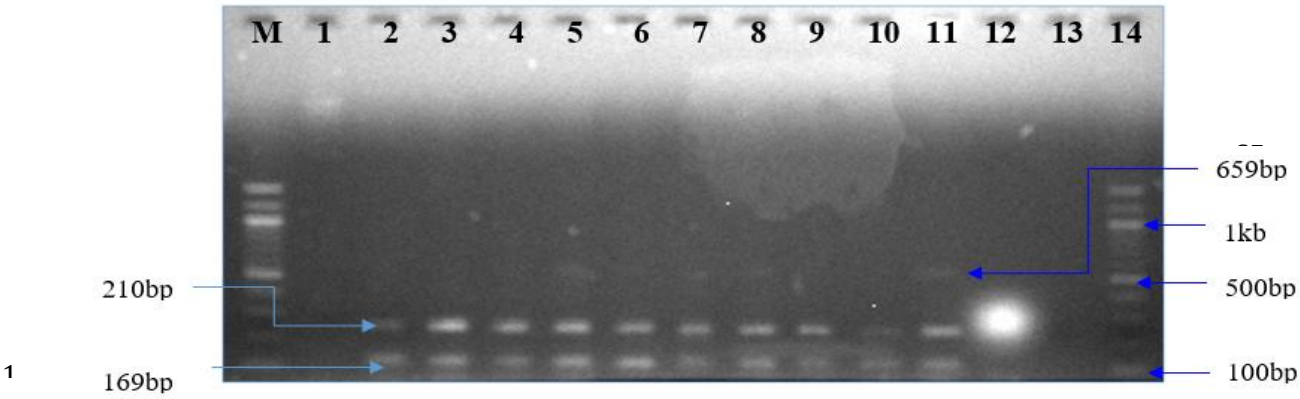


Plate I**:** Amplification of tetracycline resistance genes (*tet*A, B,C,D and K) in eleven *E. coli* isolates from the study area.
(Lane M: 1kb molecular marker, lane 1: positive control, lanes 2 to 12 [ALMA80, L_3FA57, CAMA83, CAMA84, CAMA17, CAMA71, CAFA20, ALAM19 and ALAM69]: *E. coli* isolates, lane 13: negative control, lane 14: 1kb molecular marker)

4.3.3 Detection and distribution of resistance genes amongst serotypes of*E. coli*isolates

Theresistance gene detection based on *E. coli*serotypesshowed 12(48.0%) of the total resistance genes detected were from *E. coli*O157 while 8(32.0%) were from *E. coli*non - O157 serotype. The results further indicated that 5(20.0%) of the genes detected were *tetA* and tetK from *E. coli*O157 serotype while 4(16.0%) of the genes were *tetA* and *tetK* from *E. coli*non - O157. And that only 2(8%) *tetB* were detected from *E. coli*non - O157 serotype (Table 4.6).

Table 4.6: Distribution of resistance genes according to*E. coli*strains (n=25)

E. coli strains	Number of isolates with Resistance Genes (%)			**Total**
	tetA	*tetB*	*tetK*	
Non O157	4(16)	0(0)	4(16)	**8(32)**
O157:H7	5(20)	2(8)	5(20)	**12(48)**
Total	**9(36)**	**2(8)**	**9(36)**	**20(80)**

Significant at P<0.05

4.5 Detection and distribution of virulence genes from the *Escherichia coli* isolates

4.5.1 Detection of *E. coli* virulence genes

It was observed that out of the 25 isolates subjected to multiplex PCR (mPCR), 18(72%) showed the presence of *stx1* gene, while 5(20%) showed a combination of *stx1* and *eaeA* genes. Also, 1(4.0%) of the isolates were positive for*stx1* and *stx2*(Plate 2).

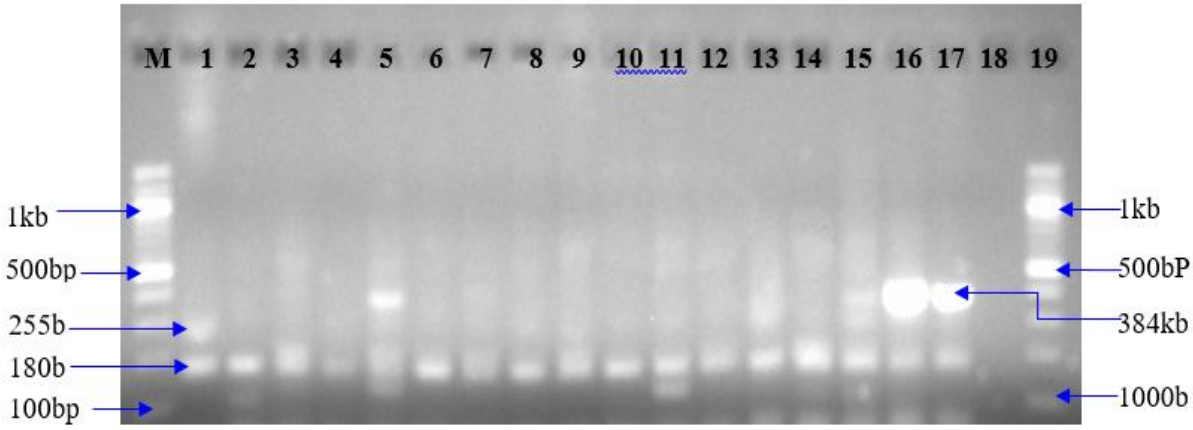


Plate 2: Multiplex PCR detection of *stx*1, *stx*2 and *eaeA* genesin 16 *Escherichia coli* O157:H7 isolates of cattle faeces from Bauchi State.
(Lane M: 1kb molecular marker, lane 1 to16 [ALMA69, CAMA60, ALAF181, CAFA25, ALAM119, ALFA173, CAMA73, ALFA192, ALAM73, AAF4, CAYM34, ALAM42, CAMA40, CAMA75, CAMA47 and ALAM65]: *E. coli* isolates, lane 17: positive control, lane 18: negative control, lane 19: 1kb molecular marker)

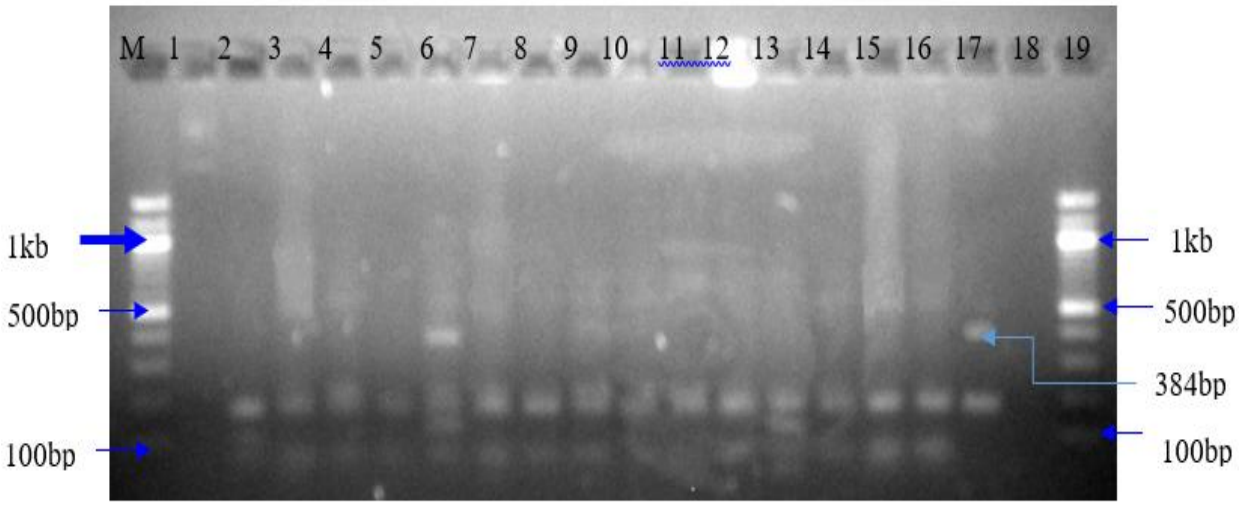


Plate 3: Multiplex PCR detection of *stx*1, *stx*2 and *eaeA* genesin 16 *E. coli* O157:H7 isolates of cattle faeces from Bauchi State
(Lane M: 1kb molecular marker, lane 1to 16: [ALMA69, CAMA60, ALAF181, CAFA25, ALAM119, ALFA173, CAMA73, ALFA192, ALAM73, AAF4, CAYM34, ALAM42, CAMA40, CAMA75, CAMA47 and ALAM65] *E. coli* isolates, lane 17: positive control, lane 18: negative control, lane 19: 1kb molecular marker)

4.5.2 Detection of virulence genes in isolates from various locations

From the study, virulence genes were harboured by bacterial isolates from cattle faeces and manure obtained from variouslocationsof selected LGAs of Bauchi state, there was only one virulence gene (*stx1*) detected from livestock market in Bauchi LGA, no detection was made from isolates that originated from the abattoir and cattle farms. Isolates from cattle faecal samples of cattle farms origin in Darazo LGA were found to harbour virulence genes whileno detection of geneswas made from isolates ofabattoir and livestock markets origin. Isolates fromKatagum LGA were found to harbour 11 *stx1* virulence genes in faecal samples from cattle farms as well as 10 *stx1*from isolates from livestock markets. Furthermore, bacterial isolates from livestock market of Katagum LGA were found to harbour one *stx2* genesand 3 *eaeA* virulence gene.

4.5.3 Detection of virulence genes according to strain of *E. coli*O157:H7

Thestudy revealed 25of the *E. coli* isolates harbouring three (3)virulence genes (*stx1, stx2 and eaeA*), out of which 17/25(68%) were *E. coli* O157 strains while 6/25(24%) were non - O157 *E. coli*. Moreover,*stx1* was detected from 17/19(89%) *E. coli* O157:H7 and 3/6(50%) from non – O157:H7, while *stx2* was detected in only 1 non – O157 *E. coli*but not detected from any of the O157:H7 isolates. *eaeA* gene was detected from two (2) *E. coli* O157 and the non – O157 each(Table 4.7).

Table 4.7: Distribution of virulence genes according to *E. coli*strain(n=25)

E. coli strain	Number of isolates with virulence genes (%)			Total
	stx1	*stx2*	*eaeA*	
O157:H7	17(68)	0(0)	2(8)	19(76)
Non O157	3(12)	1(4)	2(8)	6(24)
Total	20(80)	1(4)	4(16)	25(100%)

Significant at P<0.05

4.6 Co-occurrence of antimicrobial resistance and virulence genes in *Escherichia coli* isolates

The result showed nine (9) *E. coli* isolates were found toharbour *stx1*gene, a combination of *tetA* and *tetB* in six (6) in isolates, while *tetB* and *tetK* only in one isolate each and a combination of *tetA*, *tetB* and *tetK* in one isolate. Whereas three (3) of the isolates that are harbouring the genes are from the livestock market, while six (6) are from isolates obtained from cattle farms and none from the abattoir (Table 4.8).

4.7 Comparative occurrences of *E. coli* O157:H7 and gene detectionin isolates from cattle faeces and manure samples from Bauchi state

The result of the study showed higher occurrence of *E. coli* O157 in isolates offaecal samples 48(4.84%) than in isolates obtainedfrom manure samples 2(1.21). The result further indicated all the25(100%) *E. coli* isolates from faecal samples harbouring the virulence genes,while 20/25 (80%) of the *E. coli* isolates were found to harbour the antimicrobial resistance genes (*tet*A, *tet*B and *tet*K) (Table 4.9).

Table 4.8:Co-occurrence of antimicrobial resistance and of virulence genes amongst *E. coli* isolates

Isolate No	Source of Isolates	Sample* I.D	Resistance genes
1	ALM	ALMA80	*tetA, tetK*
2	ACF	CAFA20	*tetA, tetK*
3	ACF	CAMA17	*tetK*
4	ACF	CAMA83	*tetA, tetB, tetK*
5	BCF	L_3FA57	*tetA, tetK*
6	ACF	CAMA71	*tetA, tetK*
7	ALM	ALAM69	*tetB*
8	ALM	ALAM19	*tetA, tetK*
9	ACF	CAMA84	*tetA, tetK*

Key: *=all isolates were positive for stx1, ACF=Azare cattle farm, ALM= Azare livestock market, BCF=Bauchi cattle farm

Table 4.9 Comparative occurrences of *E. coli* O157 and gene detection in isolates from cattle faeces and manure samples from Bauchi state N=25

		Faecal samples No (%)	Manure No (%)
Total o of samples collected		990	165
No. presumptive positive		314(31.7)	7(4.24)
No.positive for*E. coli* O157		48(15.3)	2(28.6)
No. of isolates harbouring Antimicrobial resistance genes			
	tetA	9(36)	0(0)
	tetB	2(8)	0(0)
	tetK	9(36)	0(0)
No of isolates harbouring virulence genes			
	stx1	20(80)	0(0)
	stx2	1(4)	0(0)
	eaeA	4(16)	0(0)

4.8 Socio-Economic factors and the use of manure

4.8.1 Socio-Demographic Information of Respondents (Manure Users)

The results showed respondents less than 40 years of age 79(50.8%) were predominant in the study, while respondents who indicated farming as their primary occupation were also the majority 90(58.1%). It was also observed that respondents with basic education 98(63.2%) were the majority, while those who had not acquired any form of educational qualification were the least 9(5.8%). (Table 4.10).

Table 4.10: Socio-Demographic Characteristics of farmers who used Manure (Respondents)(n=155)

Factor		Frequency (%)
Age	Less than 40	79(50.8)
	40 – 59	60(38.7)
	60 and above	16(10.3)
Occupation	Farming	90(58.1)
	Farming / other occupation	21(13.5)
	Other occupations	13(8.4)
	Livestock rearing and other occupation	31(20.0)
Education Status	Non formal	24(0.6)
	Basic education	98(63.2)
	Higher education	24(15.5)
	No formal education	9(5.8)

4.8.2 Socio-Demographic Information of Respondents (Manure Producers)

The results showed that majority of respondents 149(97.4%) whosetheir animals (or animals under their custody) producethe manurewere male with only 4(2.6%)beingfemale.Furthermore, respondents less than 40 years of age 97(63.4%) were predominant compared to all the other respondents'age groups. It was also observed that respondents who had farming as their primary occupation 54(35.3%) were majority, while those whose occupation isanimal rearing and business were the least 8(5.2%) (Table 4.11).

Most respondents 90(58.8%) have acquired basic education, also observed that majority of the respondents 105(68.6%) were aware that sick animals shed pathogens in their faeces,while the majority of respondents 117(76.5%) indicated that they isolate sick animals from their herds (Table 4.11).

The results also further indicated a large number of respondents 151(98.7%) who produce manure and use it on their farm lands as against few 2(1.3%) who sold the manure to prospective buyers. The majority of respondents 97(63.4%) did not have any conventional storage facility for the manure but rather dump the manure at their backyards(Table 4.11).

Table 4.11: Socio-Demographic Information of Respondents **(Manure Producers)**

Factor	**Response**	**Frequency (%)**	***N=153**
Sex	Female	4(2.6)	
	Male	149(97.4)	
Age	less than 40 years	97(63.4)	
	40 – 59 years	47(30.8)	
	60 years and above	9(5.9)	
Occupation	Other occupation	23(7.3)	
	Farming	41(26.8)	
	Farming/ other occupation	54(35.3)	
	Rearing	27(17.6)	
	Rearing / Business	8(5.2)	
Education status	Non formal education	32(20.9)	
	Basic education	90(58.8)	
	Higher education	24(15.7)	
	None	7(4.6)	
Awareness of sick animals shedding pathogens	Yes	105(68.6)	
	No	48(31.4)	
Isolation of sick animals	Yes	117(76.5)	
	No	36(23.5)	
Separation of sick animals	Yes	59(38.6)	
	No	94(61.4)	
Manure use	Sold to buyers	2(1.3)	
	Used on farms	151(98.7)	
Storage facility	Dump in heaps at backyards	97(63.4)	
	Freely disposed in open space	38(24.8)	
	Packed in sacks	18(11.8)	

N=Number of respondents

4.8.3 Sources of manure

From the study, majority of respondents 90(58.0%) obtained their manure from their own animals, while a very few respondents 2(1.29%) indicated sourcing manure from cattle markets.Furthermore, 60(38.7%) of the respondents reported sourcing their manure from many sources (Table 4.12)

Table 4.12: Sources of manure

Source of Manure	**Frequency (%)**
Cattle farms	14**(**9.03)
Own animals	90**(**58.0)
More than one source	49**(**31.6)
Cattle markets	2**(**1.29)
Total	155(100%)

4.9 Educational Status of Respondents

4.9.1 Educational status of respondents and Isolation of sick Animals

From the study it was noted that the respondents had various levels of education which included basic education 99(63.8%), tertiary education 23(14.8%) while 24(15.4%) had non-formal education(Table 4.13). Among these respondents 116(72.9%) reported isolating their sick animals, while 39(25.2%) did not isolate their sick animals. The relationship of educational level and isolation of sick animals was statistically significant ($p<0.05$)

4.9.2 Preference for Manure Use based on Educational Status of respondents

Results showed that the majority 99(63.8%) of the respondents using manure on their farms had basic education qualification, while 9(5.8%) of the respondents who used manure on their farms did not have any form of education, but the difference was not statistically significant at ($p <0.05$) (Table 4.14)

Table 4.13: Educational status of respondents and their practices of Isolation of sick Animals

Educational status	Isolation of Sick animals		Total
	Yes	No	
*Basic education	75(48.4)	24(15.5)	99(63.9)
Tertiary education	22(14.2)	1(0.64)	23(14.8)
Non formal	16(10.3)	8(5.16)	24(15.5)
None	3(1.93)	6(3.86)	9(5.79)
Total	116(74.8)	39(25.2)	155(100%)

Basic education=Primary and secondary education combined

$\chi^2 = 22.174^a$, p-value = 0.001, Significant at p<0.05

Table 4.14: Preference for Manure Use based on Educational Status of respondents

Education Status	Preference for Manure Use				Total
	Cheaper	Easy to get	Easy to get / Cheaper	Been using themanure	
*Basic education	84(54.2)	8(5.16)	1(0.65)	6(3.87)	99(63.8)
Tertiary education	19(12.2)	4(2.58)	0(0)	0(0)	23(14.8)
Non formal	14(9.03)	8(5.16)	2(1.29)	0(0)	24(15.4)
None	8(5.16)	1(0.65)	0(0)	0(0)	9(5.80)
Total	**125(80.6)**	**21(13.5)**	**3(1.94)**	**6(3.87)**	**155(100)**

Basic education=Primary and secondary education combined

The chi-square statistic is 16.1463. The p-value is .063889. The result is not significant at p <0.05.

4.9.3 Frequency of manure collection by respondents from farms in relation to Type of housing for Animals

Result of the study showed that out of the 155 respondents17(10.9%) havefenced animal housing,while 3(2.0%)of respondent's mentioned their animals' housing have cement or concrete floor and 134(89.3%) stated their animals housing is without floor. With regard to frequency of manure collection, only 31 (20.0%) of the respondents reported collecting manure once a week, while 33 (21.2%) other respondents reported collecting manure from the animals housing twice a week (Table 4.15). It was also reported that 19 (12.2%) respondents said they collect manure once every two weeks while the remaining 72 (46.4%) respondents reported indiscriminate collection of manure. The relationship between housing types and frequency of manure collection was statistically significant ($p<0.05$).

4.9.4 Respondents' awareness of animals shedding pathogens in faeces and Isolation of sick animals

The results of the study showed that 105(67.7%) of the respondents were aware that animals shed pathogens in their faeces while 50(32.2%) reported ignorance of animals shedding pathogens. Of the 105 respondents that showed awareness onanimals shedding pathogens in their faeces, 100 of them reported that they practiced isolation of sick animals from their herds (Table 4.16). Furthermore, out of the 33(21.3%) respondents that reported being un-aware of cattle shedding pathogens, 17(10.9%) of them still reported isolating sick animals from the herd. The awareness of respondents that animals shed pathogens in faeces was statistically significant ($p<0.05$) to the practice of isolation of sick animals.

Table 4.15: Frequency of Manure collection in relation to Type of housing for Animals

Frequency of Manure collection	Type of Housing for Animals			Total
	Local fencing of the animals	Floored with cement or concrete	Non-floored housing	
Once every week	1(0.64)	0(0)	30(19.4)	31(20.0)
Twice every week	2(1.29)	0(0)	31(20.0)	33(21.3)
Once every two weeks	8(5.16)	1(0.64)	10(6.45)	19(12.2)
Indiscriminately collected	7(4.51)	2(1.29)	63(40.6)	73(47.1)
Total	**18(11.6)**	**3(1.93)**	**134(86.5)**	**155(100%)**

χ^2= 25.493[a], p-value = 0.002, Significant at p<0.05

Table 4.16: Respondents' awareness that animals can shed pathogens in faeces and Isolation of sick animals

Animals shed pathogens in their faeces	Isolation of Sick animals[No. (%)]		Total
	Yes	No	
Yes	100(64.5)	5(3.22)	105(67.7)
No	17(10.9)	33(21.3)	50(32.2)
Total	**117(75.5)**	**38(24.5)**	**155(100%)**

χ^2= 70.451[a], p-value = 0.000, Significant at p<0.05

4.9.5 Preference for Manure Use based on accessibility by the respondents

From this study, 125(80.6%) of the respondents indicated use of manure because it was cheap, while 21(13.5%) indicated use of manure due to ease of getting it, 3(1.93%) for ease of getting the manure / being cost effective as their reasons for using the manure while 6(3.9%) of respondents indicatedthat they practiced the method for a long time (Table 4.17). Among the respondents, farmers 90(58.0%) were the majority of those using the manure, and the result showed that occupation of respondents and preference for manure use was not statistically significant ($p < 0.05$)

Table 4.17: Preference for Manure Use based on accessibility byrespondents

Occupation	Preference for Manure Use				Total
	Cheaper	Easy to get	Easy to get / Cheaper	Practice the method for long	
Farming	66(42.6)	17(10.9)	3(1.93)	4(2.6)	90(58.1)
Farming and others	19(12.2)	0(0)	0(0)	2(1.3)	21(13.5)
Other occupation	9(5.8)	4(2.6)	0(0)	0(0)	13(7.4)
Rearing and others	31(20)	0(0)	0(0)	0(0)	31(20)
Total	**125(80.6)**	**21(13.5)**	**3(1.93)**	**6(3.9)**	**155(100%)**

4.9.6 Awareness of Consumer preference based on Age of respondents

The results of the study showed majority 146(94.2%) of respondents indicatedthat they were aware of consumers' preference for crops grown with manure, while 6(3.87%) of respondents reported thatconsumers did not show any preference, but the differencewas not statistically significant ($p < 0.05$) (Table 4.18).

4.9.7 Awareness of Consumer preference based on Education Status

The results showed that majority 99(63.8%) of respondents were aware of consumer preference for crops grown with manure and such respondents were found to have acquired basic education, as against few 9(5.80%) other respondents with no any educational qualification, the result was not statistically significant ($p < 0.05$) (Table 4.19).

Table 4.18: Awareness for Consumers' preference of crops grown with manure based on Age of respondents

Age	Awareness of Consumer preference over crops grown with manure			Total
	No they do not show any preference	Not aware of such preferences	Yes they do show preferences	
Less than 40	1(0.64)	3(1.93)	75(48.4)	79(50.9)
40 – 59	2(1.29)	3(1.93)	55(35.5)	60(38.7)
60 and above	0(0)	0(0)	16(10.3)	16(10.3)
Total	**3(1.93)**	**6(3.87)**	**146(94.2)**	**155(100%)**

The chi-square statistic is 1.6359. The p-value is .802326. The result is not significant at p ≤0.05

Table 4.19: Awareness for consumers' preference of crops grown with manure based on Education Status of respondents

Educational Status	Awareness of Consumer preference over crops grown with manure			Total
	No they do not show any preference	Not aware of such preferences	Yes they do show preferences	
*Basic education	2(1.29)	2(1.29)	95(61.3)	99(63.8)
None	0(0)	0(0)	9(5.8)	9(5.8)
Non formal	0(0)	4(2.58)	20(12.9)	24(15.5)
Tertiary	1(0.64)	0(0)	22(14.2)	23(14.8)
Total	**3(1.93)**	**6(3.87)**	**146(94.2)**	**155(100%)**

Basic education=Primary and secondary education combined
The chi-square statistic is 8.5097. The p-value is .203086. The result is not significant at p ≤0.05.

4.9.8 Methods of manure application and the use of protective equipment

Results of the study showed four (4) common methods of manure application by the respondents namely; application of the manure after sun drying – 115 (74.2%), followed by application of the manure after compositing – 31 (20.0%), other forms of manure application (such as slurry, manure combined with household refuse and manure combined with fertilizer) – 8 (5.3%) and the least was application of fresh manure – 1 (0.64%) (Table 4.20). With regard to the use of protective clothing, it was observed that majority of the respondents – 114 (73.5%) did not wear any form of protective equipment (such as face mask, hand gloves and coverall) while applying manure on their farms, followed by those who wore coverall – 19 (12.2%) while the least – 5 (3.22%)were those who used only hand gloves or other forms of protective equipment respectively. The different protective equipment used by respondents and the equipment included face masks 24 (14.2%) and hand gloves 13 (8.4%) while the majority 115 (74.2%) did not use any protective equipment). The type of clothing used by farmers and the methods of manure application on the farms was statistically significant ($p<0.05$).

Table 4.20: Type of protective equipment used while applying manure and Nature of manure applied on the farm

Type of personal protective equipment(PPE)used	Nature of manure applied on the farm				Total
	Freshly Applied	After composting	Sun drying	Other forms of manure application*	
Face Mask	0(0)	5(3.22)	7(4.51)	1(0.64)	13(8.38)
Hand Gloves	0(0)	2(1.29)	3(1.93)	0(0)	5(3.22)
Coverall	0(0)	1(0.64)	17(10.9)	0(0)	19(12.2)
Other forms of protection	0(0)	0(0)	5(3.22)	0(0)	5(3.22)
Not using any PPE	1(0.64)	23(14.8)	83(53.5)	7(4.51)	114(73.5)
Total	**1(0.64)**	**31(20)**	**115(74.2)**	**8(5.16)**	**155(100%)**

**** slurry, manure combined with household refuse and manure combined with fertilizer***
$\chi^2 = 11.833^a$, p-value = 0.459, Not significant at p<0.05

4.9.9 Health problems observed after manure application in relation to type of protective equipment used

The study also showed that the respondents reported having headache/fever, while - 61(39.2%) had respiratory problems and large number - 51(32.9%) did not report any condition sequel to the use of manure (Table 4.21).

Table 4.21: Health problems observed after manure application and Type of protective cloth used

LGA	Use of protective clothing				Health problems				Duration of manure use			
	Face mask	Hand gloves	Rain booth	None	Headache	Respiratory problem	Abdominal Upset	None	<5yrs	5-10 yrs	11-15 yrs	>15 years
Bauchi	4(2.6)	3(1.93)	4(2.58)	43(27.7)	7(4.51)	35(22.5)	6(3.87)	2(1.29)	0(0)	0(0)	0(0)	50(32.3)
Darazo	5(3.2)	8(5.16)	5(3.2)	36(23.2)	7(4.51)	30(19.3)	3(1.93)	14(9.03)	0(0)	0(0)	0(0)	54(34.8)
Katagum	13(8.4)	2(1.29)	13(8.38)	36(23.2)	10(6.45)	6(3.87)	0(0)	35(22.5)	4(2.58)	16(10.3)	4(2.58)	27(17.4)
Total	**22(14.2)**	**13(8.4)**	**22(14.2)**	**115(74.2)**	**24(15.5)**	**61(39.3)**	**9(5.80)**	**51(32.9)**	**4(2.58)**	**16(10.3)**	**4(2.58)**	**131(84.5)**
		155(100%)				**155(100%)**				**155(100%)**		

χ^2= 39.358[a] p-value = 0.001, Significant (p< 0.05)

CHAPTER FIVE

5.0 DISCUSSION

The results of this research found a higher occurrence rate of *E. coli* in faecal samples 314(31.7%) than in cattle manure 7(4.24%). This poses a risk of pasture and environmental contamination with a high potential for infection to animals and a public health hazard to individuals such as the herdsmen, butchers, livestock traders and veterinary professionals who have close association with cattle faeces and manure in several ways. This is similar to the work of Ferreira *et al*.(2014) which stated that the high prevalence of shiga toxin producing *E. coli* in dairy cattle poses a significant risk to public health, since these microorganisms can contaminate products intended for human consumption, such as water, raw and pasteurized milk, meat products, dairy products, and/or products of plant origin. Also as stated by Witold and Hovde (2010) that transmission of *E. coli* to people occurs primarily via ingestion of inadequately processed contaminated food or water and less frequently through contact with manure of animals or infected people. Also individuals such as crop farmers have increased risk of coming in contact with the *Escherichia coli* due to the tendency that rain water could take the organism from the crop farms to streams which could be used as a source of drinking water as stated in a document; storing manure on small farms(AMM), (2015).

Theresultsof the research documented a higher occurrence of *E. coli* in samples of male cattle 219(22.1%) than from female cattle, 95(9.6%),and this is similar to a study by Musa *et al*. (2012) in Kaduna, who reported *E. coli* prevalence of 2.1% in male and

1% female cattle. However, the prevalence of 4.48% *E. coli* from cattle faeces is a threat to the environment and to livestock management since livestock production in the state is largely under extensive system. The results are however, contrary to a report by Biruhfesta *et al.* (2017) that found no statistically significant difference ($P< 0.05$) in the prevalence of *E. coli* O157:H7 based on sex of animals. The resultsare however not in agreement with that of Montenegro *etal*. (1990) who reported *E. coli* prevalence rates of 11.6% and 3.0% in cows and bulls respectively carried out in Germany using DNA hybridization technique, but with other serogroups of *E. coli* than O157:H7. Also in another research carried out in Hawassa, Ethiopia showed no statistically significant difference in the prevalence of *E. coli* O157: H7 based on sex, origin, and breed of cattle (Atnafie*et al.,* 2017). Also Hassan *et al*. (2014)reported a higher prevalence of *E. coli* in female cattle of unorganized farming systems, and in cattle ≥3 years of age.

The research showed a higher frequency of occurrence of *E. coli*in adult animals 271 (27.4%)than from younger ones 43(4.43%)probably because of the fact that the adult animals have higher feed consumption per day and as such an opportunity to take in more of the organisms in the contaminated pasture than the younger ones. The finding is entirely different to that of Gannon *et al*. (2002) who found an apparently cyclic pattern of *E. coli* shedding where faecal prevalence was 25% from 1-7 weeks of age, declined to 0% after animals were turned out to pasture and increased to 6 – 14% by 2 weeks post weaning.

The result further indicated that out of the total faecal samples collected from the three locations, *E. coli* isolation was highest in adult cattle 103(79%) than in younger animals 27(21%), and the result also indicated that even within the adults, more positive samples were obtained from animals presented to the livestock markets 67(51%) than animals in the abattoir 1(0.76%) with no statistical significant difference ($p<0.05$).

The results of the antibiotic susceptibility testing for *E. coli* isolates indicated some of the isolates were resistant to commonly used antimicrobial agents: (Cefazolin (88%); Sulfamethoxazole (84%); Enrofloxacin (80%). Also the proportion of isolates that were susceptible various antibiotics showed higher susceptibility to Chloramphenicol (80%), Gentamicin and Imipenem (70%). The antibiotic resistances reported in this study were either different from or similar to reports by other researchers(Martin *et al.*, 2010; Ayo *et al*., 2011;Amosun*et al*., 2012),and these differences and similarities may be due to differences in the samples examined, differences in year or time of sampling and sampling procedure employed as stated by Frederick (2015).

The finding that16% of the *E. coli* isolates wereresistantto Imipenem is alarming,because the drug is not reported to be commonly used in the treatment of livestock diseases, but yet some of the *E. coli* isolates from the study area have shown varying levels of resistance to the drug. This can probably be attributed to horizontal gene transfer,and the results for this research on this aspect have also showed some similarities and differences with other researches.

In a report from Owerri Nigeria, Duru *etal.* (2013) showed *E. coli* isolates from poultry exhibiting higher susceptibility to Imipenem (100%) at the same timebeing resistant to other drugs like Ceftriaxone (90%) and Amoxillin + Clavulanic acid (90%).

Also a research conducted by Christiana *et al*. (2014) in Oyo state Nigeria reportedthat*E. coli* isolates of human and bovine origin weresusceptible to Imipenem (100%) while resistance to Ciprofloxacin and Amoxicillin + Clavulanic acid were 60% and 70% respectively.A similar research by Ajay *et al.* (2013) for*E. coli*from human specimensfrom a hospital in India showed2% resistance to Imipenem, while resistance to other antibiotics were Gentamicin (43.1%) and Ciprofloxacin (10.5%).

To the best of our knowledge, this is the first study, which describes the prevalence of antibiotic resistance among *E. coli* isolated from faecal samples of healthy cattle and manure in Bauchi state. The study showed high rates of resistance among isolates to individual antibiotics, with 98% of*E. coli* isolates being resistant to at least one antibiotic. Multiple drug resistant (MDR)was observed in 98% of the *E. coli* isolates except one isolate, a finding that is similar to Aviji and Sabuj (2016) in Khulshi Bangladesh which reported 100% MDR for *E. coli* isolates to the antimicrobial agents tested.

The 98% prevalence of MDR in*E. coli* O157 isolates observed in this research is higher than that obtained in a study conducted in Zaria by Igwe *et al*. (2016) which evaluated phenotypic virulence characters of the organism that contributes to the

expression of MDR properties of *E. coli*. The research further showed *E. coli* resistant to tetracycline, sulfonamide and othersthough with MDR prevalence of 59.3% where *E. coli* isolates were resistant to 4 and above antibiotics tested.

The MDR in this research is also higher than the 64.3% obtained in a research by Bello *et al*. (2013) where they described the situation as an indication that adequate measures need be taken to regulate drug use in both humans and animals in order to minimize the risk of increasing antimicrobial resistance. The result of the research is in agreement with a research by Tadesse*et al.* (2012) that stated antimicrobial resistance is a multivariable phenomenon not only directly related to antimicrobial usage.

The proportion of isolates (96%) with MAR index greater than 0.2 observed in this research is worrisome, and it may be an indication of indiscriminate use of different classes of the antimicrobials,and an indication of lack of control or improper monitoring of use of such antibiotics in the prevention or treatment of animals or usage as growth promoters. Alien *et al.* (2012) stated that the indiscriminate use of antibiotics in animal husbandry should be discouraged because of the resistant pathogens that can be transmitted to humans through consumption of foods harbouring such pathogens.

The occurrence of resistance genes in*E. coli* isolates fromfaecal samples of animals reared under extensive management system in this studycan be linked to the way veterinary drugs are unprofessionally and indiscriminately used for the treatment of

animal diseases, or due to prolonged under-dosing and incomplete dosing which may probably contribute to resistance development by microbes as reported by Michael *et al*. (2014).And the finding is also similar to the report of a study in Sokoto state by Abubakar *et al.* (2016) that showed*E. coli* O157:H7 isolates from cattle faeces harbouring resistance genes from cattle managed under traditional-herding system (extensive management), and observed that it can be a potential public health threat and a source of human infection in the state.

However, the results for this research is contrary to the work of Agga*et al.*(2019) which stated that animals kept in confinement (or intensive management system) can facilitate the spread of genes associated with antibiotic resistance; it is however not known how cattle beef production system that involves maximal use of pasture and forage from time calves are weaned until they are placed in feedlot affects the persistence of antibiotic resistance genes in the environment.

The detection of virulence genes in *E. coli*isolates from cattle faeces obtained from all the sampling locations (i.e. Abattoir, cattle farms and livestock markets) can be attributed to the movement of cattle from farms to livestock markets on their way to the abattoirs for slaughter as mentioned by Kaltungo *et al.*(2013) and due to the fact that livestock owners do purchase animals and mix them immediately with other livestock in the herds.

The result of the study showed the occurrence of both resistance and virulence genes in some of the *E. coli*isolates examined. Faecal samples were found to act as reservoir

of the organisms harbouring resistance and virulence genes and are potential risk to human and animal health in the study area. Similar reports were made by Wambuga*et al*. (2018) in a study carried out on faecal samples of cattle reared aroundAthi River area inKenya, where *E. coli*isolates were found harbouring resistance genes, virulence genes and integrons 1. The presence of mobile genetic elements lead to their dissemination and possible development of multiple-resistance phenotype. The report further explained that the dissemination of resistance is associated with mobile genetic elements, such as plasmids that may also carry virulence determinants.

The study revealed that only 2(1.21%) of the manure samples were positive for *E. coli* O157 and this could be due to inadequate moisture and nutrients for the organism to survive in the manure.

The results of this research observed that the ease of manure collection was dependent on the type of housing of the cattle. The more frequently the manure was collected the less the tendency of occurrence of the *E. coli* in the manure. Hence the indiscriminate manure collection observed in the study could increase the chances of higher occurrence of the *E. coli* (harbouring resistance and virulence genes) and the accompanying risk for public health. The relationship between frequency of manure collection and occurrence of *E. coli*may be similar to the report of Baker *et al*. (2019) who described a statistically significant relationship between prevalence of genes in manure and the storage, cleaning processes of materials used for manure collection and the frequency of accumulated manure collection from the farms,

The results of the present study revealed that the majority 105(67.7%) of respondents were aware that animals shed pathogens in their faeces and that a vast majority among them practiced isolation of sick animals from their herds in order to prevent further spread of any infection among the animals. This is similar with the report by Delahoy*et al*. (2018) which stated that there is need for improved understanding of which pathogens are transmitted in animal faeces.The burden of disease that may be attributable to faecal contamination in the environment under improved human sanitation scenarios is vital in explaining meaningful ways to reduce the burden of disease via the safe management of animal faeces.

The respondents' awareness of the presence of pathogens in the faeces of animals could also be attributed to their enlightenment through listening to agricultural programs on radio and their interactions with veterinary personnel.Previous studies by Kaltungo *et al.* (2013) reported that pastoralists accounted their sources of knowledge of livestock diseases from parents, friends and agricultural radio programs as well as their interactions with veterinary personnel. In another study, Christy *et al.* (2016) had reported that public campaigns to sensitize farmers, more especially farm owners on the risks associated with handling raw manure and the necessity of proper management and treatment are imperative.

Results of the study which found that respondents'usedmanure because it was a cheap alternative as well as ease of getting it, is similar to the assertion ofLeip*et al*. (2019) that manure is often traded at a price that is not necessarily linked to its fertilizer

value; the approach explains the value of an equivalent application of fertilizers, which the farmer would apply in order to achieve the same crop yield.

The results of the current study showed that majority of respondents 99(63.8%) were aware of consumer preferences for crops grown with manure agrees with a similar report by Marsh *et al.*(2017)which indicated both farmers and non-farmers believed that organic crops are safe and of high quality.

The various methods of manure application used by the respondents in this study have been a tradition by crop farmers in line with farmers trained by agricultural extension workers (Sa'idu *etal*., 1991). It was also observed that the current scarcity of fertilizer, as well as the current economic problems and high prices of the inorganic fertilizers might have led the respondents to revert to the use of manure which they believed to have more advantages on the farm lands compared to the inorganic fertilizers. The results of the study indicated that respondents who never used protective equipment were the majority 115(74.2%) and their action can be related to ignorance of the harmful effects of the manure they frequently handled while applying on their farms. The health conditions reported by some respondents such as headache/fever and respiratory problems may be a consequence of their exposure to pathogens through inhalation and ingestion of*E. coli*.

Also the health conditions observed among respondents in the study population could be due to frequent use of manure, especially as farmers do engaged in irrigation

farming during the dry season as reported by Izuogu*et. al.* (2015). The aim of using personal protective equipment (PPE) is to protect the health condition of the farmers or for safety at the farm, as enumerated by Loveday *et al*. (2014) that evidence – basedguidelines on infection prevention and proper selection and use of the PPE should be proposed for the farmers.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

1. The result of the studyindicated that 314(31.72%) of the 990 faecal samples and 7(4.24%) out of 165 manure samplesharboured*Escherichia coli.*
2. The study revealedhigh frequency of *Escherichia coli*isolation in samples obtained from male animals 219 (22.1%) compared to those from female animals 95 (9.6%); also higher occurrence of *E. coli* was observed in samples of adult animals 271 (27.4%) than in younger ones 43 (4.43%)
3. All isolates showed MDR phenotypes with some isolates showing resistance to 8 antimicrobial agents belonging to 7 classes of antimicrobial agents.
4. The proportion of *Escherichia coli* isolates having MAR index of 0.2 or higher was 96%.
5. Results of the mPCR showed the co-occurrences of resistance and virulence genes in some of the isolates examined (gene carriage), where 1isolates (CAMA83) was found to harbour 4 genes; and 6 isolates (ALMA80, CAFA20,

L_3FA57, CAMA71, ALAM19 and CAMA84)were found to harbour 3 genes each.

6. Antimicrobial resistance genes (*tetA, tetB* and *tetK*) and virulence genes (*stx1, stx2* and *eaeA)* were detected from *E. coli* isolatesin cattle faeces from the cattle farms and livestock markets.
7. Respondents whose animals produced manure and those using manure on their farmsin the study area were found to haveacquired various levels of education as it was observed thatthe relationship between those who had basiceducation 75/150(48.4%) and isolation of sick animal was statistically significant ($p<0.05$).
8. Economic reason (like the manure being cheaper) wasfound to be the major factor why majority of the respondents (125/150(80.6%) used manure on their farm lands, in addition to other reasons mentioned.
9. The lack of awareness that animals could shed pathogens 50/150(32.2%) in their faeces and the fact that some of the respondents did not practice isolation of sick animals 38/150(24.5%) from their herds were also observed in the study.
10. The study found that majority of respondents did not useany protective equipment117/150(75.5%)) and that this attitude maypredispose them to certain hazardsor illnesses due to constant contact with manure contaminated with pathogens.

6.2 Recommendations

In the light of the findings from this study, the following recommendations are beinggiven:

1. Relevant government and non-governmentalagencies should collaborate to organize stakeholders' meetings on ways to curtail the abuse and unauthorized use of antibiotics by farmers for the treatment and prevention of animal diseases,
2. A further more detailed investigation for the identification of virulence and resistance genes in *E. coli* from cattle and their environment should be instituted to safeguard the spread of such genes in the environment, animals and humans
3. Cattle herders and crop farmers should be enlightened on the advantages as well as dangers associated with the use of cattle faeces as manure on their farms, and should be encouraged on the use of protective equipment during all activities on their farms in order to avoid exposure to *E. coli* and other pathogens.

4. Further research and implementation of the research findings should be encouraged to improve on the existing manure management practices as well as to uncover new, low cost procedures of manure use that would minimize the spread of enteric pathogens from the manure to the farmers. And government at all levels should encourage the safe use of manure by farmers in a modern way as it can help in reducing the depletion of soil fertility and environmental contamination.
5. In line with the above, cottage industries should be established to handle and process manure for safe use by farmers.
6. Practices such as isolating sick animals from healthy ones in the herd should be encouraged among farmers as it may play a significant role in preventing the spread of pathogens among the animals and in the environment.

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APPENDICES

AppendixI:

A

QUESTIONNAIRE

I am a postgraduate student of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, carrying out a survey to evaluatethe practice, pattern of distribution and socio-

economic factors promoting the use of manure on farm lands in Bauchi state North – eastern Nigeria, your responses would help to achieve the above mentioned objective. All your answers will be treated with confidentiality and anonymity.

Please tick [✓] in theboxes adjacent to any suitable (s)for the following questions, your kind cooperation will be highly appreciated thank you.

A: LOCATION (Animals/Farm/Abattoir)

1. Local Government Area _____
2. Ward/Village _____
3. Address _____
4. GPS location _____

B: DEMOGRAPHIC INFORMATION

1. Sex of respondent

   (a) Male [ ] (b) Female [ ]

2. Age of Respondent

   (a) Less than 20 [ ] (b) 20-39 [ ]
   (c) 40-59 [ ] (d) 60 and above [ ]

3. Occupation of Respondent_____

4. Educational status of respondent

   (a) O'level [ ] (b) ND [ ]
   (c) HND/BSC [ ] (d) Others
   (Specify)_____

C: MANURE PRODUCTION AND MANAGEMENT INFORMATION

1. Individual's relationship with the Animals

   (a) Own animals [ ] (b) Other people's animals [ ]
   (c) Animals presented for slaughter [ ]

2. Types of Management system for the Animals

   (a) Intensive [ ]
   (b) Semi intensive [ ]
   (c) Extensive (free range) [ ]

3. Type of housing for the animals
(a) Local fencing of the animals [ ]
(b) Floored with cement or concrete [ ]
(c) Non floored housing [ ]
(d) Other (specify)_____

4. Average number of the animals
(a) Less than 50 [ ]
(b) 51 – 150 [ ]
(c) 151 – 200 [ ]
(d) More than 200 [ ]

5. How often do you collect the Manure?
(a) Once every week [ ]
(b) Twice every week [ ]
(c) Once every two weeks [ ]
(d) Indiscriminately collected [ ]

6. Who handles the manure
(a) The farmer [ ]
(b) Cattle owner/Farm manager [ ]
(c) Abattoir workers [ ]

7. Are you aware that sick Animals shed pathogens in their feaces?
(a) Yes [ ]
(b) No [ ]

8. If Yes (in Q7 above) do you isolate sick Animals from healthy ones
(a) Yes [ ] (b) No [ ]

9. Do you separate Manure of sick animals from those of healthy animals?
(a) Yes [ ] (b) No [ ]

10. What do you use Manure for?
(a) Sold to prospective buyers [ ]
(b) Used on farm lands [ ]
(c) For medicinal purposes [ ]
(d) Other (specify)_____

11. Kind of storage facility (ies)you have for the manure?

(a) _____
(b) _____
(c) _____

12. Any awareness that animals treated with drugs shed traces of such drugs in their feaces?
(a) Yes [ ]
(b) No [ ]

Appendix II:

QUESTIONNAIRE

B

I am a postgraduate student of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, carrying out a survey to evaluate the practice, pattern of distribution and socio-

economic factors promoting the use of manure on farm lands in Bauchi state North – eastern Nigeria, your responses would help to achieve the above mentioned objective. All your answers will be treated with confidentiality and anonymity.

Please tick [✓] in the boxes adjacent to anysuitable option (s) for the following questions, your kind cooperation will be highly appreciated, thank you.

A: LOCATION (Crop farm land)

5. Local Government Area _____
6. Ward/Village _____
7. Address _____
8. GPS location _____

B: DEMOGRAPHIC INFORMATION

5. Sex of respondent

   (b) Male [ ] (b) Female [ ]

6. Age of Respondent

   (b) Less than 20 [ ] (b) 20 – 39 [ ] (c) 40-59[ ] (d) 60 and above [ ]

7. Occupation of Respondent_____

8. Educational status of respondent

   (b) O'level [ ] (b) ND[ ] (c) HND/BSC [ ]
   (d) Others (Specify)_____

C: INFORMATION ON THE FARM LAND

13. Why do you prefer the use of manure?

(a) Easy to get [ ] (c) Used to it [ ]
(b) Cheaper [ ] (d) Others (specify)_____

14. Sources of Manure

(a) Own animals [ ] (c) From cattle markets [ ]
(b) From cattle farms [ ] (d) From abattoirs/slaughter slabs [ ]

15. Type(s) of Manure used?

(a) Cattle manure [ ] (b) Poultry Manure [ ]
(c) Small ruminants manure [ ] (d) Combination of more than one above [ ]
(e) Others (specify)_____

16. Nature of Manure applied on the farm.
(a) Freshly applied [ ] (b) After decomposition [ ]
(c) Sun drying [ ] (d) Others (specify)_____

17. Type of crop(s) planted on the farm land.
(a) Maize [ ] (b) Rice [ ] (c) Guinea corn [ ]
(d) Millet [ ] (e) Vegetables [ ] (f) Others (specify)_____

18. Frequency of application.
(a) Once every season or planting period [ ] (b) Twice per raining season [ ]
(c) More than twice per raining season[ ] (d) Applied indiscriminately[ ]

19. How long have you been using Manure on your farm land?
(a) Less than 5years [ ] (b) 5 – 10 years [ ]
(c) 11 – 15 years [ ] (d) More than 15years [ ]

20. What type of protective cloth do you wear while applying Manure to the farm?
(a) Face mask[ ] (b) Hand gloves [ ]
(c) Coverall [ ](d) others, specify_____

21. If you don't use any protective clothing,Why?
(a) Not available [ ] (b) Don't think it is necessary [ ]
(c) Other reasons (Specify)_____

22. What type of health related problems have you ever observed after application of manure?
(a) Headache/Fever [ ] (b) Respiratory problem/dizziness [ ]
(c) Stomach upset/vomiting [ ] (d) Others (Specify)_____

23. How do you think the use of Manure can be improved?
(a) Setting up cottage industry that will process the Manure [ ]

(b) Creating awareness on uses and dangers associated with manure use[ ]

(c) Others (Specify)_____

24. Indicate advantage(s) of the use of manure?

(a) Control of erosion [ ] (b) Improved fertility of the soil [ ]

(c) Increase crop yield [ ] (d) All of the above [ ]

(e) Others (Specify)_____

25. What kind of difficulty (ies)do you observe in conveying the manure to the farm?

(a) Lack of vehicle to convey the manure to the farm [ ]

(b) Long distance of the farm from source of the manure[ ]

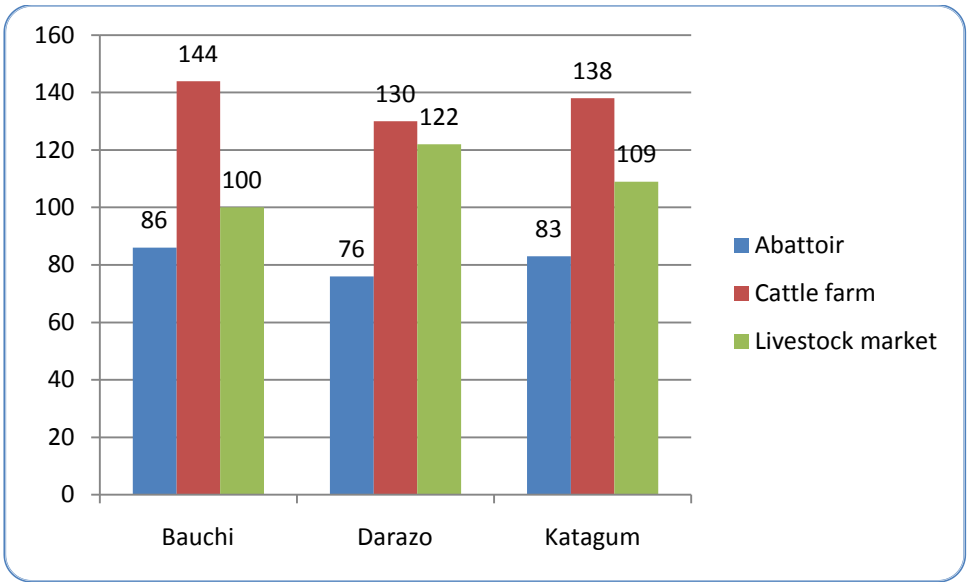
(c) All of the above [ ] (d) Others (Specify)_____

26. Are you aware of consumers preference over crops grown with Manure (organic farming) over crops which fertilizer were used

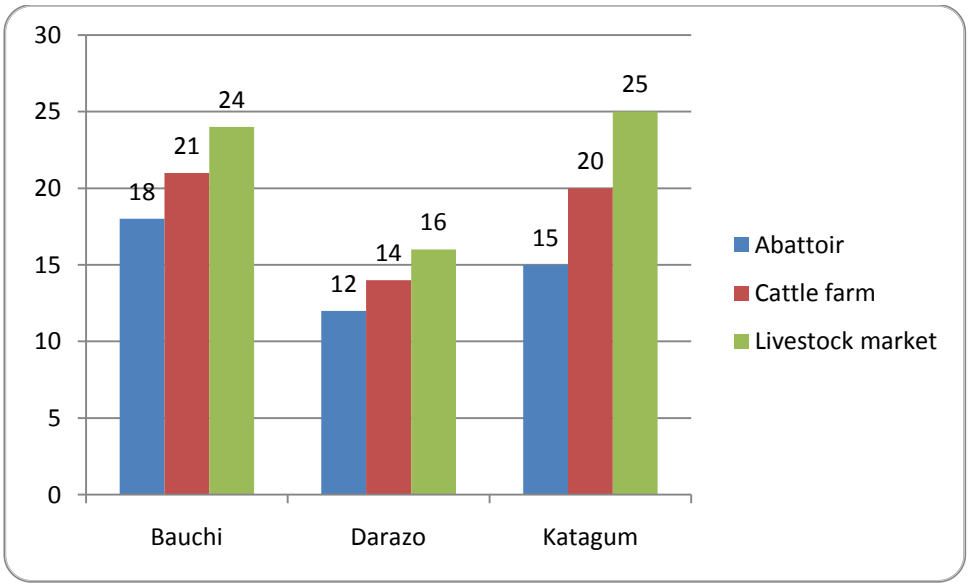
(a) Yes they do show preferences [ ](b) No they do not show any preferences[ ]

(c) Not aware of such preferences [ ]

IIIa Distribution of cattle faecal samples collected from different locations inthree LGA of Bauchi State



IIIb Distribution of cattle manure samples collected from different locations in three LGA of Bauchi State



IV Monthly distribution of cattle faecal and manure samples collected fromdifferent locations in threeLGA of Bauchi state

Month	Faecal samples									Manure samples								
	Bauchi			Darazo			Katagum			Bauchi			Darazo			Katagum		
	A	CF	LM	A	CF	LM	A	CF	LM	A	CF	LM	A	CF	LM	A	CF	LM
March	43	72	50	0	0	0	0	0	0	9	10	12	0	0	0	0	0	0
April	43	72	50	0	0	0	0	0	0	9	11	12	0	0	0	0	0	0
May	0	0	0	39	65	61	0	0	0	0	0	0	6	7	8	0	0	0
June	0	0	0	39	65	61	0	0	0	0	0	0	6	7	8	0	0	0
July	0	0	0	0	0	0	42	69	55	0	0	0	0	0	0	8	10	13
August	0	0	0	0	0	0	41	69	54	0	0	0	0	0	0	7	10	12
Total	**86**	**144**	**100**	**78**	**130**	**122**	**83**	**138**	**109**	**18**	**21**	**24**	**12**	**14**	**16**	**15**	**20**	**25**

Key: A=Abattoir;CF=Cattle farms; LM=Livestock market

V Distribution and occurrence of *Escherichia coli* in cattle faeces based on sex of animals from Bauchi LGA

Locations	Number collected	Sex		Total	χ^2 17.29	p-value 0.008
		Male (% positive)	Female (% positive)			
Abattoir	86	25 (19)	8 (6)	33 (24)		
Cattle farms	144	16 (12)	26 (19)	42 (31)		
Livestock market	100	45 (33)	15 (11)	60 (44)		
Total	**330**	**86 (64)**	**49 (36)**	**135 (100)**		

Significant at P<0.05

VI Distribution and occurrence of *Escherichia coli* in cattle faeces based on Age of animals from Bauchi LGA

Location	Number collected	Age		Total	Point prob. 1.0E-5
		Young (% Positive)	Adult (% Positive)		
Abattoir	86	0 (0)	33 (24)	33 (24)	
Cattle farms	144	9 (7)	33 (24)	42 (31)	
Livestock market	100	0 (0.0)	60 (44)	60 (44)	
Total	**330**	**10 (7)**	**125 (93)**	**135 (100)**	

Not significant at P<0.05

VII Distribution and occurrence of *Escherichia coli* in cattle faeces based on sex of animals from Darazo LGA

Location	Number collected	Sex		Total	χ^2 3.539	p-value 0.738
		Male (% positive)	Female (% positive)			
Abattoir	76	9 (18.3)	8 (16.3)	17 (35)		
Cattle farms	130	9 (18.3)	15 (30.6)	24 (49)		
Livestock market	122	6 (12.2)	2 (4.0)	8 (16)		
Total	**330**	**24 (49)**	**25 (51)**	**49 (100)**		

Not Significant at P<0.05

VIII Distribution and occurrence of *Escherichia coli* in cattle faeces based on Age of animals from Darazo LGA

Location	Number	Age		Total	Point probability
		Young	Adult		

	collected	(% positive)	(% positive)		0.01254
Abattoir	76	0 (0.0%)	17 (35)	17 (35)	
Cattle farms	130	6 (12)	18 (37)	24 (49)	
Livestock market	122	1 (2.0)	7 (14)	8 (16)	
Total	**330**	**7 (14)**	**42 (86)**	**49 (100)**	

Not Significant at P<0.05

IX Distribution and occurrence of *Escherichia coli* in cattle faeces based on sex of animals from Darazo LGA

Location	Number collected	Sex		Total	Point probability 0.03299
		Male (% Positive)	Female (% Positive)		
Abattoir	83	0 (0)	1 (0.76)	1 (0.76)	
Cattle farms	138	35 (26.9)	6 (4.6)	41 (31.5)	
Livestock market	109	74 (56.9)	14 (10.7)	88 (67.6)	
Total	**330**	**109 (84)**	**21 (16)**	**130 (100)**	

Not Significant at P<0.05

X Distribution and occurrence of *E. coli* in cattle faeces by age of animals from Katagum LGA

Location	Number collected	Age		Total	Point probability 0.07463
		Young (% Positive)	Adult (% Positive)		
Abattoir	83	0(0.0)	1 (0.76)	1 (0.76)	
Cattle farms	138	6(4.6)	35(26.9)	41 (31.5)	
Livestock market	109	21(16.1)	67(51.5)	88 (67.6)	
Total	**330**	**27(21)**	**103(79)**	**130 (100)**	

Not Significant at P<0.05

XI All Samples positive for *E. coli* on presumptive test

LGA	**Location**	**Sample type**	**No. screened**	**No. positive**	**(%)**
Bauchi	Abattoir	Faeces	86	36	13.07
	Cattle farms	Faeces	144	43	

	Livestock market	Faeces	100	72	
	Abattoir	Manure	18	0	
	Cattle farms	Manure	21	0	
	Livestock market	Manure	24	0	
Darazo	Abattoir	Faeces	78	2	1.04
	Cattle farms	Faeces	130	8	
	Livestock market	Faeces	122	1	
	Abattoir	Manure	12	0	
	Cattle farms	Manure	14	0	
	Livestock market	Manure	16	1	
Azare	Abattoir	Faeces	83	4	18.3
	Cattle farms	Faeces	108	63	
	Livestock market	Faeces	139	138	
	Abattoir	Manure	15	0	
	Cattle farms	Manure	20	0	
	Livestock market	Manure	25	6	
	Total		**1155**	**374**	**32.4**

XII Results of Biochemical tests

LGA	**Location**	**Sample type**	**No. screened**	**No. positive**	**(%)**
Bauchi	Abattoir	Faeces	0	0	5
	Cattle farms	Faeces	5	2	
	Livestock market	Faeces	4	2	
Darazo	Abattoir	Faeces	2	1	6.25
	Cattle farms	Faeces	8	4	
	Livestock market	Faeces	1	0	

	Livestock market	Manure	1	0	
Azare	Abattoir	Faeces	1	1	48.7
	Cattle farms	Faeces	18	16	
	Livestock market	Faeces	36	20	
	Livestock market	Manure	5	2	
	Total		**80**	**48**	**60**

XIII Bacterial species identified by Microbact 12E from various locations in Bauchi state

S/No	Location	Faeces							
		No. tested	***E.C***	***S.A***	***K.D***	***K.O***	***C.D***	***S.M***	***S.L***
1	B.C.F	5	2	1	1	1	0	0	0
2	B.L.M	4	2	0	0	0	2	0	0
3	D. A	2	1	1	0	0	0	0	0
4	D.C.F	8	4	1	0	0	2	1	0
5	D. L.M	1	0	0	0	0	1	0	0
6	A. A	1	1	0	0	0	0	0	0
7	A. C.F	18	17	0	0	0	1	0	0

8	A. L.M	36	22	4	0	0	8	1	1
Total		75	48	7	1	1	14	2	1

KEY: *N.T= BCF = Bauchi cattle farm, BLM = Bauchi livestock market, DA = Darazo abattoir, DCF = Darazo cattle farm, DLM = Darazo livestock market, AA = Azare abattoir, ACF = Azare cattle farm, ALM = Azare livestock market. E. C = Escherichia coli, S. A = Serratia arizonae, K. D = Klebsiella diversus, K. O =Klebsiella oxytoca, C. D= Citrobacter diversus, S.M = Serratia marcancens, S.L = Liquefaciens*

XIV Serological test results for *Escherichia coli* isolates from cattle faeces

S/No	Sample location	No. tested	Latex Agglutination		Total
			O157:H7	Non O157	
1	Bauchi Cattle Farms	5	0	2	2
2	Bauchi Livestock Market	4	1	1	2
3	Darazo Abattoir	2	0	1	1
4	Darazo Cattle Farms	8	2	2	4
5	Darazo Livestock Market	1	0	0	0
6	Azare Abattoir	1	0	1	1
7	Azare Cattle Farms	18	11	5	16

8	Azare Livestock Market	36	13	7	20
Total		**75**	**28**	**20**	**48**

XV Serological test results for *Escherichia coli* isolates from cattle manure

S/No	Sample location	No. tested	Latex Agglutination	
			O157:H7	Non O157
1	Bauchi Cattle Farms	0	0	0
2	Bauchi Livestock Market	0	0	0
3	Darazo Abattoir	0	0	0
4	Darazo Cattle Farms	0	0	0
5	Darazo	0	0	0
6	Azare Abattoir	0	0	0
7	Azare Cattle Farms	0	0	0
8	Azare Livestock Market	5	2	0
Total		**5**	**2**	**2**

XVI Serological tests results for *Escherichia coli*O157:H7 strains

SAMPLE POSITIVE FOR *E. COLI* O157:H7 STRAIN					
STRAIN	**I.D**	**LOCATION**	**SAMPLE TYPE**	**No.**	**(%)**
O157:H7	BLAM72	Bauchi	Livestock market faeces	1	**3.4**
O157:H7	DCAM18	Darazo	cattle farms faeces	2	**6.9**
O157:H7	DCAM73	Darazo	cattle farms faeces		
O157:H7	ACAF20	Azare	cattle farms faeces	**26**	**89.7**
O157:H7	ACAF25	Azare	cattle farms faeces		
O157:H7	ACYM35	Azare	cattle farms faeces		
O157:H7	ACYM43	Azare	cattle farms faeces		
O157:H7	ACAM45	Azare	cattle farms faeces		
O157:H7	ACAM47	Azare	cattle farms faeces		
O157:H7	ACAM60	Azare	cattle farms faeces		
O157:H7	ACAM65	Azare	cattle farms faeces		
O157:H7	ACAM71	Azare	cattle farms faeces		
O157:H7	ACAM75	Azare	cattle farms faeces		
O157:H7	ACAM84	Azare	cattle farms faeces		
O157:H7	ALAM4	Azare	cattle farms faeces		
O157:H7	ALAF11	Azare	cattle farms faeces		
O157:H7	ALAF18	Azare	cattle farms faeces		
O157:H7	ALAM33	Azare	cattle farms faeces		
O157:H7	ALAM37	Azare	cattle farms faeces		
O157:H7	ALAM39	Azare	cattle farms faeces		
O157:H7	ALAM42	Azare	cattle farms faeces		

O157:H7	ALAM44	Azare	cattle farms faeces		
O157:H7	ALAM64	Azare	cattle farms faeces		
O157:H7	ALAF73	Azare	cattle farms faeces		
O157:H7	ALAM74	Azare	cattle farms faeces		
O157:H7	ALAM80	Azare	cattle farms faeces		
O157:H7	ALAM86	Azare	cattle farms faeces		
O157:H7	ALM9	Azare	cattle farms faeces		
O157:H7	ALM13	Azare	cattle farms faeces		
Total				**29**	**100**

XVII Serological tests results for *Escherichia coli*Non O157 strains

SAMPLE POSITIVE FOR *E. COLI* NON O157 STRAIN					
STRAIN	**I.D**	**LOCATION**	**SAMPLE TYPE**	**No**	**%**
Non O157	BCAF10	Bauchi	Cattle farm faeces	3	**15.8%**
Non O157	BCAM15	Bauchi	Cattle farm faeces		
Non O157	BLAF57	Bauchi	Cattle farm faeces		
Non O157	DAYM35	Darazo	Abattoir faeces	3	**15.8%**
Non O157	DCAM16	Darazo	Cattle farm faeces		
Non O157	DCAM17	Darazo	Cattle farm faeces		
Non O157	AAAF4	Azare	Abattoir faeces		**68.4%**
Non O157	ACYM34	Azare	Cattle farm faeces		
Non O157	ACAM56	Azare	Cattle farm faeces		
Non O157	ACYM71	Azare	Cattle farm faeces		
Non O157	ACAM83	Azare	Cattle farm faeces		
Non O157	ACAM95	Azare	Cattle farm faeces		

Non O157	ALAF19	Azare	Livestock mkt faeces	13	
Non O157	ALAF20	Azare	Livestock mkt faeces		
Non O157	ALAM62	Azare	Livestock mkt faeces		
Non O157	ALAM69	Azare	Livestock mkt faeces		
Non O157	ALAM75	Azare	Livestock mkt faeces		
Non O157	ALAM93	Azare	Livestock mkt faeces		
Non O157	ALAM109	Azare	Livestock mkt faeces		
Total				**19**	**100**

XVIII Occurrence of other gram negative organisms

I.D	ORGANISMS TYPE
BCAM28	Serratia arizonae
BCAF58	Klebsiella diversus
BCAM60	Klebsiella oxytoca
BLAM2	Citrobacter diversus
BLAM45	Citrobacter diversus
DAAM14	Serratia arizonae
DCAF5	Citrobacter diversus
DCAF27	Serratia arizonae
DCYF50	Citrobacter diversus
DCYM53	S. morcancens
DLAM2	Citrobacter diversus
ACAF45	Citrobacter diversus
ALAF3	Citrobacter diversus
ALAM7	Serratia arizonae
ALAM8	Citrobacter diversus
ALAM14	Serratia arizonae
ALYM15	S. liquefaciens
ALAM17	Serratia arizonae
ALAF23	S. morcancens
ALAM40	Citrobacter diversus
ALAF41	Citrobacter diversus
ALYM61	Citrobacter diversus
ALAM66	Citrobacter diversus

ALAM76	Serratia arizonae
ALAF96	Citrobacter diversus
ALM4	Citrobacter diversus
ALM7	Serratia arizonae
ALM18	Citrobacter diversus

XIXa Antimicrobial susceptibility test of *Escherichia coli* isolates from cattle faeces and manure

LGA	Sample Type	No. Isolates	Antimicrobial agents											
			Imipenem (IPM) (Disc content = 10µg)			Tetracyclines (TE) (Disc content = 30µg)			Sulfamethoxazole (SXT) (Disc content = 23.75µg)			Ciprofloxazole (CIP) (Disc content = 5µg)		
			S ≥23	I 20-22	R ≤19	S ≥15	I 12-14	R ≤11	S ≥16	I 11-15	R ≤10	S ≥21(30)	I 16-20	R ≤15(20)
Bauchi	Faeces	5	5	0	0	0	0	5	0	0	5	4	0	1
	Manure	0	_	_	_	_	_	_	_	_	_	_	_	_
Darazo	Faeces	6	4	2	0	1	0	5	0	1	5	4	2	0
	Manure	0	_	_	_	_	_	_	_	_	_	_	_	_
Katagum	Faeces	35	24	5	7	19	4	13	0	8	28	14	8	14
	Manure	4	1	1	2	3	0	1	0	0	4	1	3	0
Total		**50**	_	_	_	_	_	_	_	_	_	_	_	_

Key: S =Sensitive, I=Intermediate, R=Resistance, ≥ = Greater than or equal to, ≤ = less than or equal to

XIXb Antimicrobial susceptibility test of *Escherichia coli* isolates from cattle faeces and manure

LGA	Sample Type	No. Isolates	Antimicrobial agents											
			Chloramphenicol (C) (Disc content = 30µg)			Amoxicillin clavulanate (AMC) (Disc content = 10µg+20µg)			Ticarcillinclavulanate (TIM) (Disc content = 75 +10µg)			Gentamycin (CN) (Disc content + 10µg)		
			S ≥18	I 13-17	R ≤12	S ≥18	I 14-17	R ≤13	S ≥20	I 15-19	R ≤14	S ≥15	I 13-14	R ≤12
Bauchi	Faeces	5	4	1	0	1	3	1	2	1	2	5	0	0
	Manure	0	-	-	-	-	-	-	-	-	-	-	-	-
Darazo	Faeces	6	4	2	0	2	0	4	4	1	1	3	1	2
	Manure	0	-	-	-	-	-	-	-	-	-	-	-	-
Katagum	Faeces	35	29	4	3	13	10	13	15	11	10	25	4	7
	Manure	4	3	0	1	0	2	2	2	1	1	2	1	1
Total		50	-	-	-	-	-	-	-	-	-	-	-	-

Key : S =Sensitive, I=Intermediate, R=Resistance, ≥ = Greater than or equal to, ≤ = less than or equal to

XIXc Antimicrobial susceptibility test of *Escherichia coli* isolates from cattle faeces and manure

LGA	Sample Type	No. Isolates	Antimicrobial agents											
			Erythromycin (E) (Disc content = 50µg)			Enrofloxacin (ENR) (Disc content = 5µg)			Ceftriaxone (CRO) (Disc content = 30µg)			Cefazolin (KZ) (Disc content = 30µg)		
			S ≥18	I 13-17	R ≤12	S ≥18	I 14-17	R ≤13	S ≥20	I 15-19	R ≤14	S ≥15	I 13-14	R ≤12
Bauchi	Faeces	5	0	2	3	0	1	4	4	1	0	0	0	5
	Manure	0	-	-	-	-	-	-	-	-	-	-	-	-
Darazo	Faeces	6	0	3	3	0	1	5	2	3	1	0	0	6
	Manure	0	-	-	-	-	-	-	-	-	-	-	-	-
Katagum	Faeces	35	0	10	26	0	9	27	22	3	11	0	**7**	29
	Manure	4	0	1	3	0	0	4	2	1	1	0	**0**	4
Total		50												

Key: S =Sensitive, I=Intermediate, R=Resistance, ≥ = Greater than or equal to, ≤ = less than or equal to

XX Multi-drug resistance pattern of resistant *Escherichia coli* isolates to antimicrobial agents and their classes

S/N	Class/Groups	Drugs	Drug Resistance (%)
1	β lactams	Amoxicillin clavulanate	11(22)
		Ticarcillin clavulanate	5(10)
2	Cephelosporins	Ceftriaxone	6(12)
		Cefazolin	23(46)
3	Aminoglycosides	Gentamicin	5(10)
		Erythromycin	17(34)
4	Macrolides Fluoroquinolones	Ciprofloxacin	6(12)
		Enrofloxacin	21(42)
5	Carbapenems	Imipenem	3(6)
6	Tetracyclines	Tetracycline	24(48)
7	Folate Pathway Inhibitors	Sulfamethoxazole	19(38)
8	Phenicols	Chloramphenicol	3(6)

XXI PCRresults for E. coli isolates

Location	Sample	Detection of Virulence gene		Detection of Resistance gene	
		No. tested	%	No. tested	No positive
BLM	Faeces	1	4	1	11.1
DCF	Faeces	2	8	1	11.1
AA	Faeces	1	4	0	0
ACF	Faeces	11	44	5	55.6
ALM	Faeces	10	40	2	22.2
Total		**25**	**100**	**9**	**100**

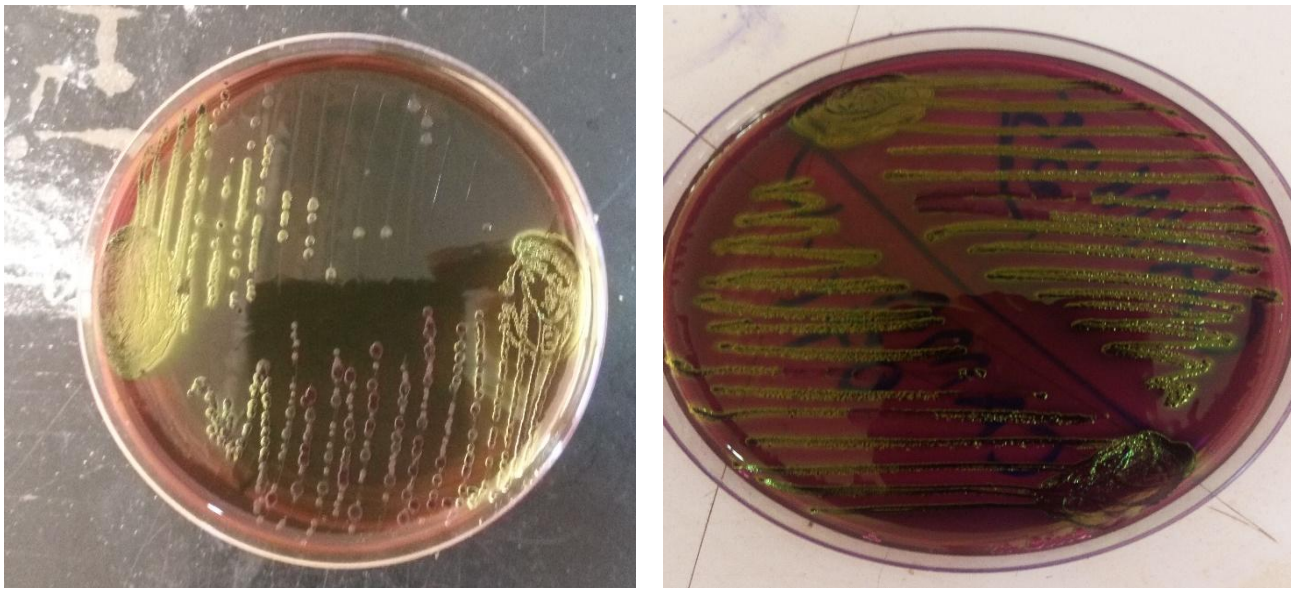
KEY: BLM= Bauchi livestock market, DCF= Darazo cattle farms, AA= Azare abattoir, ACF= Azare cattle farm, ALM= Azare livestock market

XXII *Escherichia coli* strains and distribution of genes detected

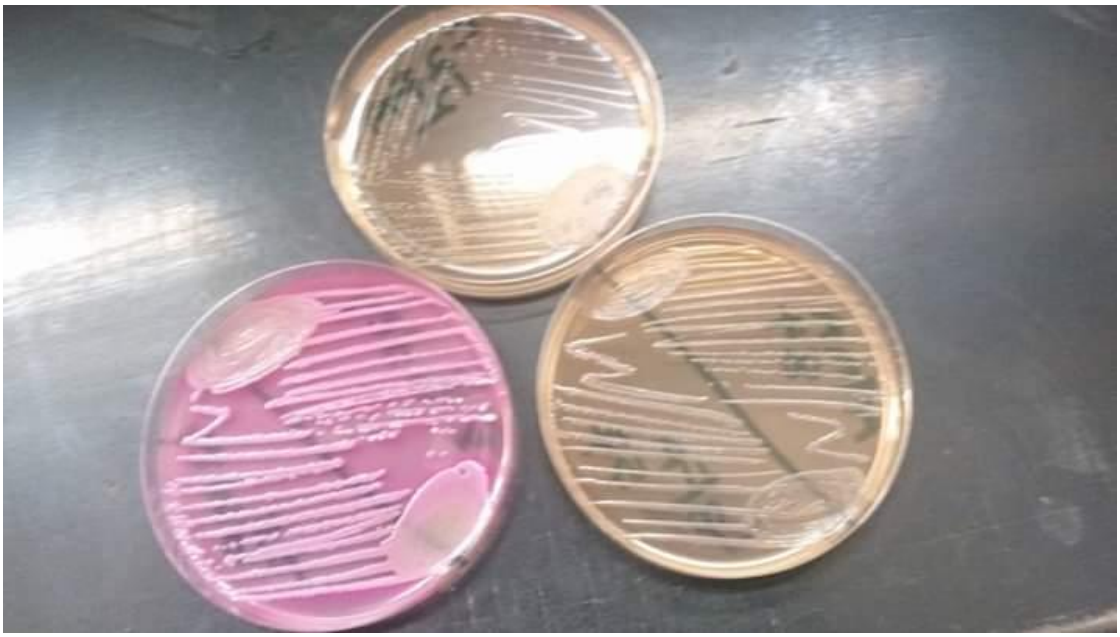
Sample	Location	Organism	Strain of	Virulence	Resistance

I.D			organism	genes	genes
ACAM45	Cattle market	*Escherichia coli*	O157:H7	*stx1*	*tetK*
BLAF57	Livestock market	*Escherichia coli*	Non O157	*stx1*	*tetA, tetK*
DCAM17	Cattle market	*Escherichia coli*	Non O157	*stx1*	*tetA, tetK*
ACAF20	Cattle market	*Escherichia coli*	O157:H7	*stx1*	*tetA, tetK*
ACAM71	Cattle market	*Escherichia coli*	O157:H7	*stx1*	*tetA, tetK*
ACAM84	Cattle market	*Escherichia coli*	O157:H7	*stx1*	*tetA, tetK*
ALAM80	Livestock market	*Escherichia coli*	O157:H7	*stx1, eaeA*	*tetA, tetK*
ACAM83	Cattle market	*Escherichia coli*	Non O157	*stx1*	*tetA, tetB, tetK*
ALAM69	Livestock market	*Escherichia coli*	Non O157	*stx1*	*tetA, tetB, tetK*
DCAM73	Cattle market	*Escherichia coli*	O157:H7	*stx1*	Nil
AAAF4	Abattoir	*Escherichia coli*	Non O157	*stx1, eaeA*	Nil
ACAF25	Cattle market	*Escherichia coli*	O157:H7	*stx1*	Nil
ACYM43	Cattle market	*Escherichia coli*	O157:H7	*stx1*	Nil
ACAM47	Cattle market	*Escherichia coli*	O157:H7	*stx1*	Nil
ACAM60	Cattle market	*Escherichia coli*	O157:H7	*stx1*	Nil
ACAM65	Cattle market	*Escherichia coli*	O157:H7	*stx1*	Nil
ACAM75	Cattle market	*Escherichia coli*	O157:H7	*stx1*	Nil
ALAF18	Livestock market	*Escherichia coli*	O157:H7	*stx1*	Nil
ALAF19	Livestock market	*Escherichia coli*	Non O157	*stx1, eaeA*	Nil
ALAF20	Livestock market	*Escherichia coli*	Non O157	*stx1*	Nil
ALAM37	Livestock market	*Escherichia coli*	O157:H7	*stx1, eaeA*	Nil
ALAM42	Livestock market	*Escherichia coli*	O157:H7	*stx1*	Nil
ALAF73	Livestock market	*Escherichia coli*	O157:H7	*stx1*	Nil
ALAM109	Livestock market	*Escherichia coli*	Non O157	*stx1, eaeA*	Nil
ALAM64	Livestock market	*Escherichia coli*	O157:H7	*stx1, stx2*	Nil

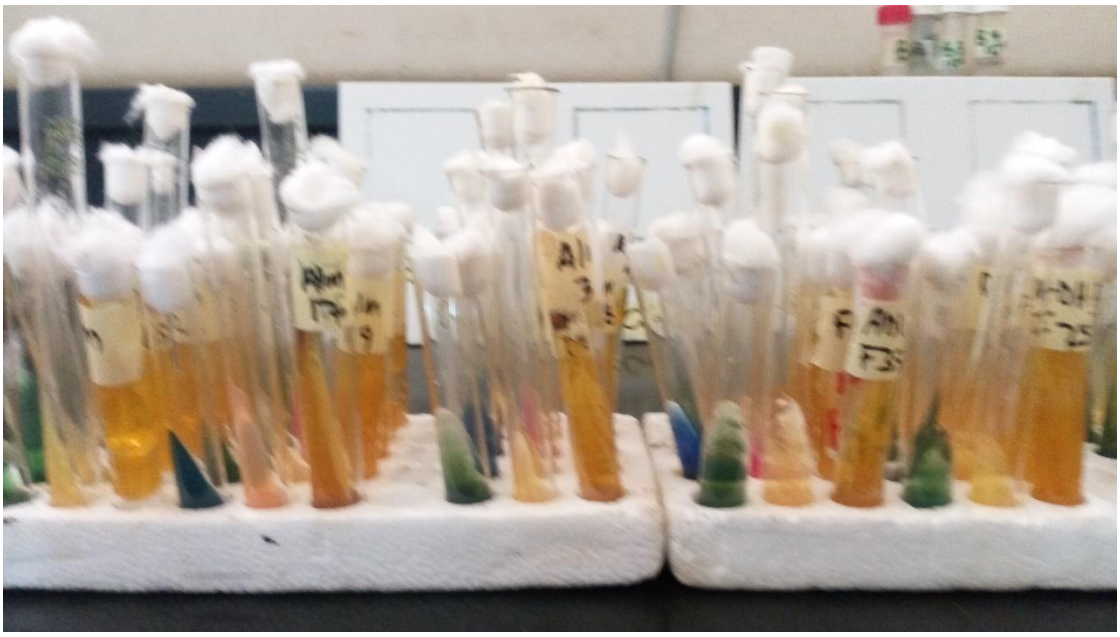
XXIII: Morphological appearance of *E. coli*showing Greenish metallic sheen on Eosin methylene blue Agar



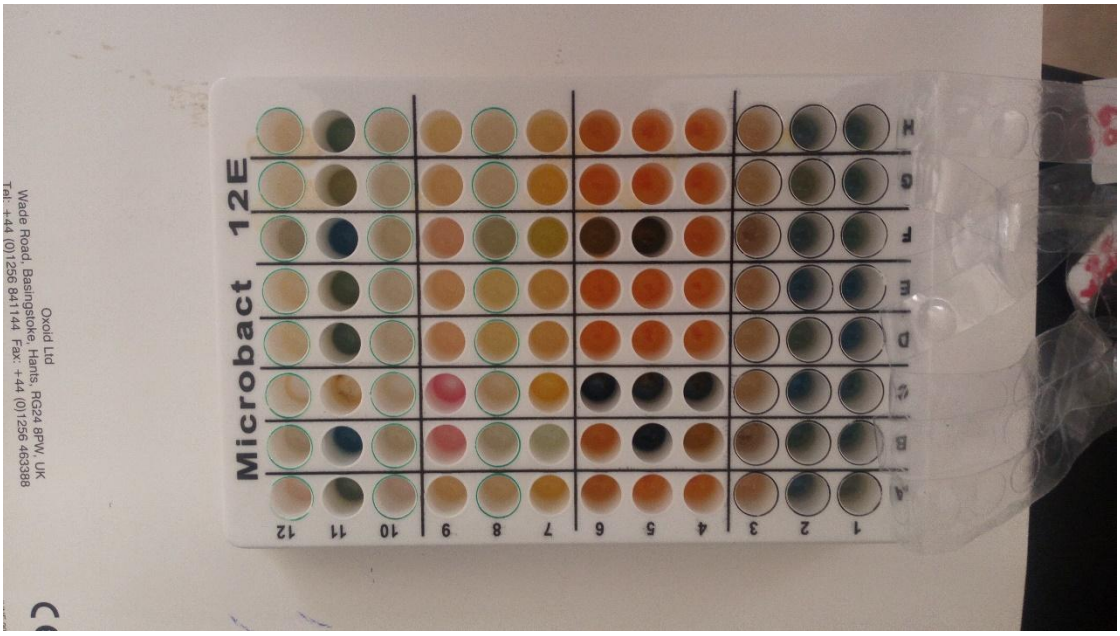
XXIV:Morphological appearance of *E. coli* O157 on CT-Sorbitol MacConkey



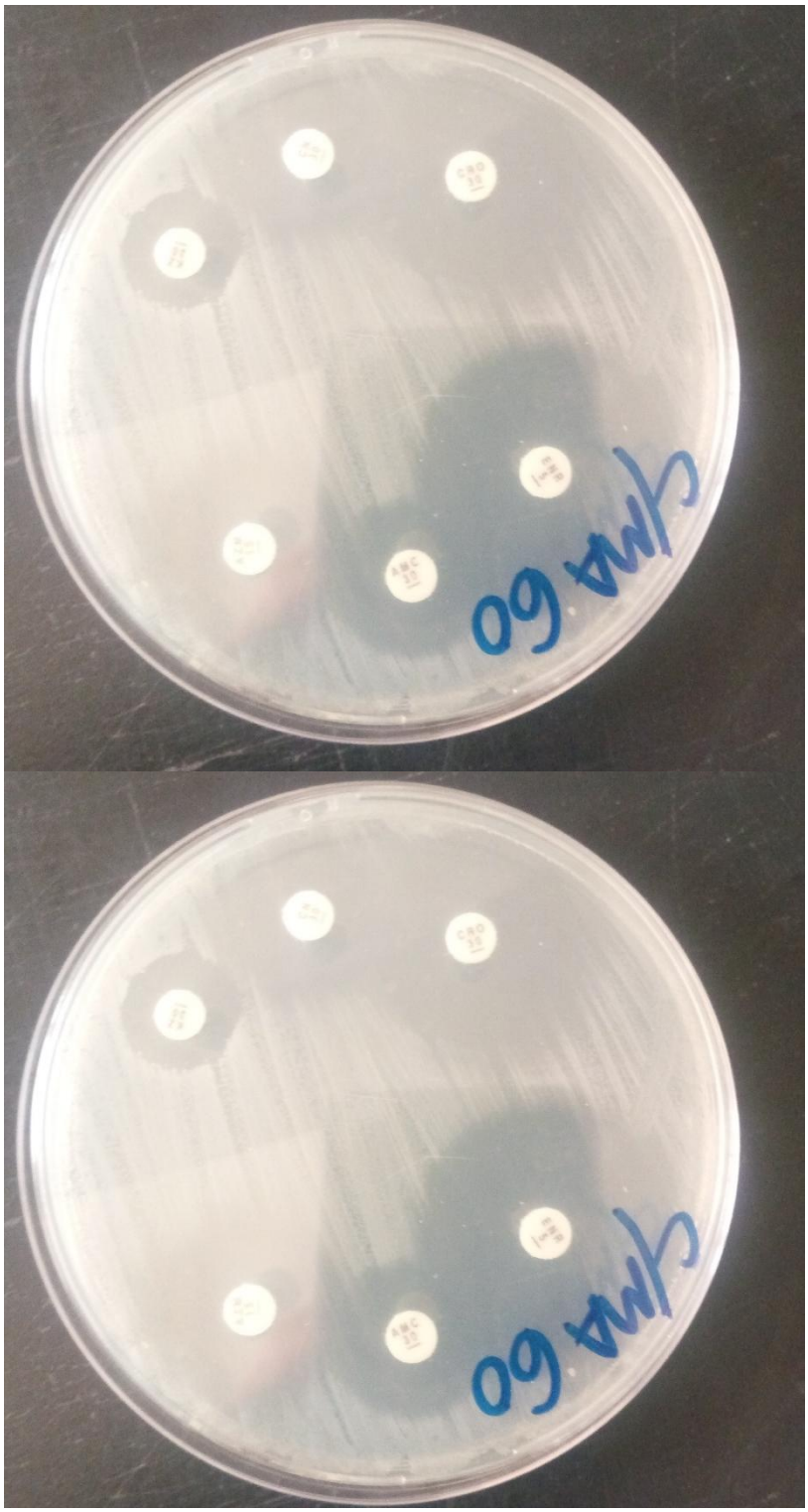
XXV: Plate showing test tubes for conventional Biochemical test results of the *E. coli*isolates



XXVI: Plate showing Biochemical test result for *E. coli* isolates using Microbact 12E



XXVII: Plates showing appearance of zone of inhibition around the Antibiotic disc on Mueller Hinton agar



XXVIII: Conventio4444nal Biochemical Test Results

S/N	SAMPLE NO	EMB	SMC	GMS	TSI	U	C	I	M	H_2S	MR	VP

1	ALMA 84	-	+	-	A/A + G	-	-	+	+	-	+	-
2	L3MA 72	-	+	-	A/A + G	-	-	+	+	-	+	-
3	C2MA 28	-	+	-	A/A + G	-	-	-	+	-	+	-
4	L1MA 45	-	+	-4	A/A + G	-	±	-	+	-	+	-
5	L_3FA 57	-	+	-	A/A + G	-	-	+	+	-	+	-
6	ALMA 44	-	+	-	A/A + G	-	-	+	+	-	+	-
7	ALMA 64	-	+	-	A/A + G	-	-	+	+	-	+	-
8	CAMA 71	-	+	-	A/A + G	-	-	+	+	-	+	-
9	ALAM 42	-	-	-	A/A + G	-	-	+	+	-	+	-
10	ALMA 80	-	-	-	A/A + G	-	-	+	+	-	+	-
11	ALMA 174	-	-	-	A/A + G	-	±	-	+	-	+	-
12	CIFA 10	-	-	-	A/A + G	-	-	-	+	-	+	-
13	CIMA 60	-	-	-	A/A + G	-	±	+	+	-	+	-
14	ALMA 66	-	-	-	A/A + G	-	±	+	+	-	+	-
15	CIFA 58	-	-	-	A/A + G	-	-	-	+	-	+	-
16	CAYM 35	-	-	-	A/A + G	-	+	-	+	-	+	-
17	CAMA 95	+	-	-	A/A + G	-	-	+	+	-	+	-
18	ALMA 75	-	-	-	A/A + G	-	-	+	+	-	+	-
19	CAYMA 71	-	-	-	A/A + G	-	-	+	+	-	+	-
20	ALMA 162	-	-	-	A/A + G	-	-	+	+	-	+	-
21	CAM_2BS	-	-	-	A/A + G	-	-	+	+	-	+	-
22	CAM 17	-	-	-	A/A + G	-	-	+	+	-	+	-
23	CAMA 84	-	-	-	A/A + G	-	-	+	+	-	+	-
24	ALAM 73	-	-	-	A/A + G	-	-	+	+	-	+	-
25	CAFA 20	-	-	-	A/A + G	-	-	+	+	-	+	-
26	CAYM 34	+	-	-	A/A + G	-	-	+	+	-	+	-
27	ALFA 208	+	-	-	A/A + G	-	-	+	+	-	+	-
28	CAMA 75	+	-	-	A/A + G	-	-	+	+	-	+	-
29	DAYM 35	+	-	-	A/A + G	-	-	+	+	-	+	-
30	DAM 18	+	-	-	A/A + G	-	-	+	+	-	+	-
31	ALMA 93	+	-	-	A/A + G	-	-	+	+	-	+	-
32	$CAMA\ 47_5$	+	-	-	A/A + G	-	-	+	+	-	+	-
33	CAM 73	+	-	-	A/A + G	-	-	+	+	-	+	-
34	$CAM16_5$	+	-	-	A/A + G	-	-	+	+	-	+	-
35	ALAM 79	+	-	-	A/A + G	-	-	+	+	-	+	-
36	$CAMA\ 4^05$	+	-	-	A/A + G	-	-	+	+	-	+	-
37	CAMA 56	+	-	-	A/A + G	-	-	+	+	-	+	-
38	CAFA 25	+	-	-	A/A + G	-	-	+	+	-	+	-
39	ALAM 133	+	-	-	A/A + G	-	-	+	+	-	+	-
S/N	**SAMPLE NO**	**EMB**	**SMC**	**GS**	**TSI**	**U**	**C**	**I**	**M**	**H_2S**	**MR**	**VP**
40	$CAMA\ 65_5$	+	-	-	A/A + G	-	-	+	+	-	+	-
41	CAMA 53	+	-	-	A/A + G	-	-	+	+	-	+	-
42	$CAYM34_5$	+	-	-	A/A + G	-	-	+	+	-	+	-
43	CAMA 83	+	-	-	A/A + G	-	-	+	+	-	+	-
44	$CAMA\ 60_5$	+	-	-	A/A + G	-	-	+	+	-	+	-
45	$ALM\ 9_5$	+	-	-	A/A + G	-	-	+	+	-	+	-
46	ALAF 119	+	-	-	A/A + G	-	-	+	+	-	+	-

47	ALMA 139	+	-	-	A/A + G	-	-	+	+	-	+	-
48	ALAMA 86	+	-	-	A/A + G	-	-	+	+	-	+	-
49	ALMA 4	+	-	-	A/A + G	-	-	+	+	-	+	-
50	ALMA 18	+	-	-	A/A + G	-	-	+	+	-	+	-
51	ALAM 69	+	-	-	A/A + G	-	-	+	+	-	+	-
52	ALFA 19_2		-	-	A/A + G	-	-	+	+	-	+	-
53	AAAF 4	+	-	-	A/A + G	-	-	+	+	-	+	-
54	ALAF 181	+	-	-	A/A + G	-	-	+	+	-	+	-
55	ALM 13_5	+	-	-	A/A + G	-	-	+	+	-	+	-
56	ALAFA 173	+	-	-	A/A + G	-	-	+	+	-	+	-
57	ALMA 174	+	-	-	A/A + G	-	-	+	+	-	+	-
58	DAAM 14	+	-	-	A/A + G	-	-	+	+	-	+	-
59	ALMA 114	+	-	-	A/A + G	-	-	+	+	-	+	-
60	ALAF 96	+	-	-	A/A + G	-	-	+	+	-	+	-
61	ALMA 142	+	-	-	A/A + G	-	-	+	+	-	+	-
62	ALFA 144	+	-	-	A/A + G	-	-	+	+	-	+	-
63	YF 50	+	-	-	A/A + G	-	-	+	+	-	+	-
64	DLAM 2	+	-	-	A/A + G	-	-	+	+	-	+	-
65	C3AM 2	+	-	-	A/A + G	-	-	+	+	-	+	-
66	DCAF 5	+	-	-	A/A + G	-	-	+	+	-	+	-
67	ALMA 177	+	-	-	A/A + G	-	-	+	+	-	+	-
68	DCYM 53	+	-	-	A/A + G	-	-	+	+	-	+	-
69	ALYM 61	+	-	-	A/A + G	-	-	+	+	-	+	-
70	ALAF 33	+	-	-	A/A + G	-	-	+	+	-	+	-
71	ALAM 76	+	-	-	A/A + G	-	-	+	+	-	+	-
72	ALYM 156	+	-	-	A/A + G	-	-	+	+	-	+	-
73	ADF 27	+	-	-	A/A + G	-	-	+	+	-	+	-
74	ALM 185	+	-	-	A/A + G	-	-	+	+	-	+	-
75	ALM 466	+	-	-	A/A + G	-	-	+	+	-	+	-
76	ALFA 231	+	-	-	A/A + G	-	-	+	+	-	+	-
77	ALAM 80	+	-	-	A/A + G	-	-	+	+	-	+	-
78	ALM 7s	+	-	-	A/A + G	-	-	+	+	-	+	-
79	ALMA 199	+	-	-	A/A + G	-	-	+	+	-	+	-
80	CAMA122	+	-	-	A/A + G	-	-	+	+	-	+	-

XXIX: Microbact 12E Positive Result for *E. COLI*

S/N	SAMPLE NO	CODE	IDENTIFICATION
1	ALMA 84	6760	*Escherichia coli*
2	L_3MA 72	6760	*Escherichia coli*
3	L_3FA 57	6760	*Escherichia coli*
4	ALMA 44	6760	*Escherichia coli*

5	ALMA 64	6760	*Escherichia coli*
6	CAMA 71	6760	*Escherichia coli*
7	ALAM 42	6760	*Escherichia coli*
8	ALMA 80	6760	*Escherichia coli*
9	ALMA 174	6462	*Escherichia coli*
10	CIFA 10	6760	*Escherichia coli*
11	CAYM 35	6762	*Escherichia coli*
12	CAMA 95	6760	*Escherichia coli*
13	ALMA 75	6762	*Escherichia coli*
14	CAYMA 71	6760	*Escherichia coli*
15	ALMA 162	6760	*Escherichia coli*
16	CAM $2B_S$	6760	*Escherichia coli*
17	CAM 17	6760	*Escherichia coli*
18	CAMA 84	6762	*Escherichia coli*
19	ALAM 73	6760	*Escherichia coli*
20	CAFA 20	6762	*Escherichia coli*
21	CAYM 34	6760	*Escherichia coli*
22	ALFA 208	6760	*Escherichia coli*
23	CAMA 75	6760	*Escherichia coli*
24	DAYM 35	6760	*Escherichia coli*
25	DAM 18	6760	*Escherichia coli*
S/N	**SAMPLE NO**	**CODE**	**IDENTIFICATION**
26	ALAM 93	6760	*Escherichia coli*
27	CAMA 47_s	6760	*Escherichia coli*
28	CAMA 73	6760	*Escherichia coli*
29	CAMA 16_s	6760	*Escherichia coli*
30	ALAM 79	6760	*Escherichia coli*
31	CAMA 45	6760	*Escherichia coli*
32	CAMA 56	6762	*Escherichia coli*
33	CAFA 25	6760	*Escherichia coli*
34	ALAM 133	6762	*Escherichia coli*

35	CAMA 65_s	6760	*Escherichia coli*
36	CAMA 53	6760	*Escherichia coli*
37	CAYM 34_s	6760	*Escherichia coli*
38	CAMA 83	6760	*Escherichia coli*
39	CAMA 60_s	6762	*Escherichia coli*
40	ALM 9_s	6762	*Escherichia coli*
41	ALAF 119	6762	*Escherichia coli*
42	ALMA 139	6760	*Escherichia coli*
43	AMA 86	6762	*Escherichia coli*
44	ALMA 4	6760	*Escherichia coli*
45	ALMA 18	6760	*Escherichia coli*
46	ALAM 69	6762	*Escherichia coli*
47	ALFA 19	6762	*Escherichia coli*
48	AAAF 04	6762	*Escherichia coli*
49	ALAF 181	6762	*Escherichia coli*
50	ALM 13_s	6762	*Escherichia coli*
51	ALAFA 173	6760	*Escherichia coli*

XXX: WELCOLEX *E. COLI* O157:H7 Serological Test Result

S/N	SAMPLE NO	O157	H7	Sorbitol MacConkey
1	ALMA 84	-	-	-
2	L_3MA 72	+	+	+
3	L_3FA 57	-	-	+
4	ALMA 44	+	+	+
5	ALMA 64	+	+	+
6	CAMA 71	+	+	+
7	ALAM 42	+	+	-

8	ALMA 80	+	+	-
9	ALMA 174	+	+	+
10	CIFA 10	-	-	-
11	CAYM 35	+	+	-
12	CAMA 95	-	-	+
13	ALMA 75	-	-	-
14	CAYMA 71	-	-	-
15	ALMA 162	-	-	-
16	CAM $2B_S$	-	-	+
17	CAM 17	-	-	-
18	CAMA 84	+	+	+
19	ALAM 73	+	+	-
20	CAFA 20	+	+	-
S/N	**SAMPLE NO**	**O157**	**H7**	**Sorbitol MacConkey**
21	CAYM 34	+	+	-
22	ALFA 208	-	-	-
23	CAMA 75	+	+	-
24	DAYM 35	-	-	-
25	DAM 18	+	+	-
26	ALAM 93	-	-	-
27	CAMA 47_s	+	+	+
28	CAMA 73	+	+	-
29	CAMA 16_s	-	-	+

30	ALAM 79	-	+	-
31	CAMA 40_s	+	+	+
32	CAMA 56	-	-	-
33	CAFA 25	+	+	-
34	ALAM 133	+	+	-
35	CAMA 65_s	+	+	+
36	CAMA 53	-	-	-
37	CAYM 34_s	-	-	+
38	CAMA 83	-	-	-
39	CAMA 60_s	+	+	+
40	ALMA 9_s	+	+	+
41	ALAF 119	+	+	-
S/N	**SAMPLE NO**	**O157**	**H7**	**Sorbitol MacConkey**
42	ALMA 139	+	+	-
43	ALMA 86	+	+	-
44	ALMA 4	+	+	-
45	ALMA 18	-	-	-
46	ALAM 69	-	-	-
47	ALFA 19s	-	-	-
48	AAAF 04	-	-	-
49	ALAF 181	+	+	-
50	ALMA 13_s	+	+	+
51	ALAFA 173	+	+	-

XXX: List of Antibiotics Used and their Classes

S/No	Names		Class	Sensitive ≥	Intermediate	Resistance ≤
1	Imipenem	**IPM**	**Carbapenems**	23	20-22	19
2	Tetracycline	**TE**	**Tetracyclines**	15	12-14	11
3	Sulfamethoxazole	**SXT**	**Folate Pathway Inhibitors**	16	11-15	10
4	Ciprofloxacin	**CIP**	**Fluoroquinolones**	21 (31)	16-20	15 (20)
5	Chloramphenicol	**C**	**Phenicols**	18	13-17	12
6	Amoxicillin clavulanate	**AMC**	**β lactamase**	18	14-17	13
7	Ticarcillin clavulanate	**TIM**		20	15-19	14
8	Gentamicin	**CN**	**Aminoglycosides**	15	13-14	12
9	Erythromycin	**E**		23	14-22	13
10	Enrofloxacin	**ENR**		17	18-21	22
11	Ceftriaxone	**CRO**	**Cephems**	23	20-22	19
12	Cefazolin	**KZ**		23	20-22	19

XXXII: Antibiotic Susceptibility Result for the *E. coli*Isolates,zone of Inhibition (mm)

S/N	SAMPLE NO	IPM	TE	C	SXT	E	CIP	ENR	CRO	CN	KZ	AMC	TIM
1	CAMA 71	25	10	25	0	12	30	24	24	18	15	15	20
2	ALMA 44	21	17	19	19	11	22	32	33	24	18	22	23
3	L3MA 72	26	10	22	22	15	25	21	26	17	14	16	20
4	L3FA 57	31	22	20	24	18	0	22	25	15	12	15	14
5	CIFA 10	32	7	25	0	8	30	27	21	16	10	16	17
6	CIMA 60	23	0	25	0	10	31	32	25	21	15	16	20
7	ALMA 80	25	0	22	0	14	30	17	18	18	12	18	22
8	ALAM 42	0	18	23	15	12	29	21	11	16	0	10	14
9	ALMA 174	32	0	0	0	0	0	22	27	32	0	0	22
10	CAYM 35	24	20	22	20	12	25	30	27	23	19	25	28
11	ALMA 64	25	20	24	20	12	25	21	16	14	13	16	15
12	CAMA 95	24	17	20	10	0	30	18	20	17	0	16	14
13	ALMA 75	34	10	36	14	12	24	25	14	0	10	12	20
14	CYMA 71	23	20	20	12	10	29	32	10	20	17	10	18
15	ALMA 162	0	19	22	0	15	24	30	21	10	15	16	14
16	CAM $2B_5$	35	10	16	0	13	28	17	25	21	12	22	10
17	CAM 17	27	0	19	0	12	31	13	27	14	10	22	10
18	CAMA 84	29	0	24	17	10	33	24	30	28	14	13	20
19	ALAM 73	26	15	27	20	0	22	19	14	20	17	10	24
20	CAFA 20	36	10	0	12	0	26	31	19	13	0	10	24
21	CAMYM34	23	13	0	10	17	18	22	24	20	0	11	17
22	ALFA 208	20	12	24	19	13	33	20	19	16	0	0	15
23	CAMA 75	23	17	20	0	0	0	30	12	12	0	17	28
24	DAYM 35	21	10	16	0	20	27	29	20	10	0	20	23
25	DAM 18	30	0	18	0	22	29	34	22	0	0	0	22
26	ALAM 93	29	22	22	26	10	28	28	26	18	15	20	15
27	$CAMA47_5$	28	19	25	23	19	19	30	30	19	21	19	12
28	CAM 73	30	0	27	22	18	19	30	27	21	13	20	17
29	$CAMA\ 16_5$	22	10	16	15	14	17	23	20	17	10	0	20
30	ALAM 79	20	18	17	0	10	15	20	22	20	20	18	15
31	$CAMA\ 40_5$	25	10	22	21	0	17	19	28	0	26	17	27

S/N	SAMPLE NO	IPM	TE	C	SXT	E	CIP	ENR	CRO	CN	KZ	AMC	TIM
32	CAMA 56	31	26	20	0	0	18	27	21	9	0	16	22
33	CAFA 25	28	22	21	0	20	19	34	30	15	10	21	28
34	ALAM 133	23	16	28	20	15	15	30	27	12	12	28	10
35	CAMA 65_3	30	20	25	22	17	18	31	25	15	21	20	13
36	CAMA 53	22	19	30	28	12	18	31	25	15	21	20	13
37	CAYM 43_5	21	16	24	16	10	33	23	29	18	20	20	14
38	CAMA 83	29	10	20	13	0	12	22	31	16	17	17	17
39	CAMA 60_5	34	20	16	0	13	15	30	28	14	0	15	20
40	ALM 9_5	19	0	26	0	15	17	28	35	17	14	16	13
41	ALAF119	29	0	27	18	11	14	20	10	20	23	13	15
42	ALMA139	27	18	17	0	15	20	29	18	21	0	27	28
43	ALMA 86	21	17	20	11	20	22	35	23	19	19	21	28
44	ALMA 4	36	0	28	28	10	20	30	18	15	23	20	16
45	ALAM 18	30	20	21	20	0	11	19	30	0	17	15	27
46	ALAM 69	27	13	30	0	20	15	27	29	22	13	0	14
47	ALFA 192	29	10	18	14	0	13	33	23	10	21	13	20
48	AAAF 4	31	0	31	10	11	14	36	31	13	0	12	17
49	ALAF 181	25	13	20	15	0	21	20	23	15	12	14	18
50	ALM 13_5	28	16	26	10	13	16	34	29	14	10	0	15
51	ALAFA173	30	15	17	16	14	19	27	31	16	20	0	22

XXXIII: Antibiotic Sensitivity Test Result for *E. coli*

SAMPLE NO	IPM	TE	C	SXT	E	CIP	ENR	CRO	CN	KZ	AMC	TIM	NO.	MAR Index
CAMA 71	S	R	S	R	R	S	R	S	S	R	I	S	**5**	0.4
ALMA 44	I	S	S	R	R	S	R	S	S	R	R	S	**5**	0.4
L3MA 72	S	R	S	R	I	S	I	S	S	R	I	S	**3**	0.2
L3FA 57	S	R	S	R	I	R	R	S	S	R	R	R	**7**	0.6
CIFA 10	S	R	S	R	R	S	R	I	S	R	I	I	**5**	0.4
CIMA 60	S	R	S	R	R	S	R	S	S	R	I	S	**5**	0.4
ALMA 80	S	R	S	R	I	S	R	R	S	R	S	R	**6**	0.5
ALAM 42	R	S	S	I	R	S	I	R	S	R	R	R	**6**	0.5
ALMA 174	S	R	R	R	R	R	R	S	S	R	R	S	**8**	0.7
CAYM 35	S	S	S	R	R	R	R	S	S	R	S	S	**5**	0.4
ALMA 64	S	S	S	R	R	S	I	R	I	R	I	I	**4**	0.3
CAMA 95	S	S	S	R	R	S	I	I	S	R	I	R	**4**	0.3
ALMA 75	S	R	S	I	R	S	R	R	R	R	R	S	**7**	0.6
CYMA 71	S	S	S	I	R	S	R	R	S	R	R	I	**5**	0.4
ALMA 162	R	S	S	R	I	S	R	I	R	R	I	R	**6**	0.5
CAM $2B_5$	S	R	I	R	R	S	R	S	S	R	S	R	**6**	0.5
CAM 17	S	R	S	R	R	S	R	S	I	R	S	R	**6**	0.5
CAMA 84	S	R	S	R	R	S	R	S	S	R	R	S	**6**	0.5
ALAM 73	S	S	S	R	R	S	I	R	S	R	R	S	**5**	0.4
CAFA 20	S	R	R	I	R	S	R	R	I	R	R	S	**7**	0.6
CAMYM34	S	I	R	R	I	I	R	S	S	R	R	I	**5**	0.4
ALFA 208	I	I	S	R	R	S	I	R	S	R	R	I	**5**	0.4
CAMA 75	S	S	S	R	R	R	R	R	R	R	I	S	**7**	0.6
DAYM 35	I	R	I	R	R	S	R	I	R	R	R	S	**7**	0.6
DAM 18	S	R	S	R	I	S	R	I	R	R	R	S	**6**	0.5
SAMPLE NO	**IPM**	**TE**	**C**	**SXT**	**E**	**CIP**	**ENR**	**CRO**	**CN**	**KZ**	**AMC**	**TIM**	**NO.**	**MAR Index**

ALAM 93	S	R	S	R	R	S	R	S	S	R	S	I	**5**	0.4
CAMA47$_5$	S	S	S	R	I	I	R	S	S	I	S	R	**3**	0.2
CAM 73	S	R	S	R	I	I	R	S	S	R	S	I	**4**	0.3
CAMA 16$_5$	I	R	I	I	I	I	R	I	S	R	R	S	**4**	0.3
ALAM 79	I	S	I	R	R	R	I	I	S	I	S	I	**3**	0.2
CAMA 40$_5$	S	R	S	R	R	I	I	S	R	R	I	S	**5**	0.4
CAMA 56	S	S	S	R	R	I	R	I	R	R	I	S	**5**	0.4
CAFA 25	S	S	S	R	R	R	R	S	S	R	S	S	**5**	0.4
ALAM 133	S	S	S	R	I	R	R	S	R	R	S	R	**6**	0.5
CAMA 65$_3$	S	S	S	R	I	I	R	S	S	I	S	R	**3**	0.2
CAMA 53	I	S	S	R	R	I	R	S	S	I	S	R	**4**	0.3
CAYM 43$_5$	I	S	S	R	R	S	R	S	S	I	S	R	**4**	0.3
CAMA 83	S	R	S	I	R	R	R	S	S	R	I	I	**5**	0.4
CAMA 60$_5$	S	S	I	R	R	R	R	S	I	R	I	S	**5**	0.4
ALM 9$_5$	R	R	R	R	I	I	R	R	S	R	I	R	**8**	0.7
ALAF119	S	R	S	R	R	R	I	R	S	R	R	I	**7**	0.6
ALMA139	S	S	I	R	I	I	R	R	S	R	S	S	**4**	0.3
ALMA 86	I	S	S	I	I	S	R	S	S	R	S	S	**2**	
ALMA 4	S	R	S	R	R	I	R	R	S	R	S	I	**6**	0.5
ALAM 18	S	S	S	R	R	R	I	S	R	R	I	S	**5**	0.4
ALAM 69	S	I	S	R	I	R	R	S	S	R	R	R	**6**	0.5
ALFA 192	R	R	S	I	R	R	R	S	R	I	R	S	**7**	0.6
AAAF 4	R	R	S	R	R	R	R	S	I	R	R	I	**8**	0.7
ALAF 181	R	I	S	I	R	R	I	S	S	R	I	I	**4**	0.3
ALM 13$_5$	R	S	S	R	R	I	R	S	I	R	R	I	**6**	0.5
ALFA173	R	S	I	R	I	I	R	S	S	I	R	S	**4**	0.3

0.2=5, 0.3=9, 0.4=15, 0.5=11, 0.6=7, 0.7=3

XXXIV: Summary of Antibiotics Showing Resistance to Isolates

SAMPLE NO	**IPM**	**TE**	**C**	**SXT**	**E**	**CIP**	**ENR**	**CRO**	**CN**	**KZ**	**AMC**	**TIM**	

CAMA 71	S	R	S	R	R	S	R	S	S	R	I	S	TE, SXT, ENR, KZ
ALMA 44	I	S	S	R	R	S	R	S	S	R	R	S	SXT, E, ENR, KZ, AMC
L3MA 72	S	R	S	R	I	S	I	S	S	R	I	S	TE, SXT, KZ,
L3FA 57	S	R	S	R	I	R	R	S	S	R	R	R	TE, SXT, CIP, ENR, KZ, AMC, TIM
CIFA 10	S	R	S	R	R	S	R	I	S	R	I	I	TE, SXT, E, ENR, KZ
CIMA 60	S	R	S	R	R	S	R	S	S	R	I	S	TE, SXT, E, ENR, KZ
ALMA 80	S	R	S	R	I	S	R	R	S	R	S	R	TE, SXT, ENR, CRO, KZ, TIM
ALAM 42	R	S	S	I	R	S	I	R	S	R	R	R	IPM, E, CRO, KZ, AMC, TIM
ALMA 174	S	R	R	R	R	R	R	S	S	R	R	S	TE, C, SXT, E, CIP, ENR, KZ, AMC
CAYM 35	S	S	S	R	R	R	R	S	S	R	S	S	SXT, E, CIP, ENR, KZ
ALMA 64	S	S	S	R	R	S	I	R	I	R	I	I	SXT, E, CRO, KZ,
CAMA 95	S	S	S	R	R	S	I	I	S	R	I	R	SXT, E, KZ, TIM
ALMA 75	S	R	S	I	R	S	R	R	R	R	R	S	TE, E, ENR, CRO, CN, KZ, AMC
CYMA 71	S	S	S	I	R	S	R	R	S	R	R	I	E, ENR, CRO, KZ, AMC
ALMA 162	R	S	S	R	I	S	R	I	R	R	I	R	IPM, SXT, ENR, CN, KZ, TIM
CAM $2B_5$	S	R	I	R	R	S	R	S	S	R	S	R	TE, SXT, E, ENR, KZ, TIM
CAM 17	S	R	S	R	R	S	R	S	I	R	S	R	TE, SXT, E, ENR, KZ, TIM
CAMA 84	S	R	S	R	R	S	R	S	S	R	R	S	TE,SXT, E, ENR, KZ, AMC
ALAM 73	S	S	S	R	R	S	I	R	S	R	R	S	SXT, E, CRO, KZ, AMC
CAFA 20	S	R	R	I	R	S	R	R	I	R	R	S	TE, C, E, ENR, CRO, KZ, AMC
CAMYM34	S	I	R	R	I	I	R	S	S	R	R	I	C, SXT, ENR, KZ, AMC
ALFA 208	I	I	S	R	R	S	I	R	S	R	R	I	SXT, E, CRO, KZ, AMC
CAMA 75	S	S	S	R	R	R	R	R	R	R	I	S	SXT, E, CIP, ENR, CRO, CN, KZ,
DAYM 35	I	R	I	R	R	S	R	I	R	R	R	S	TE, SXT, E, ENR, CN, KZ, AMC
DAM 18	S	R	S	R	I	S	R	I	R	R	R	S	TE, SXT, ENR, CN, KZ, AMC
SAMPLE NO	**IPM**	**TE**	**C**	**SXT**	**E**	**CIP**	**ENR**	**CRO**	**CN**	**KZ**	**AMC**	**TIM**	
ALAM 93	S	R	S	R	R	S	R	S	S	R	S	I	TE, SXT, E, ENR, KZ
CAMA 47_5	S	S	S	R	I	I	R	S	S	I	S	R	SXT, ENR, TIM
CAM 73	S	R	S	R	I	I	R	S	S	R	S	I	TE, SXT, ENR, KZ,
CAMA 16_5	I	R	I	I	I	I	R	I	S	R	R	S	TE, ENR, KZ, AMC

ALAM 79	I	S	I	R	R	R	I	I	S	I	S	I	SXT, E, CIP,
CAMA 40_5	S	R	S	R	R	I	I	S	R	R	I	S	TE, SXT, E, CN, KZ,
CAMA 56	S	S	S	R	R	I	R	I	R	R	I	S	SXT, E, ENR, CN, KZ,
CAFA 25	S	S	S	R	R	R	R	S	S	R	S	S	SXT, E, CIP, ENR, KZ
ALAM 133	S	S	S	R	I	R	R	S	R	R	S	R	SXT, CIP, ENR, CN, KZ, TIM
CAMA 65_3	S	S	S	R	I	I	R	S	S	I	S	R	SXT, ENR, TIM
CAMA 53	I	S	S	R	R	I	R	S	S	I	S	R	SXT, E, ENR,TIM
CAYM 43_5	I	S	S	R	R	S	R	S	S	I	S	R	SXT, E, ENR, TIM
CAMA 83	S	R	S	I	R	R	R	S	S	R	I	I	TE, E, CIP, ENR, KZ,
CAMA 60_5	S	S	I	R	R	R	R	S	I	R	I	S	SXT, E, CIP, ENR, KZ,
ALM 9_5	R	R	R	R	I	I	R	R	S	R	I	R	IPM, TE, C, SXT, ENR, CRO, KZ, TIM
ALAF119	S	R	S	R	R	R	I	R	S	R	R	I	TE, SXT, E, CIP, CRO, KZ, AMC
ALMA139	S	S	I	R	I	I	R	R	S	R	S	S	SXT, ENR, CRO, KZ
ALMA 86	I	S	S	I	I	S	R	S	S	R	S	S	ENR, KZ,
ALMA 4	S	R	S	R	R	I	R	R	S	R	S	I	TE, SXT, E, ENR, CRO, KZ,
ALAM 18	S	S	S	R	R	R	I	S	R	R	I	S	SXT, E, CIP, CN, KZ,
ALAM 69	S	I	S	R	I	R	R	S	S	R	R	R	SXT, CIP, ENR, KZ, AMC, TIM
ALFA 192	R	R	S	I	R	R	R	S	R	I	R	S	IPM, TE, E, CIP, ENR, CN, AMC
AAAF 4	R	R	S	R	R	R	R	S	I	R	R	I	IPM, TE, SXT, E, CIP, ENR, KZ, AMC
ALAF 181	R	I	S	I	R	R	I	S	S	R	I	I	IPM, E, CIP, KZ,
ALM 13_5	R	S	S	R	R	I	R	S	I	R	R	I	IPM, SXT, E, ENR, KZ, AMC
ALAFA173	R	S	I	R	I	I	R	S	S	I	R	S	IPM, SXT, ENR, AMC

XXXV: Isolates Sent for PCR

S/N	SAMPLE NO	SAMPLE I.D	Serotypes
1	CAMA 73	11	O157
2	ALMA 80	6	O157
3	CAFA 20	17	O157
4	CAMA 83	8	O157
5	CAFA 25	4	O157
6	CAMA 71	13	O157
7	CAMA 47	23	O157
8	CAYM 34	18	O157
9	ALAF 181	3	O157
10	ALAM 73	15	O157
11	ALFA 173	10	O157
12	CAMA 75	22	O157
13	ALAM 42	20	O157
14	ALAM 119	5	O157
15	CAMA 84	9	O157
16	ALMA 65	1	O157
17	CAMA 40	21	O157
1	CAMA 17	12	NonO157
2	ALAM 65	25	NonO157
3	L_3FA 57	7	NonO157
4	ALAM 69	19	NonO157
5	ALAM 69	24	NonO157
6	CAMA 60	2	NonO157
7	ALFA 192	14	NonO157
8	AAF 4	16	NonO157

XXXV: PCR Results showing genes distribution

LGA	Source	*Virulence*	Resistance	Total
Katagum	Market	*stx1*	*tetA* , *tetK*	3
Katagum	Farms	*stx1*	*tetA* , *tetK*	3
Katagum	Farms	*stx1*	*tetA* , *tetK*	3
Katagum	Farms	*stx1*	*tetA* , *tetK*, *tetB*	4
Bauchi	Market	*stx1*	*tetA* , *tetK*	3
Katagum	Farms	*stx1*	*tetA* , *tetK*	3
Katagum	Market	*stx1*	*tetB*	2
Katagum	Market	*stx1*	*tetA*, *tetK*	3
Katagum	Farms	*stx1*	*tetA*, *tetK*	3