UNIVERSITI PUTRA MALAYSIA

OCCURRENCE OF ANTIBIOTIC RESISTANT PATHOGENIC Escherichia coli IN CATTLE, FARM ENVIRONMENT, MILK AND BEEF

MIAN KHAQAN SHAH

FPV 2017 4

OCCURRENCE OF ANTIBIOTIC RESISTANT PATHOGENIC *Escherichia coli* IN CATTLE, FARM ENVIRONMENT, MILK AND BEEF

By

MIAN KHAQAN SHAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

January 2017

DEDICATION

This thesis is especially dedicated to:
My beloved parents,
MIAN USMAN SHAH (Late)
and
RIFFAT BIBI

My beloved siblings,
MARIA USMAN
SUMAYYA USMAN
MIAN HAROON SHAH

My grandmother and aunt
RAHAT BIBI (Late)
BUSHRA NOSHEEN

And my precious friend
COL LIN LEE

Who always pray, trust, support and encourage me to do the best

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

OCCURRENCE OF ANTIBIOTIC RESISTANT PATHOGENIC *Escherichia coli* IN CATTLE, FARM ENVIRONMENT, MILK AND BEEF

By

MIAN KHAQAN SHAH

January 2017

Chairman: Professor Saleha Abdul Aziz, PhD
Faculty : Veterinary Medicine

Antibiotic usage is one of the key factor which has been widely reported to cause the emergence and spread of antibiotic resistant microorganisms in both humans and animals. Animals may be affected with antibiotic resistant pathogenic *E. coli* being resistant to one or more antibiotics. The increase in multidrug drug resistance (MDR) among bacterial pathogens is a global threat for both human and animal health. Due to the limited data on the occurrence of multidrug resistant pathogenic *E. coli* in cattle, farm environment, milk and beef in Malaysia, the current study was carried out. The specific objectives were to determine the occurrence of pathogenic *E. coli* in cattle, farm environment, milk and beef retailed in the markets and to determine the antibiotic resistance and MDR profiles of the isolates.

A total of 222 samples were collected from eight farms and 60 beef samples from seven markets. In each farm, 12 faecal samples and 12 environmental samples were collected which included three samples each of feed, water from drinking buckets, swabs of the floor of stall and pooled samples of house flies which consisted, 5-7 flies per pooled sample. Thirty milk samples were collected from five dairy farms which consisted six milk samples in each farm. Beef samples were randomly chosen from retail stalls in each market. The number of beef samples collected in each visit to the markets varied from 6 to 18 per market, depending on the number of beef retailers in that market. *Escherichia coli* were isolated using conventional isolation methods. Immunomagnetic separation (IMS) technique was used to obtain higher concentration of *E. coli* O157 and then cultured on Sorbitol MacConkey agar supplemented with Cefexime Tellurite and on Chromagar O157. The suspected colonies were subjected to biochemical tests to identify *E. coli*.

A total of 55.2% (53/96) faecal samples and 43.7% (42/96) farm environmental samples were positive for *E. coli*. Among the environmental samples, *E. coli* were isolated from 37.5% (9/24) of feed, 62.5% (15/24) of floor of stalls, 33.3% (8/24) of pooled flies and 41.6% (10/24) of water. *Escherichia coli* were isolated from 33.3% (10/30) milk samples and 10% (6/60) of beef samples. The *E. coli* isolates were then

screened for *E. coli* O157 using Dry Spot Latex agglutination test (Oxoid). A total of 33.3% (20/60) isolates from dairy cattle farms, 40% (18/45) isolates from beef cattle farms and 33.3% (2/6) beef in the markets were presumptively identified as *E. coli* O157. The confirmation of the *E. coli* O157 and pathogenic *E. coli* isolates was done by multiplex PCR (m-PCR) using five sets of primers, which were optimized to detect the presence of pathogenic *E. coli* genes namely *st, lt, Ial, rfb*O157 and *fli*CH7 in one single reaction tube. However, all isolates were found negative for *st* and *lt* (ETEC), *Ial* (EIEC), *rfb*O157 (*E. coli* O157) genes indicating the absence of pathogenic *E. coli* while four (3.6%) isolates from cattle were positive for *fli*CH7 (H7). The findings of this study showed high antibiotic resistance and MDR in *E. coli* isolated from beef cattle, dairy cattle, farm environment, milk and market beef. *Escherichia coli* isolates from cattle and farm environment showed resistance to all 12 antibiotics tested. The antibiotic resistance of *E. coli* isolates from cattle faeces and farm environment was observed to penicillin at 93.7%, erythromycin 61%, gentamicin 2.1% and streptomycin 8.4%, while no resistance was observed to ciprofloxacin, norfloxacin and enroflocxacin. Among *E. coli* isolates from milk, the resistance observed to erythromycin and penicillin was 100%, to ampicillin and tetracycline at 50%, streptomycin, amoxicilline-clavalunic acid and trimethoprim-sulfamethoxazole at 20% while no resistance was observed against ciprofloxacin, gentamicin, cefotaximine, norfloxacine and enroflocxacine. *Escherichia coli* isolates from beef showed resistance to all antibiotics. The resistance against ampicillin, erythromycin, penicillin and amoxicilline-clavalunic acid was 100% while to ciprofloxacin, gentamicin and norfloxacin was 16.7 %. The multidrug resistance among *E. coli* isolates from cattle faeces, farm environment and milk was 44.2 %, 52.5% and 70% respectively. The highest MDR observed was 100% in *E. coli* isolates from beef.

The absence of pathogenic *E. coli* may possibly indicate that dairy and beef cattle in these farms are not reservoirs of ETEC, EIEC, EHEC (*E. coli* O157). However, the risk of other zoonotic strains of pathogenic *E. coli* for humans is a rising concern because they may be present in the food chain. The presence of high MDR *E. coli* species could pose a serious threat towards compromising the treatment in humans, as bacteria are resistant to the drugs of choice and therefore is of an important concern and significant public health risk. Certainly, the prevalence of MDR *E. coli* in cattle farms, milk and market meat should be monitored. The awareness of farmers and meat sector workers towards hygienic measures should be practiced and there should be prudent use of antibiotics to control antibiotic resistance.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

KEHADIRAN PATOGEN *Escherichia coli* RINTANG ANTIBIOTIK PADA LEMBU, PERSEKITARAN LADANG, SUSU DAN LEMBU

Oleh

MIAN KHAQAN SHAH

Januari 2017

Pengerusi : Professor Saleha Abdul Aziz, PhD
Fakulti : Perubatan Veterinar

Penggunaan antibiotik adalah salah satu faktor utama yang telah banyak dilaporkan menyebabkan kemunculan dan merebak mikroorganisma rintang antibiotik pada manusia dan haiwan. Haiwan mungkin terjejas oleh patogen *E. coli* rintang antibiotik yang rintang terhadap satu atau lebih antibiotik. Peningkatan kerintangan pelbagai drug (MDR) antara bakteria patogen mempakan ancaman global terhadap kesihatan manusia dan haiwan. Oleh kerana terdapat data yang terhad berkaitan kejadian *E. coli* patogen yang rintang pelbagai drug pada lembu, persekitaran ladang, susu dan daging lembu di Malaysia, kajian tersebut dijalankan. Objektif khusus adalah untuk menentukan kehadiran *E. coli* patogen pada lembu, persekitaran ladang, susu dan daging lembu yang dijual di pasar dan untuk menentukan rintangan antibiotik dan profil MDR bakteria yang diasingkan.

Sebanyak 222 sampel telah dikumpul daripada lapan ladang dan 60 sampel daging lembu daripada tujuh gerai jualan daging lembu di pasar. Dalam setiap ladang, 12 sampel najis dan 12 sampel persekitaran telah dikumpul yang terdiri deripada tiga sampel setiap satu bagi makanan, air dalam baldi minuman, swab lantai kandang dan kumpulan lalat rumah yang terdiri daripada lima-tujuh lalat dalam setiap sekumpulan sampel. Tiga puluh sampel susu dikumpulkan daripada lima ladang tenusu yang terdiri enam sampel susu di setiap ladang. Sampel daging lembu telah dipilih secara rawak daripada gerai jualan daging lembu di setiap pasar. Bilangan sampel daging lembu yang dikumpul dalam setiap lawatan ke pasar adalah 6 hingga 18 sampel bagi setiap pasar, bergantung kepada bilangan gerai jualan daging lembu di pasar tersebut. *Escherichia coli* telah diasingkan dengan menggunakan kaedah pengasingan konvensional. Teknik pemisahan immunomagnetik (IMS) telah digunakan untuk mendapatkan konsentrasi *E. coli* O157 yang lebih tinggi dan kemudian dikultur pada Sorbitol MacConkey agar yang tambah dengan Cefexime Tellurite dan pada Chromagar O157. Koloni yang disyaki dikenakan ujian biokimia untuk mengenal pasti *E. coli.*

Sebanyak 55.2% (53/96) sampel najis dan 43.7% (42/96) sampel persekitaran ladang adalah positif untuk *E. coli.* Antara sampel persekitaran ladang, *E. coli* telah diasingkan daripada 37.5% (9/24) makanan, 62.5% (15/24) daripada lantai kandang, 33.3% (8/24) daripada kumpulan lalat dan 41.6% (10/24) daripada air. *Escherichia coli* telah diasingkan daripada 33.3% (10/30) sampel susu dan 10% (6/60) sampel daging lembu. Pencilan *E. coli* kemudiannya disaring untuk *E. coli* O157 dengan menggunakan ujian Dry Spot Latex agglutination (Oxoid). Sebanyak 33.3% (20/60) pencilan daripada ladang lembu tenusu, 40% (18/45) pencilan daripada ladang daging lembu dan 33.3% (2/6) daging lembu daripada pasar, kemungkinan dikenalpasti sebagai *E. coli* O157. Pengesahan untuk pencilan *E. coli* O157 dan patogenik *E. coli* dilakukan dengan multipleks PCR (m-PCR) menggunakan lima set primers, yang dioptimumkan untuk mengesan kehadiran gen patogenik *E. coli* iaitu *st, lt, Ial, rfb*O157 and *fli*CH7 dalam satu tiub reaksi tunggal. Walaubagaimanapun, semua pencilan didapati negatif untuk *st* dan *lt* (ETEC), *Ial* (EIEC), *rfb*O157 (*E. coli* O157) menunjukkan kemungkinan tiada *E. coli* patogen dikesan hanya empat (3.6%) pencilan yang diasingkan daripada lembu adalah positif untuk gen *fli*CH7 (H7). Kajian ini menunjukkan rintangan antibiotik dan MDR yang tinggi pada *E. coli* yang telah dipencilkan daripada lembu daging, lembu tenusu, persekitaran ladang, susu dan daging lembu di pasar. *Escherichia coli* yang diasingkan daripada lembu dan persekitaran ladang menunjukkan rintangan terhadap semua 12 antibiotik yang diuji. Rintangan antibiotik yang diperhatikan di kalangan pencilan *E. coli* yang diasingkan daripada najis lembu dan persekitaran ladang terhadap *penicillin* adalah 93.7%, *erythromycin* 61.0%, *gentamicin* 2.1% dan *streptomycin* 8.4%, manakala tiada rintangan diperhatikan terhadap *ciprofloxacin*, *norfloxacin* dan *enroflocxacin*. Antara *E. coli* diasingkan daripada susu, rintangan diperhatikan terhadap *erythromycin* dan *penicillin* adalah 100%, terhadap *ampicillin* dan *tetracycline* pada 50.0%, *streptomycin*, *amoxicilline-clavalunic acid* dan *trimethoprim-sulfamethoxazole* pada 20.0% manakala tiada rintangan ditunjukkan terhadap *ciprofloxacin*, *gentamicin*, *cefotaximine*, *norfloxacine* dan *enroflocxacine*. *Escherichia coli* diasingkan daripada daging lembu menunjukkan rintangan terhadap semua antibiotik. Rintangan terhadap *ampicillin*, *erythromycin*, *penicillin* dan *amoxicilline-clavalunic acid* adalah 100% manakala untuk *ciprofloxacin*, *gentamicin* dan *norfloxacin* adalah 16.7%. Rintangan pelbagai drug di kalangan *E. coli* yang diasingkan daripada najis lembu, persekitaran ladang dan susu adalah masing-masing pada 44.2%, 52.5%, dan 70%. MDR tertinggi diperhatikan adalah 100% pada *E. coli* yang diasingkan daripada daging lembu.

Ketiadaan *E. coli* patogen mungkin menunjukkan bahawa tenusu dan daging lembu di ladang-ladang ini adalah bukan reservoir untuk ETEC, EIEC, EHEC (*E. coli* O157). Walau bagaimanapun, risiko strain zoonotik *E. coli* patogen yang lain tarhadap manusia adalah satu kebimbangan yang semakin meningkat kerana mereka boleh hadir dalam rantaian makanan. Kehadiran spesies *E. coli* MDR tinggi boleh menimbulkan ancaman serius terhadap rawatan pada manusia, oleh kerana bakteria rintang terhadap ubat pilihan dan oleh itu adalah suatu kebimbangan yang penting dan merupakan risiko kesihatan awam yang bererti. Sudah tentu, prevalen MDR *E. coli* dalam ladang lembu, susu dan daging di pasar perlu dipantau. Kesedaran petani dan pekerja sektor daging ke arah langkah-langkah kebersihan perlu diamalkan dan perlu berhemat dalam penggunaan antibiotik untuk mengawal rintangan antibiotik.

ACKNOWLEDGEMENTS

All praises be to Allah (SWT), the creator, nourisher, cherisher, sustainer and provider of one and all who bestowed the ability in me making my dreams a reality. I would like to express my sincere appreciation and profound gratitude to the Chairman of my supervisory committee Prof. Dr. Saleha Abdul Aziz for her unwavering support, scholarly criticisms throughout the research and the program as a whole. Her thorough scrutiny and suggestions made this reality. I am grateful and indebted to supervisory committee member, AP. Dr. Zunita Binti Zakaria for her valuable suggestions throughout this study. I also thank AP. Dr. Malaika Watanabe, AP. Dr. Siti Khairani-Bejo, Prof. Hussni Omar Mohammed, Dr. Siti Zubaidah Ramanoon, AP. Dr. Goh Yong Meng, AP. Dr. Latiffah Hassan and AP. Dr. Abdul Rahim Mutalib for their valuable assistance during my studies. Special thanks to Prof. Son Radu of Microbiology Lab, Faculty of Food Sciences and Technology, UPM for providing positive control for my research work.

Many thanks to Puan Fauziah Nordin of Veterinary Public Health Lab, Mohd Azri Roslan and Krishnammah Kuppusamy of Bacteriology Lab, Puan Siti Khatijah Muhammad of Virology Lab, Puan Maizatul Akmal Mokhtar of Parasitology Lab and Md Nazim Razali Kanini of Large Animal Unit of the Faculty of Veterinary Medicine for their kind support, assistance and cooperation throughout my laboratory work and sampling.

I am indeed very grateful to my lab mates Dauda Goni, Jalo Ibrahim, Abdur Rasheed, Yousif, Emelia and Abdelrahman for their friendly support and cooperation which I cherish a lot. I am very thankful to my cousins and friends Mian Shahab Shah, Mian Samiullah Jan, Mian Farhan Shah, Saad Zulkifl, Syed Rizwan Shah, Mian Mohd Umer, Mohd Hayat Khan, Sumaira Farid, Shahfaisal Mian, Yasir Gohar, Adnan Umer, Shahzeb, Inayat Ullah Zeb, Ibrar Zeb, Col Lin Lee, Liew Pit Sze, Lim Jin Nee, Faiz Fawzi Jalil, Shoaib Khan, Danish Ali, Chang Wei San, Loo Yuet Ying, Hameed Ullah, Nabeel Khattak, Omar Ijaz, Haseeb Fazal, Shakeeb Fazal, Munir Khan, Saad Akbar, Sufiyan Mohammad, Hamadh Imam, Dr. Konto, Sani Yahaya, Asinamai Bitrus, Maqsood Kasi, Wasay Khan, Anwar Khan, Ali Asif Raza, Aamir Hussain and many others whom space would not permit me to mention.

My special gratitude and thanks go to my uncles Mian Qasim Shah, Mian Bukhari Shah, Faridullah Shah Mian, Mian Mohd Asif, Mian Mohd Wisal, Mian Sakhawat Shah and Mian Mohd Nasir for their well wishes, prayers and support.

I certify that a Thesis Examination Committee has met on 13 January 2017 to conduct the final examination of Mian Khaqan Shah on his thesis entitled "Occurrence of Antibiotic Resistant Pathogenic *Escherichia coli* in Cattle, Farm Environment, Milk and Beef" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Abdul Aziz bin Saharee, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Jalila binti Abu, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Sharifah Aminah Syed Mohamad, PhD
Associate Professor
Universiti Teknologi MARA
Malaysia
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 22 March 2017

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Saleha Abdul Aziz, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairperson)

Zunita Binti Zakaria, PhD
Professor
Faculty of Veterinary Medicine
Univeristi Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: ________________________ Date: _____________________

Name and Matric No: Mian Khaqan Shah,GS39541

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: ____________________

Name of Chairman of Supervisory Committee: Professor Dr. Saleha Abdul Aziz

Signature: ____________________

Name of Member of Supervisory Committee: Professor Dr. Zunita Binti Zakaria

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

E. coli	*Escherichia coli*
ExPEC	extraintestinal *E. coli*
ETEC	enterotoxigenic *E. coli*
EHEC	enterohaemorrhagic *E. coli*
EPEC	enteropathogenic *E. coli*
EIEC	enteroinvasive *E. coli*
EAEC	enteroaggregative *E. coli*
DAEC	diffusely adherent *E. coli*
AIEC	adherent invasive *E. coli*
STEC	Shiga-toxin
VTEC	Vero toxin producing
HUS	hemolytic uremic syndrome
PCR	polymerase chain reaction
UTI	urinary tract infection
Bfp	Bundle-forming pili
LEE	Locus for enterocyte effacement
CFA	colonization factor antigen
AAF	aggregative adherence fimbria
Daa	diffuse adhesion
AIDA	adhesion involved in diffuse adherence
Ipa	Invasion plasmid antigen
LA	localized adherence
AA	aggregative adherence
ST/*st*	heat stable
LT/*lt*	heat labile
H_2O	Water
NA^+	Sodium
Cl	Chloride
CT	cholera enterotoxin
CFs	colonization factors
IECs	intestinal epithelial cells
BFP/*bfp*	bundle forming pulse
AE/*eae*	attaching and effacing
ESP	*E. coli* secreted proteins
HC	hemorrhagic colitis
Stx-1	shiga toxin 1
Stx-2	shiga toxin 2
VT1	Vero toxins 1
VT2	Vero toxins 2
HEp-2	HeLa cells
DAF	decay accelerating factor
CD	Crohn's disease
IBD	inflammatory bowel disease
CAECAM6	carcino-embryonic antigen-related cell adhesion molecule 6
OMVs	outer membrane vesicles
OmpA	outer membrane protein A
ER	endoplasmic reticulum
EMB	Eosin Methylene Blue
CT	Cefixime and Tellurite

SMAC	Sorbitol MacConkey
VP	Voges-proskauer
TSI	Triple sugar iron
SIM	Sulfide Indole Motality
H_2S	Hydrogen Sulfide
IMS	Immunomagnetic separation
DNA	Deoxyribonucleic acid
PFGE	pulsed-field gel electrophoresis
m-PCR	multiplex polymerase chain reaction
GEMS	Global Enteric Multicenter Study
ECDC	European Centre for Disease Prevention and Control
CDC	Centre for Disease Control and Prevention
USA	United States of America
WHO	World Health Organization
S	susceptible
R	Resistant
I	Intermediate
EU	European Union
UPM	University Putra Malaysia
BPW	Buffered Peptone Water
TSB	Trypton Soy Broth
ml	milliliter
µm	micrometer
˚C	degree centigrade
PBS	Phosphate buffer saline
µl	microliter
min	minute
mins	minutes
TBE	Tris-Borate-EDTA
UV	Ultra violet
bp	base pair
P	probability
SPSS	Statistical Package for the Social Sciences
M	marker
L	Ladder
MDR	multidrug resistance
CLSI	Clinical and Laboratory Standards Institute
BSAC	British Society for Antimicrobial Chemotherapy
Amp	ampicillin
Te	tetracycline
E	erythromycin
Cn	gentamicin
Ctx	cefotaxime
Nor	norfloxicin
Enr	enrofloxacin
Amc	amoxicillin-clavalunic acid
Sxt	trimetophrim-sulfamethoxazole
NaCl	Sodium chloride
ATCC	American Type Culture Collection

CHAPTER 1

INTRODUCTION

Foodborne illness is an unavoidable public health concern worldwide. Consumption of contaminated food may lead to foodborne illness in humans (Jianghong and Carl, 2007). Most pathogens cause infection while a number produce toxins inside the host after ingestion which may lead to mild to severe diarrhea and even death in severe infection. The occurrence of death is mostly due to septicemia and/or hypovolemic shock in which there is an excessive loss of body fluids due to diarrhea and vomiting. Over 250 bacterial species are reported to cause foodborne illnesses among which *Escherichia coli* (*E. coli*) is considered to be causing most of these illnesses (Carlos *et al*., 2003). *Escherichia coli* is divided in to two types, pathogenic *E. coli* and non-pathogenic *E. coli.* The non-pathogenic strains of *E. coli* described as commensal *E. coli* are present in the normal microflora of intestine which are harmless, hinders the growth of harmful bacteria and produces vitamins (Nataro and Kaper, 1998; Beauchamp and Sofos, 2010). The pathogenic *E. coli* strains can be further classified into intestinal diarrheagenic *E. coli* which causes diarrhea and extraintestinal *E. coli* (ExPEC) which causes wide range of illnesses in humans such as the neonatal meningitis, chronic urinary tract infections, septicemia and hemolytic uremic syndrome (Nataro and Kaper, 1998; Chomvarin *et al*., 2005; Beauchamp and Sofos, 2010; Croxen and Finlay, 2010).

Intestinal diarrheagenic *E. coli* strains are known to be the major contributor of diarrheal diseases worldwide, leading to mortality among children especially under the age of 5 years (Croxen *et al*., 2013). Based on the mechanism of the disease and presence of virulence factors, at least seven classes of diarrheagenic *E. coli* are identified, namely, enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC) include *Shigella*, enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) (Beauchamp and Sofos, 2010; Jafari *et al*., 2012; Allocati *et al*, 2013;) and the recently emerged, adherent invasive *E. coli* (AIEC) (Allocati *et al*., 2013; Martinez-Medina and Garcia-Gil, 2014).

Of all diarrheagenic *E. coli* identified, Shiga-toxin or Vero toxin producing (STEC/VTEC) EHEC is the most important pathotype in human diseases (Wani *et al*., 2003). There are many serotypes in STEC and among them, the EHEC serotype O157:H7 is found to be highly virulent, responsible for causing outbreaks of bloody diarrhea and hemolytic uremic syndrome (HUS) around the globe. Ruminants are recognized as natural reservoir hosts for *E. coli* O157:H7 (Nataro and Kaper, 1998). No treatment has yet been found for the infections caused by EHEC (Goldwater and Bettelheim, 2012). The non-availability of treatment of EHEC imparts more attention towards the study of epidemiology, pathology and control measures in case of outbreak.

Pathogenic *E. coli* can be found in contaminated environment (water and soil) because they are being shed in the faeces of infected animals and humans. Contamination of animal products may be due to inappropriate practices during slaughtering and dressing process, especially from intestinal contents and faeces during evisceration (Bhunia, 2007).

It is of utmost public health importance to differentiate between the pathogenic and nonpathogenic *E. coli*. The techniques and selective media can be rather expensive. Special selective media are used with the addition of antibiotics for good growth of pathogenic strains of *E. coli*. Immunomagnetic separation is one of the important techniques used in clinical isolation of *E. coli*. The technique increase the sensitivity of detection of pathogenic *E. coli* using special magnetic beads coated with the specific antigen for specific pathogens which produce beadbacteria complex by binding with the target bacteria (Weagant and Bound, 2001; Wright *et al*., 1994). Some other media are also used to produce abundant growth of the pathogenic *E. coli* such as Sorbitol MacConkey agar with Cefixime Tollurite and Chromagar O157 (Khanjar and Alwan, 2014). For characterization of different strains of *E. coli*, polymerase chain reaction (PCR) method is used and is based on amplification of specific virulent genes (Nataro & Kaper, 1998). Watterworth *et al.,* (2005) designed a multiplex PCR assay by using six sets of primers to identify four different types of pathogenic *E. coli*. Chang *et al*., (2013) designed a duplex PCR assay by using two sets of primers for the detection of *E. coli* O157:H7.

Bacterial enteric pathogens showed resistance to antimicrobial drugs that are used in food producing animals as well as humans and is an unavoidable consequence in both developed and developing countries (Bogaard and Stobberingh, 2000; Threlfall *et al.,* 2000). The pathogens which are resistant to antibiotics are being spread in the environment through contaminated faeces from infected animals (Fofana *et al*., 2006; Diarrassouba *et al*., 2007; Pallecchi *et al*., 2007; Ahmed *et al*., 2010). The antibiotic resistant genes carried by antibiotic resistant bacteria decrease the therapeutic effects of antibiotics which leads to increase in morbidity and mortality among animals and humans and may lead to outbreaks (Da Costa *et al*., 2009).

In United States, a study by Aarestrup *et al.,* (1999) showed 20% of pathogenic *E. coli* isolates showed resistance to more than one antibiotic. Later on, Lee (2009) reported 70% of strains were resistant to more than one antibiotic. The study revealed the most predominant resistance was to ampicillin (88%) and streptomycin (81%) which are the most widely used antibiotics in human medicine. In Spain, a study by Mora *et al*., (2005), reported most of the isolates of STEC serotype O157 from humans, sheep, cattle and food showed antibiotic resistance to more than one antibiotic especially to tetracycline and ampicillin.

In Malaysia, not many studies were reported on the occurrence of pathogenic *E. coli* in beef and milk, however very limited studies on pathogenic *E. coli* in cattle and farm environment and their antibiotic resistance patterns. The occurrence of pathogenic *E. coli* and their antibiotic resistance patterns were reported among many workers to vary because of many factors such as management and environmental differences.

Therefore, it is of great importance to determine the occurrence of pathogenic *E. coli* in cattle farms through the detection of virulent genes and the antibiotic resistance patterns of the isolates.

The hypothesis of this study was: There is a low prevalence of pathogenic *E. coli* in cattle, farms environment, milk and beef and the occurrence of multiple drug resistance (resistant to three or more classes of antibiotics) in *E. coli* isolates is low.

Therefore the objectives for the current study were:

1. to determine the occurrence of pathogenic *E. coli* in cattle, farm environment, milk and beef.
2. to detect the virulent genes of pathogenic *E. coli* isolates using multiplex polymerase chain reaction.
3. to determine the antibiotic resistance patterns of the pathogenic *E. coli* isolates.

REFERENCES

Aarestrup, F. (2012). Sustainable farming: Get pigs off antibiotics. *Nature*, *486*(7404), 465-466.

Aarestrup, F. M., and Wegener, H. C. (1999). The effects of antibiotic usage in food animals on the development of antimicrobial resistance of importance for humans in *Campylobacter* and *Escherichia coli*. *Microbes and Infection*, *1*(8), 639-644.

Abike, T. O., Olufunke, O. A., and Oriade, K. D. (2015). Prevalence of multipl e antibiotic resistant *Escherichia coli* serotypes in cow raw milk samples and traditional dairy products in Osun State, Nigeria. *British Microbiology Reviews Journal*, *5*, 117-125.

Adam, M. R. and Moss, M. O. (2008). *Food Microbiology*. 3rd Edition. Royal Society of Chemistry, Cambridge. Pp. 216-224.

Adetunji, V. O., and Odetokun, I. A. (2011). Bacterial hazards and critical control points in goat processing at a typical tropical abattoir in Ibadan, Nigeria. *International Journal of Animal and Veterinary Advances*, *3*, 349-354.

Adzitey, F., Huda, N., and Rahmat Ali, G. R. (2012). Prevalence and antibiotic resistance of *Campylobacter*, *Salmonella*, and *L. monocytogenes* in ducks: a review. *Foodborne Pathogens and Disease*, *9*(6), 498-505.

Adzitey, F., N. Huda and G. Rusul, (2010). Prevalence of *Escherichia coli* in duck intestines, faeces, soil and wash water samples in Penang, Malaysia. Proceedings of the 4th International Conference Postgraduate Education: Globalisation and Liberalisation of Postgraduate Education, Nov. 26-28, Cititel Mid Valley, Kuala Lumpur, pp: 165-167.

Afset, J. E., Bergh, K., and Bevanger, L. (2003). High prevalence of atypical enteropathogenic *Escherichia coli* (EPEC) in Norwegian children with diarrhoea. *Journal of Medical Microbiology*, *52*(11), 1015-1019.

Ahmed, M. O., Clegg, P. D., Williams, N. J., Baptiste, K. E., and Bennett, M. (2010). Antimicrobial resistance in equine faecal *Escherichia coli* isolates from North West England. *Annals of Clinical Microbiology and Antimicrobials*, *9*(1), 12.

Alhaj, N., Mariana, N. S., Raha, A. R., and Ishak, Z. (2007). Prevalence of antibiotic resistance among *Escherichia coli* from different sources in Malaysia. *International Journal of Poultry Science*, *6*(4), 293-297.

Alikhani M. Y., Mirsalehian A., and Aslani M. M. (2006). Detection of typical and atypical enteropathogenic *Escherichia coli* (EPEC) in Iranian children with and without diarrhoea. *Journal of Medical Microbiology. 55*:1159–1163.

Allocati, N., Masulli, M., Alexeyev, M. F., and Di Ilio, C. (2013). *Escherichia coli* in Europe: An overview. *International Journal of Environmental Research and Public Health*, *10*(12).

Allos, B. M., Moore, M. R., Griffin, P. M., and Tauxe, R. V. (2004). Surveillance for sporadic foodborne disease in the 21st century: the FoodNet perspective. *Clinical Infectious Diseases*, *38*(Supplement 3), S115-S120.

Angulo, F. J., and Scallan, E. (2007). Activities, achievements, and lessons learned during the first 10 years of the Foodborne Diseases Active Surveillance Network: 1996–2005. *Clinical Infectious Diseases*, *44*(5), 718-725.

Angulo, F. J., Nargund, V. N., and Chiller, T. C. (2004). Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. *Journal of Veterinary Medicine, Series B*, *51*(8-9), 374-379.

Apun, K., Chang, P. P., Sim, E. U. H., and Micky, V. (2006). Clonal diversity of *Escherichia coli* isolates from marketed beef in East Malaysia. *World Journal of Microbiology and Biotechnology*, *22*(7), 661-667.

Apun, K., Kho, K.L., Chong, Y.L., Hashimatul, F.H., Abdullah, M.T., Rahman, M.A. and Samuel, L. (2010). Detection of *Escherichia coli 0157:H7* in wildlife from distributed habitats in Sarawak, Malaysia. *Research Journal of Microbiology 6*:132-139

Arslan, H., Azap, Ö. K., Ergönül, Ö., Timurkaynak, F. and Urinary Tract Infection Study Group. (2005). Risk factors for ciprofloxacin resistance among *Escherichia coli* strains isolated from community-acquired urinary tract infections in Turkey. *Journal of Antimicrobial Chemotherapy*, *56*(5), 914-918.

Arumugaswamy, R. K., Rusul, G., Hamid, S. A., and Cheah, C. T. (1995). Prevalence of *Salmonella* in raw and cooked foods in Malaysia. *Food Microbiology*, *12*, 3-8.

Awadallah, M. A., Merwad, A. M., and Mohamed, R. E. (2013). Prevalence of zoonotic *Escherichia coli* and Salmonellae in wild birds and humans in Egypt with emphasis on RAPD-PCR Fingerprinting of *E. coli*. *Global Veterinaria*, *11*, 781-788.

Bagré, T. S., Kagambèga, A., Bawa, H. I., Tchamba, G. B., Dembélé, R. E., Zongo, C., Savadogo, A., Aggad, H., Traoré, A. S. and Barro, N. (2014). Antibiotic susceptibility of *Escherichia coli* and *Salmonella* strains isolated from raw and curds milk consumed in Ouagadougou and Ziniar, Burkina Faso. *African Journal of Microbiology Research*, *8*(10), 1012-1016.

Bakhshi, B., Fallahzad, S., and Pourshafie, M. R. (2013). The occurrence of atypical enteropathogenic *Escherichia coli* strains among children with diarrhea in Iran. *Journal of Infection and Chemotherapy*, *19*(4), 615-620.

Barrett, T. J., Lior, H., Green, J. H., Khakhria, R., Wells, J. G., Bell, B. P., Greene, K. D., Lewis, J. and Griffin, P. M. (1994). Laboratory investigation of a multistate food-borne outbreak of *Escherichia coli* O157: H7 by using pulsed-field gel electrophoresis and phage typing. *Journal of Clinical Microbiology*, *32*(12), 3013-3017.

Bartoloni, A., Pallecchi, L., Benedetti, M., Fernandez, C., Vallejos, Y., Guzman, E., Villagran, A. L., Mantella, A., Lucchetti, C., Bartalesi, F. and Strohmeyer, M. (2006). Multidrug-resistant commensal *Escherichia coli* in children, Peru and Bolivia. *Emerging Infectious Diseases*, *12*(6), 907-13.

Beatty M. E., Adcock P. M, Smith S. W., Quinlan K., Kamimoto L. A., Rowe S. Y., Scott K., Conover C., Varchmin T., Bopp C. A., Greene K. D., Bibb B, Slutsker L., and Mintz E. D. (2006). Epidemic diarrhea due to enterotoxigenic *Escherichia coli*. *Clinical Infectious Diseases*, *42*(3), 329-334.

Beauchamp, C. S., and Sofos, J. N. (2010). Diarrheagenic *Escherichia coli. Pathogens and Toxins in Foods, ASM Press, Washington DC*, 71-94.

Bélanger, L., Garenaux, A., Harel, J., Boulianne, M., Nadeau, E., and Dozois, C. M. (2011). *Escherichia coli* from animal reservoirs as a potential source of human extraintestinal pathogenic *Escherichia coli*. *FEMS Immunology & Medical Microbiology*, *62*(1), 1-10.

Bell, C., and Kyriakides, A. (2002). Pathogenic *Escherichia coli*. *Foodborne Pathogens: Hazards, Risk Analysis and Control*, 279-306.

Bentancor, A. B., Ameal, L. A., Calviño, M. F., Martinez, M. C., Miccio, L., and Degregorio, O. J. (2011). Risk factors for Shiga toxin-producing *Escherichia coli* infections in preadolescent schoolchildren in Buenos Aires, Argentina. *The Journal of Infection in Developing Countries*, *6*(05), 378-386.

Berg, J., McAllister, T., Bach, S., Stilborn, R., Hancock, D., and LeJeune, J. (2004). *Escherichia coli* O157: H7 excretion by commercial feedlot cattle fed either barley-or corn-based finishing diets. *Journal of Food Protection®*, *67*(4), 666-671.

Bettelheim, K., and Beutin, L. (2003). Rapid laboratory identification and characterization of verocytotoxigenic (shiga toxin producing) *Escherichia coli* (VTEC/STEC). *Journal of Applied Microbiology, 95*(2): 205-217.

Beutin, L. (1999). *Escherichia coli* O157 and other types of verocytotoxigenic *E. coli* (VTEC) isolated from humans, animals and food in Germany. *Escherichia coli O*, *157*, 121-145.

Bhunia, A. K. (2007). *Escherichia coli*. In Dennis R. Heldman (Ed.), Foodborne microbial pathogens: Mechanisms and pathogenesis USA: Springer Science & Business Media. 183 – 200.

Bogaard, V. D. E., and Stobberingh, E. E. (2000). Epidemiology of resistance to antibiotics links between animals and humans. *International Journal of Antimicrobial Agents*, *14*:327-35.

Boisen N., Scheutz F., Rasko D. A., Redman J. C., Persson S., Simon J., Kotloff K. L., Levine M. M., Sow S., Tamboura B., Toure A., Malle D., Panchalingam S., Krogfelt K. A., and Nataro J. P. (2011). Genomic characterization of enteroaggregative *Escherichia coli* from children in Mali. *Journal of Infectious Diseases*, jir757.

Borczyk, A. A., Lior, H., and Ciebin, B. (1987). False positive identifications of *Escherichia coli* 0157 in foods. *International Journal of Food Microbiology*, *4*(4), 347-349.

Bosca-Watts, M. M., Tosca, J., Anton, R., Mora, M., Minguez, M., and Mora, F. (2015). Pathogenesis of Crohn's disease: Bug or no bug. *World journal of Gastrointestinal Pathophysiology*, *6*(1), 1.

Cagle, P. T. (2009). Polymerase Chain Reaction and Reverse Transcription-Polymerase Chain Reaction. Basic concepts of molecular pathology, Springer Verlag. pp 72-85

Carlos, E., Jorge V., Ulises, H., and Alejandro, C. (2003). *Escherichia coli*. In M. D. Miliotis, and J. W. Bier (Eds.), *International Hand Book of Foodborne Pathogen* CRC.

Centers for Disease Control and Prevention (CDC), 2009. Recommendations for Diagnosis of Shiga Toxin–Producing *Escherichia coli* Infections by Clinical Laboratories; *Morbidity and Mortality Weekly Report* October 16, 2009 / Vol. 58 / No. RR-12 http://www.cdc.gov/mmwr/pdf/rr/rr5812.pdf

Centers for Disease Control and Prevention (CDC), 2013. Surveillance for foodborne disease outbreaks—United States, 2009-2010. *Morbidity and Mortality Weekly Report. 62*:41–47.

Chang, W. S., Afsah Hejri, L., Rukayadi, Y., Khatib, A., Lye, Y. L., Loo, Y. Y., Mohd Shahril, N., Soopna, P., Kuan, C. H., Goh, S. G. and Tang, J. Y. H. (2013). Quantification of *Escherichia coli* O157: H7 in organic vegetables and chickens. *International Food Research Journal*, *20*(2), 1023-1029.

Chapman, P. A., Siddons, C. A., Gerdan Malo, A. T., and Harkin, M. A. (1997): A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. *Epidemiology and Infection, 119*:245-50.

Chapman, P. A., Wright, D. J., and Siddons, C. A. (1994). A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* 0157 from bovine faeces. *Journal of Medical Microbiology*, *40*(6), 424-427.

Cheng, A. C., Turnidge, J., Collignon, P., Looke, D., Barton, M., and Gottlieb, T. (2012). *Control of Fluoroquinolone Resistance through Successful Regulation, Australia* (Doctoral dissertation, US Department of Health and Human Services).

Chomvarin, C., Ratchtrachenchai, O. A., Chantarasuk, Y., and Srigulbutr, S. (2005). Characterization of diarrheagenic *Escherichia coli* isolated from food in Khon Kaen, Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, *36*(4), 931.

Chowdhury, A., Iqbal, A., Uddin, M. G., and Uddin, M. (2011). Study on Isolation and Identification of *Salmonella* and *Escherichia coli* from Different Poultry Feeds of Savar Region of Dhaka, Bangladesh. *Journal of Scientific Research*, *3*(2), 403-411.

Chye, F. Y., Abdullah, A., and Ayob, M. K. (2004). Bacteriological quality and safety of raw milk in Malaysia. *Food Microbiology*, *21*(5), 535-541.

Clarke, R. C., McEwen, S. A., Gannon, V. P., Lior, H., and Gyles, C. L. (1989). Isolation of verocytotoxin-producing *Escherichia coli* from milk filters in south-western Ontario. *Epidemiology and Infection*, *102*(2), 253.

Clinical and Laboratory Standards Institute. (CLSI) (2010). Performance standards for antimicrobial susceptibility testing: *Twentieth Information Supplement M100-S20 Wayne, PA. USA*.

Cooley, M., Carychao, D., Crawford-Miksza, L., Jay, M. T., Myers, C., Rose, C., Keys, C., Farrar, J., and Mandrell, R. E. (2007). Incidence and tracking of *Escherichia coli* O157: H7 in a major produce production region in California. *PloS one*, *2*(11), e1159.

Coombes, B. K., Gilmour, M. W., and Goodman, C. D. (2011). The evolution of virulence in non-O157 Shiga toxin-producing *Escherichia coli*. *Frontiers in Microbiology*, *2*, 90.

Costa, D., Poeta, P., Sáenz, Y., Coelho, A. C., Matos, M., Vinué, L and Torres, C. (2008). Prevalence of antimicrobial resistance and resistance genes in faecal *Escherichia coli* isolates recovered from healthy pets. *Veterinary Microbiology*, *127*(1), 97-105.

Croxen, M. A., and Finlay, B. B. (2010). Molecular mechanisms of *Escherichia coli* pathogenicity. *Nature Reviews Microbiology*, *8*(1), 26-38.

Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M., and Finlay, B. B. (2013). Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clinical Microbiology Reviews*, *26*(4), 822-880.

Da Costa, P. M., Belo, A., Goncalves, J., and Bernardo, F. (2009) Field trial evaluating changes in prevalence and patterns of antimicrobial resistance among *Escherichia coli* and *Enterococcus* spp isolated from growing broilers medicated with enrofloxacin, apramycin, and amoxicillin. *Veterinary Microbiology. 18:* 284-292.

Dalla-Costa, L. M., Irino, K., Rodrigues, J., Rivera, I. N. G., and Trabulsi, L. (1998). Characterisation of diarrhoeagenic *Escherichia coli* clones by ribotyping and ERIC-PCR. *Journal of Medical Microbiology, 47*(3): 227-234.

Darfeuille-Michaud A, Boudeau J, Bulois P, Neut C, Glasser A. L, Barnich N, Bringer M. A, Swidsinski A, Beaugerie L, and Colombel J. F. (2004). High prevalence of adherent-invasive *Escherichia coli* associated with ileal mucosa in Crohn's disease. *Gastroenterology 127*:412–421.

Dhama, K., Rajagunalan, S., Chakraborty, S., Verma, A. K., Kumar, A., Tiwari, R. and Kapoor, S. (2013a). Food-borne pathogens of animal origin – diagnosis, prevention and control and their zoonotic significance: A review. *Pakistan Journal of Biological Sciences, 16*(20): 1076-1085.

Diarrassouba, F.; Diarra, M. S.; Bach, S.; Delaquis, P.; Pritchard, J.; Topp, E.; and Skura, B. J. (2007). Antibiotic resistance and virulence genes in commensal *Escherichia coli* and *Salmonella* isolates from commercial broiler chicken farms. *Journal for Food Protection 70*(6):1316- 1327.

Dickinson, B. L., and Clements, J. D. (1995). Dissociation of *Escherichia coli* heat-labile enterotoxin adjuvanticity from ADP-ribosyltransferase activity. *Infection and Immunity*, *63*(5), 1617-1623.

Dodd, C. C., Sanderson, M. W., Sargeant, J. M., Nagaraja, T. G., Oberst, R. D., Smith, R. A., and Griffin, D. D. (2003). Prevalence of *Escherichia coli* O157 in cattle feeds in Midwestern feedlots. *Applied and Environmental Microbiology*, *69*(9), 5243-5247.

Donaldson, S. C., Straley, B. A., Hegde, N. V., Sawant, A. A., DebRoy, C., Jayarao, and B. M., (2006). Molecular Epidemiology of Ceftiofur-Resistant *Escherichia Coli* Isolates from Dairy Calves. *Applied and Environmental Microbiology. 72*, 3940-3948.

Dow M. A., Tóth I, Malik A., Herpay M., Nógrády N., Ghenghesh K. S., and Nagy B. (2006). Phenotypic and genetic characterization of enteropathogenic *Escherichia coli* (EPEC) and entero-aggregative *E. coli* (EAEC) from diarrhoeal and non-diarrhoeal children in Libya. *Comparative Immunology, Microbiology and Infectious Diseases. 29*:100–113.

Drago, L., Nicola, L., Mattina, R., and De Vecchi, E. (2010). In vitro selection of resistance in *Escherichia coli* and *Klebsiella spp*. at in vivo fluoroquinolone concentrations. *BMC Microbiology*, *10*(1), 119.

Dulo, F., Feleke, A., Szonyi, B., Fries, R., Baumann, M. P., and Grace, D. (2015). Isolation of Multidrug-Resistant *Escherichia coli* O157 from Goats in the Somali Region of Ethiopia: A Cross-Sectional, Abattoir-Based Study.*PloS one*, *10*(11), e0142905.

Dundas, S., Todd, W. A., Stewart, A. I., Murdoch, P. S., Chaudhuri, A. K. R., and Hutchinson, S. J. (2001). The central Scotland *Escherichia coli* O157: H7 outbreak: risk factors for the hemolytic uremic syndrome and death among hospitalized patients. *Clinical Infectious Diseases*, *33*(7), 923-931.

Duse, A. (2015). *Antimicrobial Resistant Escherichia coli in Faeces from Preweaned Dairy Calves* (Vol. 2015, No. 47).

Effler, E., Isaäcson, M., Arntzen, L., Heenan, R., Canter, P., Barrett, T., Lee, L., Mambo, C., Levine, W., Zaidi, A. and Griffin, P. M. (2001). Factors contributing to the emergence of *Escherichia coli* O157 in Africa. *Emerging Infectious Diseases*, *7*(5), 812.

Enne, V. I., Cassar, C., Sprigings, K., Woodward, M. J., and Bennett, P. M. (2008). A high prevalence of antimicrobial resistant *Escherichia coli* isolated from pigs and a low prevalence of antimicrobial resistant E. coli from cattle and sheep in Great Britain at slaughter. *FEMS Microbiology Letters*, *278*(2), 193-199.

Estrada-Garcia, T., and Navarro-Garcia, F. (2012). Enteroaggregative *Escherichia coli* pathotype: a genetically heterogeneous emerging foodborne enteropathogen. *FEMS Immunology & Medical Microbiology*, *66*(3), 281-298.

Ethelberg, S., Lisby, M., Bottiger, B., Schultz, A.C., Villif, A., Jensen, T., Olsen, K. E., Scheutz, F., Kjelso, C. and Muller, L. (2010). Outbreaks of gastroenteritis linked to lettuce, Denmark, January 2010. *Euro Surveillance*, *15*(6).

European Centre for Disease Prevention and Control and European Food Safety Authority. (2011). Shiga toxin/verotoxin-producing *Escherichia coli* in humans, food and animals in the EU/EEA, with special reference to the German outbreak strain STEC O104. European Centre for Disease Prevention and Control and European Food Safety Authority, Stockholm, Sweden.

Fanaro, S., Chierici, R., Guerrini, P., and Vigi, V. (2003). Intestinal microflora in early infancy: composition and development. *Acta Paediatrica*, *92*(s441), 48-55.

Finney, M., Smullen, J., Foster, H., Brokx, S., and Storey, D. (2003). Evaluation of chromocult coliform agar for the detection and enumeration of enterobacteriaceae from faecal samples from healthy subjects. *Journal of Microbiological Methods, 54*(3): 353-358.

Fluckey, W. M., Loneragan, G. H., Warner, R., and Brashears, M. M. (2007). Antimicrobial drug resistance of *Salmonella* and *Escherichia coli* isolates from cattle feces, hides, and carcasses. *Journal of Food Protection*, *70*(3), 551-556.

Fofana, A., Bada Alambedji, R., Seydi, M., and Akakpo, A. J. (2006). Antibiotic resistance of *Escherichia coli* strains isolated from raw chicken meat in Senegal. *Dakar Medicine, 51(3):* 145-150.

Fujioka, R. S. (2001). Monitoring coastal marine waters for spore-forming bacteria of faecal and soil origin to determine point from non-point source pollution. *Water Science and Technology*, *44*(7), 181-181.

Gagliardi, J. V., and Karns, J. S. (2002). Persistence of *Escherichia coli* O157: H7 in soil and on plant roots. *Environmental Microbiology*, *4*(2), 89-96.

Gansheroff, L. J., and O'Brien, A. D. (2000). *Escherichia coli* O157: H7 in beef cattle presented for slaughter in the US: higher prevalence rates than previously estimated. *Proceedings of the National Academy of Sciences*, *97*(7), 2959-2961.

Gillespie, S. H., and Hawkey, P. M. (2006). *Principles and Practice of Clinical Bacteriology.* John Wiley & Sons, 347-365.

Girón J. A., Jones T., Millán-Velasco F., Castro-Muñoz E., Zárate L., Fry J., Frankel G., Moseley S. L., Baudry B., and Kaper J. B. (1991). Diffuse-adhering *Escherichia coli* (DAEC) as a putative cause of diarrhea in Mayan children in Mexico. *Journal of Infectious Diseases*, *163*(3), 507-513.

Goldberg, M. B., and Sansonetti, P. J. (1993). Shigella subversion of the cellular cytoskeleton: a strategy for epithelial colonization. *Infection and Immunity*, *61*(12), 4941.

Goldwater, P. N., and Bettelheim, K. A. (2012). Treatment of enterohemorrhagic *Escherichia coli* (EHEC) infection and hemolytic uremic syndrome (HUS). *BMC Medicine*, *10*(1), 1.

Gomes, T. A., Vieira, M. A., Abe, C. M., Rodrigues, D., Griffin, P. M., and Ramos, S. R. (1998). Adherence patterns and adherence-related DNA sequences in *Escherichia coli* isolates from children with and without diarrhea in São Paulo city, Brazil. *Journal of Clinical Microbiology*, *36*(12), 3609-3613.

Gordillo, M. E., Reeve, G. R., Pappas, J. A. N. E. E. N. E., Mathewson, J. J., DuPont, H. L., and Murray, B. E. (1992). Molecular characterization of strains of enteroinvasive *Escherichia coli* O143, including isolates from a large outbreak in Houston, Texas. *Journal of Clinical Microbiology*, *30*(4), 889-893.

Grad Y. H., Lipsitch M., Feldgarden M., Arachchi H. M., Cerqueira G. C., Fitzgerald M., Godfrey P., Haas B. J., Murphy C. I., Russ C., Sykes S., Walker B. J., Wortman J. R., Young S., Zeng Q., Abouelleil A., Bochicchio J., Chauvin S., Desmet T., Gujja S., McCowan C., Montmayeur A., Guinée, P., Veldkamp, J., and Jansen, W. (1977). Improved minca medium for the detection of K99 antigen in calf Enterotoxigenic strains of *Escherichia coli*. *Infection and Immunity, 15*(2): 676-678.

Gunzburg, S. T., Chang, B. J., Elliott, S. J., Burke, V., and Gracey, M. (1993). Diffuse and enteroaggregative patterns of adherence of enteric *Escherichia coli* isolated from aboriginal children from the Kimberley region of Western Australia. *Journal of Infectious Diseases*, *167*(3), 755-758.

Gyles, C. (2007). Shiga toxin-producing *Escherichia coli*: An overview. *Journal of Animal Science, 85*(Number 13 Electronic Supplement 1), 45.

Hajian, S., Rahimi, E., and Mommtaz, H. (2011). A 3-year study of *Escherichia coli* O157: H7 in cattle, camel, sheep, goat, chicken and beef minced meat. In *Proceedings of the International Conference on Food Engineering and Biotechnology (IPCBEE'11)*.

Hammerum, A. M., and Heuer, O. E. (2009). Human health hazards from antimicrobial-resistant *Escherichia coli* of animal origin. *Clinical Infectious Diseases*, *48*(7), 916-921.

Hammerum, A. M., Heuer, O. E., Emborg, H. D., Bagger-Skjøt, L., Jensen, V. F., and Rogues, A. M. (2007). Danish integrated antimicrobial resistance monitoring and research program. *Emerging Infectious Diseases, 13*(11): 1632-1639.

Hancock, D. D., Besser, T. E., Rice, D. H., Ebel, E. D., Herriott, D. E., and Carpenter, L. V. (1998). Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the Northwestern USA. *Preventive Veterinary Medicine. 35*, 11–19.

Harwood, V. J., Whitlock, J., and Withington, V. (2000). Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: use in predicting the source of fecal contamination in subtropical waters. *Applied and Environmental Microbiology*, *66*(9), 3698-3704.

Heuvelink, A. E. (2003). Review of media for the isolation of diarrhoeagenic *Escherichia coli*. Progress in *Industrial Microbiology, 37*: 229-247.

Heuvelink, A. E., Van Den Biggelaar, F. L. A. M., Zwartkruis-Nahuis, J. T. M., Herbes, R. G., Huyben, R., Nagelkerke, N., Melchers, W. J. G., Monnens, L. A. H. and De Boer, E. (1998). Occurrence of verocytotoxin-producing *Escherichia coli* O157 on Dutch dairy farms. *Journal of Clinical Microbiology*, *36*(12), 3480-3487.

Hicks, S., Candy, D. C., and Phillips, A. D. (1996). Adhesion of enteroaggregative *Escherichia coli* to pediatric intestinal mucosa in vitro. *Infection and Immunity*, *64*(11), 4751-4760.

Hill, W. E., and Wachsmuth, K. (1996). The polymerase chain reaction: applications for the detection of foodborne pathogens. *Critical Reviews in Food Science & Nutrition*, *36*(1-2), 123-173.

Ho, W. S., Balan, G., Puthucheary, S., Kong, B. H., Lim, K. T., Tan, L. K., Koh, X. P., Yeo, C. C. and Thong, K. L. (2012). Prevalence and characterization of multidrug-resistant and extended-spectrum beta-lactamase-producing *Escherichia coli* from pediatric wards of a Malaysian hospital. *Microbial Drug Resistance*, *18*(4), 408-416.

Hopkins, K., and Hilton, A. (2000). Simultaneous molecular subtyping and shiga toxin gene detection in *Escherichia coli* using multiplex polymerase chain reaction. Letters in *Applied Microbiology, 30*(2): 122-125.

Hordijk, J., Veldman, K., Dierikx, C., van Essen-Zandbergen, A., Wagenaar, J. A. and Mevius, D. (2012). Prevalence and characteristics of quinolone resistance in *Escherichia coli* in veal calves. *Veterinary Microbiology*, *156*(1–2), pp 136–142.

Hossain, M. K., Rahman, M., Nahar, A., Khair, A., and Alam, M. M. (2014). Isolation and identification of diarrheagenic *Escherichia coli* causing colibacillosis in calf in selective areas of Bangladesh. *Bangladesh Journal of Veterinary Medicine*, *11*(2), 145-149.

House, S. S. I. A. S. (2010). *Onwumere Onyine Stella PG/M. Sc/07/73955* (Doctoral dissertation, University of Nigeria, Nsukka).

Howie, H., Mukerjee, A., Cowden, J., Leith, J., and Reid, T. (2003). Investigation of an outbreak of *Escherichia coli* O157 infection caused by environmental exposure at a scout camp. *Epidemiology and Infection*, *131*(03), 1063-1069.

Hoyle, D. V., Yates, C. M., Chase-Topping, M. E., Turner, E. J., Davies, S. E., Low, J. C., Gunn, G. J., Woolhouse, M. E. J. and Amyes, S. G. B. (2005). Molecular epidemiology of antimicrobial-resistant commensal *Escherichia coli* strains in a cohort of newborn calves. *Applied and Environmental Microbiology, 71*(11), pp 6680–6688.

Huerta M., Grotto I., Gdalevich M., Mimouni D., Gavrieli B., Yavzori M., Cohen D., and Shpilberg O. (2000). A waterborne outbreak of gastroenteritis in the Golan Heights due to enterotoxigenic *Escherichia coli*. *Infection*, *28*(5), 267-271.

Irino K., Vaz T. M. I., Kato M. A. M. F., Naves Z. V. F., Lara R. R., Marco M. E. C., Rocha M. M. M., Moreira T. P., Gomes T. A. T., and Guth B. E. C. (2002). O157: H7 Shiga toxin-producing *Escherichia coli* strains associated with sporadic cases of diarrhea in São Paulo, Brazil. *Emerging Infectious Disease Journal*, *8*.

Jafari, A., Aslani, M. M., and Bouzari, S. (2012). *Escherichia coli*: a brief review of diarrheagenic pathotypes and their role in diarrheal diseases in Iran. *Iranian Journal of Microbiology*, *4*(3), 102.

Jain, S., Chen, L., Dechet, A., Hertz, A. T., Brus, D. L., Hanley, K., Wilson, B., Frank, J., Greene, K. D., Parsons, M. and Bopp, C. A. (2008). An outbreak of enterotoxigenic *Escherichia coli* associated with sushi restaurants in Nevada, 2004. *Clinical Infectious Diseases*, *47*(1), 1-7.

Jiang, X., Morgan, J., and Doyle, M. P. (2002). Fate of *Escherichia coli* O157: H7 in manure-amended soil. *Applied and Environmental Microbiology*, *68*(5), 2605-2609.

Jianghong Meng, and Carl M. Schroeder. (2007). *Escherichia coli*. In S. Simjee (Ed.), *Foodborne Diseases Humana* Pr Inc. 1-25

Jose L. Puente, and B. Brett Finlay. (2001). Pathogenic *Escherichia coli*. In E. A. Groisman (Ed.), Principles of Bacterial Pathogenesis. Academic Press., 401 - 405

Kalnauwakul, S., Phengmak, M., Kongmuang, U., Nakaguchi, Y., and Nishibuchi, M. (2007). Examination of diarrheal stools in Hat Yai city, south Thailand, for *Escherichia coli* O157 and other diarrheagenic *Escherichia coli* using immunomagnetic separation and PCR method. *Southeast Asian Journal of Tropical Medicine and Public Health, 38*(5): 871-880.

Karch, H., Tarr, P. I., and Bielaszewska, M. (2005). Enterohaemorrhagic *Escherichia coli* in human medicine. *International Journal of Medical Microbiology*, *295*(6), 405-418.

Karmali, M. A., Gannon, V., and Sargeant, J. M. (2010). Verocytotoxin-producing *Escherichia coli* (VTEC). *Veterinary Microbiology*, *140*(3), 360-370.

Khanjar, A. F., and Alwan, M. J. (2014). Genotypic Study of *Escherichia coli* O157: H7 Isolated from Stool Samples of Humans and Cattle. *International Journal*, *2*(6), 204-212.

Kikuvi, G. M., Ole-Mapenay, I. M., Mitema, E. S., and Ombui, J. N. (2006). Antimicrobial resistance in *Escherichia coli* isolates from faeces and carcass samples of slaughtered cattle, swine and chickens in Kenya. *Israel Journal of Veterinary Medicine*, *61*(3/4), 82.

Knutton, S., Shaw, R., Phillips, A. D., Smith, H. R., Willshaw, G. A., Watson, P., and Price, E. (2001). Phenotypic and genetic analysis of diarrhea-associated *Escherichia coli* isolated from children in the United Kingdom. *Journal of Pediatric Gastroenterology and Nutrition*, *33*(1), 32-40.

Konishi N., Obata H., Monma C., Nakama A., Kai A., and Tsuji T. (2011). Bacteriological and epidemiological characteristics of enterotoxigenic *Escherichia coli* isolated in Tokyo, Japan, between 1966 and 2009. *Journal of Clinical Microbiology. 49*:3348–3351.

Kotloff, K. L., Nataro, J. P., Blackwelder, W. C., Nasrin, D., Farag, T. H., Panchalingam, S., Wu, Y., Sow, S. O., Sur, D., Breiman, R. F. and Faruque, A. S. (2013). Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *The Lancet*, *382*(9888), 209-222.

Krumperman, P. H. (1983). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Applied Environmental Microbiology. 46*:165–170

Kudva, I. T., Hatfield, P. G., and Hovde, C. J. (1996). *Escherichia coli* O157: H7 in microbial flora of sheep. *Journal of Clinical Microbiology*, *34*(2), 431-433.

Kudva, I. T., Hatfield, P. G., and Hovde, C. J. (1997). Characterization of *Escherichia coli* O157: H7 and other shiga toxin-producing *Escherichia coli* serotypes isolated from sheep. *Journal of Clinical Microbiology, 35*(4): 892-899.

Le Bouguénec, C., and Servin, A. L. (2006). Diffusely adherent *Escherichia coli* strains expressing Afa/Dr adhesins (Afa/Dr DAEC): hitherto unrecognized pathogens. *FEMS Microbiology Letters*, *256*(2), 185-194.

Lee, J. H. (2009). Antimicrobial resistance of *Escherichia coli* O26 and O111 isolates from cattle and their characteristics. *Veterinary Microbiology, 135*(3-4): 401-405.

LeJeune, J. T., Besser, T. E., Merrill, N. L., Rice, D. H., and Hancock, D. D. (2001). Livestock drinking water microbiology and the factors influencing the quality of drinking water offered to cattle. *Journal of Dairy Science*, *84*(8), 1856-1862.

LeJeune, J. T., Besser, T. E., Rice, D. H., Berg, J. L., Stilborn, R. P., and Hancock, D. D. (2004). Longitudinal study of fecal shedding of *Escherichia coli* O157: H7 in feedlot cattle: predominance and persistence of specific clonal types despite massive cattle population turnover. *Applied and Environmental Microbiology*, *70*(1), 377-384.

LeJeune, J. T., Hancock, D. D., and Besser, T. E. (2006). Sensitivity of *Escherichia coli* O157 detection in bovine feces assessed by broth enrichment followed by immunomagnetic separation and direct plating methodologies. *Journal of Clinical Microbiology*, *44*(3), 872-875.

Leverstein-van Hall, M. A., Dierikx, C. M., Cohen Stuart, J., Voets, G. M., Van Den Munckhof, M. P., van Essen-Zandbergen, A., Platteel, T., Fluit, A. C., van de Sande-Bruinsma, N., Scharinga, J. and Bonten, M. J. M. (2011). Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clinical Microbiology and Infection*, *17*(6), 873-880.

Levine M. M., Ferreccio C., Prado V., Cayazzo M., Abrego P., Martinez J., Maggi L., Baldini M. M., Martin W., and Maneval D. (1993). Epidemiologic studies of *Escherichia coli* diarrheal infections in a low socioeconomic level peri-urban community in Santiago, Chile. *American Journal of Epidemiology*, *138*(10), 849-869.

Levine, M. M., and Edelman, R. O. B. E. R. T. (1983). Enteropathogenic *Escherichia coli* of classic serotypes associated with infant diarrhea: epidemiology and pathogenesis. *Epidemiologic Reviews*, *6*, 31-51.

Li, X., Watanabe, N., Xiao, C., Harter, T., McCowan, B., Liu, Y., and Atwill, E. R. (2014). Antibiotic-resistant *Escherichia coli* in surface water and groundwater in dairy operations in Northern California. *Environmental Monitoring and Assessment*, *186*(2), 1253-1260.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B. and Paterson, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, *18*(3), 268-281.

Mainda, G., Bessell, P. B., Muma, J. B., McAteer, S. P., Chase-Topping, M. E., Gibbons, J., Stevens, M. P., Gally, D. L. and Barend, M.. (2015). Prevalence and patterns of antimicrobial resistance among *Escherichia coli* isolated from Zambian dairy cattle across different production systems. *Scientific Reports*, *5*.

Mainil, J. G., and Daube, G. (2005). Verotoxigenic *Escherichia coli* from animals, humans and foods: who's who? *Journal of Applied Microbiology*, *98*(6), 1332-1344.

Manning, S. D., Motiwala, A. S., Springman, A. C., Qi, W., Lacher, D. W., Ouellette, L. M., Mladonicky, J. M., Somsel, P., Rudrik, J. T., Dietrich, S. E. and Zhang, W. (2008). Variation in virulence among clades of *Escherichia coli* O157: H7 associated with disease outbreaks. *Proceedings of the National Academy of Sciences*, *105*(12), 4868-4873.

Mansan-Almeida, R., Pereira, A. L., and Giugliano, L. G. (2013). Diffusely adherent *Escherichia coli* strains isolated from children and adults constitute two different populations. *BMC Microbiology*, *13*(1), 1.

Marchese, A., Coppo, E., Barbieri, R., Zoppi, S., Pruzzo, C., Rossi, F., Bergagna, S., Dondo, A. and Debbia, E. (2012). Characterization of fluoroquinolone-resistant *Escherichia coli* causing septicemic colibacillosis in calves in Italy: emergence of a multiresistant O78 clonal group. *Microbial Drug Resistance (Larchmont, N.Y.), 18*(1), pp 94–99.

Martin H. M., Campbell B. J., Hart C. A., Mpofu C., Nayar M., Singh R., Englyst H., Williams H. F., and Rhodes J. M. (2004). Enhanced *Escherichia coli* adherence and invasion in Crohn's disease and colon cancer. *Gastroenterology 127*:80–93.

Martinez-Medina, M., and Garcia-Gil, L. J. (2014). *Escherichia coli* in chronic inflammatory bowel diseases: An update on adherent invasive *Escherichia coli* pathogenicity. *World journal of Gastrointestinal Pathophysiology*, *5*(3), 213.

McKellar, Q. A. (1999). Antibiotics and resistance in farm animals. *Nutrition and Food Science, 99*(4): 178-180.

McPherson, M., and Moller, S. (2000). An introduction to PCR. *BIOS Scientific*, *Oxford* 1-2.

Mellmann, A., Harmsen, D., Cummings, C. A., Zentz, E. B., Leopold, S. R., Rico, A., Prior, K., Szczepanowski, R., Ji, Y., Zhang, W. and McLaughlin, S. F. (2011). Prospective genomic characterization of the German enterohemorrhagic *Escherichia coli* O104: H4 outbreak by rapid next generation sequencing technology. *PloS one*, *6*(7), e22751.

Mifflin, T. E. (2003). Setting up a PCR laboratory. *PCR primer: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY*, 5-14.

Mohammed, O., Shimelis, D., Admasu, P., and Feyera, T. (2014). Prevalence and antimicrobial susceptibility pattern of *Escherichia coli* isolates from raw meat samples obtained from abattoirs in Dire Dawa City, eastern Ethiopia. *International Journal of Food Microbiology, 5*(1), 35-39.

Moon, H., Whipp, S., Argenzio, R., Levine, M., and Giannella, R. (1983). Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines. *Infection and Immunity, 41*(3): 1340-1351.

Mora, A., Blanco, J. E., Blanco, M., Alonso, M. P., Dhabi, G., and Echeita, A., (2005). Antimicrobial resistance of shiga toxin (verotoxin)-producing *Escherichia coli* O157: H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. *Research in Microbiology, 156*(7): 793-806.

Muller, E. E., Grabow, W. O. K., and Ehlers, M. M. (2004). Immunomagnetic separation of *Escherichia coli* O157: H7 from environmental and wastewater in South Africa. *Water SA*, *29*(4), 427-432.

Muto, T., Matsumoto, Y., Yamada, M., Ishiguro, Y., Kitazume, H., Sasaki, K., and Toba, M. (2008). Outbreaks of enterohemorrhagic *Escherichia coli* O157 infections among children with animal contact at a dairy farm in Yokohama City, Japan. *Japanese Journal of Infectious Diseases*, *61*(2), 161.

Nagy, B., and Fekete, P. Z. (2005). Enterotoxigenic *Escherichia coli* in veterinary medicine. *International Journal of Medical Microbiology, 295*(6-7): 443-454.

Nanu, E., Latha, C., Sunil, B., Prejit, M. T., & Menon, K. V. (2007). Quality assurance and public health safety of raw milk at the production point. *American Journal of Food Technology*, *2*(3), 145-152.

Nash J. H., Villegas A., Kropinski A. M., Aguilar-Valenzuela R., Konczy P., Mascarenhas M., Ziebell K., Torres A. G., Karmali M. A., and Coombes B. K. (2010). Genome sequence of adherent-invasive *Escherichia coli* and comparative genomic analysis with other *Escherichia coli* pathotypes. *BMC Genomics*, *11*(1), 1.

Nataro J. P., Mai V., Johnson J., Blackwelder W. C., Heimer R., Tirrell S., Edberg S. C., Braden C. R., Glenn Morris J., Jr., and Hirshon J. M. (2006). Diarrheagenic *Escherichia coli* infection in Baltimore, Maryland, and New haven, Connecticut. *Clinical Infectious Diseases*, *43*(4), 402-407.

Nataro, J. P., and Kaper, J. B. (1998). Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews*, *11*(1), 142-201.

National Enteric Surveillance Program. 2010. National Enteric Surveillance Program (NESP) annual summary 2010. The National Microbiology Laboratory (NML) and Centre for Food-borne, Environmental and Zoonotic Infectious Diseases (CFEZID), Public Health Agency of Canada and Provincial Public Health Microbiology Laboratories, Winnipeg, Canada.

Nazmul, M. H. M. (2008). Molecular characterization of verotoxin gene in enteropathogenic *Escherichia coli* isolated from Miri Hospital, Sarawak, Malaysia. *Biomedical Research*, *19*(1), 9-12.

Nguyen, R. N., Taylor, L. S., Tauschek, M., and Robins-Browne, R. M. (2006). Atypical enteropathogenic *Escherichia coli* infection and prolonged diarrhea in children. *Emerging Infectious Diseases*, *12*(4), 597-603.

Ochoa, T. J., Barletta, F., Contreras, C., and Mercado, E. (2008). New insights into the epidemiology of enteropathogenic *Escherichia coli* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *102*(9), 852-856.

Ogden, I. D., Hepburn, N. F., and MacRae, M. (2001). The optimization of isolation media used in immunomagnetic separation methods for the detection of *Escherichia coli* O157 in foods. *Journal of Applied Microbiology*, *91*(2), 373-379.

Ogden, I. D., Hepburn, N. F., MacRae, M., Strachan, N. J. C., Fenlon, D. R., Rusbridge, S. M., and Pennington, T. H. (2002). Long-term survival of *Escherichia coli* O157 on pasture following an outbreak associated with sheep at a scout camp. *Letters in Applied Microbiology*, *34*(2), 100-104.

Okeke, I. N. (2009). Diarrheagenic *Escherichia coli* in sub-Saharan Africa: status, uncertainties and necessities. *The Journal of Infection in Developing Countries*, *3*(11), 817-842.

Okeke, I. N., Wallace-Gadsden, F., Simons, H. R., Matthews, N., Labar, A. S., Hwang, J., and Wain, J. (2010). Multi-locus sequence typing of enteroaggregative *Escherichia coli* isolates from Nigerian children uncovers multiple lineages. *PLoS One*, *5*(11), e14093.

Olsvik, Ø., Wasteson, Y., Lund, A., and Hornes, E. (1991). Pathogenic *Escherichia coli* found in food. *International Journal of Food Microbiology, 12*(1): 103-113.

Omisakin, F., MacRae, M., Ogden, I. D., and Strachan, N. J. C. (2003). Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. *Applied and Environmental Microbiology*, *69*(5), 2444-2447.

Onono, J. O., Kangethe, E. K., and Ogara, W. O. (2010). Antimicrobial susceptibility of non-sorbitol fermenting *Escherichia coli* isolated from cattle feaces and milk samples. *African Journal of Microbiology Research*, *4*(16), 1703-1707.

Orden, J. A., Ruiz-Santa-Quiteria, J. A., Cid, D., Díez, R., Martínez, S., and de la Fuente, R. (2001). Quinolone resistance in potentially pathogenic and non-pathogenic *Escherichia coli* strains isolated from healthy ruminants. *Journal of Antimicrobial Chemotherapy*, *48*(3), 421-424.

Osek, J. (2001). Multiplex polymerase chain reaction assay for identification of Enterotoxigenic *Escherichia coli* strains. *Journal of Veterinary Diagnostic Investigation, 13*(4): 308-311.

Oteo, J., Campos, J., Baquero, F., and Spanish members of the European Antimicrobial Resistance Surveillance System. (2002). Antibiotic resistance in 1962 invasive isolates of *Escherichia coli* in 27 Spanish hospitals participating in the European Antimicrobial Resistance Surveillance System (2001). *Journal of Antimicrobial Chemotherapy*, *50*(6), 945-952.

Oteo, J., Lázaro, E., de Abajo, F. J., Baquero, F., Campos, J., and Spanish EARSS Group. (2005). Antimicrobial-resistant invasive *Escherichia coli*, Spain. *Emerging Infectious Diseases*, *11*(4), 546-553.

Pallecchi, L., Lucchetti, C., Bartoloni, A., Bartalesi, F., Mantella, A., Gamboa, H., Carattoli, A., Paradisi, F., and Rossolini, G. M. (2007). Population structure and resistance genes in Antibiotic-resistant bacteria from a remote community with minimal antibiotic exposure. *Antimicrobial Agents and Chemotheraphy 51(4):*1179-1184.

Parry, S., and Salmon, R. (1998). Sporadic STEC O157 infection: Secondary household transmission in wales. *Emerging Infectious Diseases, 4*(4): 657-661.

Parveen, S., R. L. Murphree, L. Edmiston, C. W. Kaspar, K. M. Portier, and M. L. Tamplin. (1997). Association of multiple-antibiotic-resistance profiles with point and nonpoint sources of *Escherichia coli* in Apalachicola Bay. *Applied Environmental Microbiology. 63*:2607–2612.

Peralta, G., Sánchez, M. B., Garrido, J. C., De Benito, I., Cano, M. E., Martínez-Martínez, L., and Roiz, M. P. (2007). Impact of antibiotic resistance and of adequate empirical antibiotic treatment in the prognosis of patients with *Escherichia coli* bacteraemia. *Journal of Antimicrobial Chemotherapy*, *60*(4), 855-863.

Pereira, A. L., and Giugliano, L. G. (2013). Adhesion of diarrheagenic *Escherichia coli* and inhibition by glycocompounds engaged in the mucosal innate immunity. *Biology*, *2*(2), 810-831.

Pérez, C., Gómez-Duarte, O. G., and Arias, M. L. (2010). Diarrheagenic *Escherichia coli* in children from Costa Rica. *The American Journal of Tropical Medicine and Hygiene*, *83*(2), 292-297.

Pfeiffer, M. L., DuPont, H. L., and Ochoa, T. J. (2012). The patient presenting with acute dysentery–a systematic review. *Journal of Infection*, *64*(4), 374-386.

Poitrineau, P., Forestier, C., Meyer, M., Jallat, C., Rich, C., Malpuech, G., and De Champs, C. (1995). Retrospective case-control study of diffusely adhering *Escherichia coli* and clinical features in children with diarrhea. *Journal of Clinical Microbiology*, *33*(7), 1961-1962.

Preston, M. A., Johnson, W., Khakhria, R., and Borczyk, A. (2000). Epidemiologic subtyping of *Escherichia coli* serogroup O157 strains isolated in Ontario by phage typing and pulsed-field gel electrophoresis. *Journal of Clinical Microbiology, 38*(6): 2366-2368.

Quinn, P. J., Carter, M. E., Markey, B., and Carter, G. R. (1994). Enterobacteriaceae. *Clinical Veterinary Microbiology. Wolfe Publishing, London*, 209-236.

Quinn, P., Markey, B., Carter, M., Donnelly, W., Leonard, F., and Maghire, D. (2001). Enterobacteriaceae. Veterinary Microbiology and Microbial Disease, *Blackwell Science*, 107- 109.

Radu, S., Ling, O. W., Rusul, G., Karim, M. I. A., and Nishibuchi, M. (2001). Detection of *Escherichia coli* O157: H7 by multiplex PCR and their characterization by plasmid profiling, antimicrobial resistance, RAPD and PFGE analyses. *Journal of Microbiological Methods*, *46*(2), 131-139.

Rahman, S., Parvez, A. K., Islam, R., and Khan, M. H. (2011). Antibacterial activity of natural spices on multiple drug resistant *Escherichia coli* isolated from drinking water, Bangladesh. *Annals of Clinical Microbiology and Antimicrobials*, *10*(1), 1.

Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M., and Swerdlow, D. L. (2005). Epidemiology of *Escherichia coli* O157: H7 outbreaks, United States, 1982–2002.

Rasheed, M. U., Thajuddin, N., Ahamed, P., Teklemariam, Z., and Jamil, K. (2014). Antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources. *Revista do Instituto de Medicina Tropical de São Paulo*, *56*(4), 341-346.

Ratchtrachenchai, O. A., Subpasu, S., Hayashi, H., and Ba-Thein, W. (2004). Prevalence of childhood diarrhoea-associated *Escherichia coli* in Thailand. *Journal of Medical Microbiology*, *53*(3), 237-243.

Riley, L., Remis, R., Helgerson, S., McGee, H., Wells, J., and Davis, B., (1983). Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine, 308*(12): 681- 685.

Rivero, M. A., Passucci, J. A., Rodriguez, E. M., and Parma, A. E. (2010). Role and clinical course of verotoxigenic *Escherichia coli* infections in childhood acute diarrhoea in Argentina. *Journal of Medical Microbiology*, *59*(3), 345-352.

Rodloff, A. C., Leclercq, R., Debbia, E. A., Cantón, R., Oppenheim, B. A., and Dowzicky, M. J. (2008). Comparative analysis of antimicrobial susceptibility among organisms from France, Germany, Italy, Spain and the UK as part of the tigecycline evaluation and surveillance trial. *Clinical Microbiology and Infection*, *14*(4), 307-314.

Rolhion, N., Barnich, N., Bringer, M. A., Glasser, A. L., Ranc, J., Hébuterne, X., Hofman, P. and Darfeuille-Michaud, A. (2010). Abnormally expressed ER stress response chaperone Gp96 in CD favours adherent-invasive *Escherichia coli* invasion. *Gut*, *59* (10), 1355-1362.

Roopnarine, R., Ammons, D., and Adesiyun, A. A. (2009). Frepuency of antimicrobial resistance of *Escherichia coli* isolates from dairy farms in Trinidad by source and presence of virulence markers. *Veterinarski Arhiv*, *79*(3), 229-243.

Šafaříková, M., and Šafařík, I. (2001). Immunomagnetic separation of *Escherichia coli* O26, O111 and O157 from vegetables. *Letters in Applied Microbiology*, *33*(1), 36-39.

Sahilah, A. M. (1998). Plasmid profiling and antibiotic resistance of *Escherichia coli* and *E. coli* O157 strains. *Food Science and Biotechnology Department, University Putra Malaysia, Serdang Selangor*.

Sahilah, A. M., Nor'Aishah, H., and Azuhairi, A. A. (2010). Detection of shiga toxin 1 and 2 (stx1 and stx2) genes in *Escherichia coli* 0157: H7 isolated from retail beef in Malaysia by multiplex polymerase chain reaction (PCR). *Sains Malaysiana*, *39*(1), 57-63.

Saleha, A. A. (2002). Isolation and characterization of *Campylobacter jejuni* from broiler chickens in Malaysia. *International Journal of Poultry Science 1*:94-97.

Sallam, K. I. (2007). Prevalence of Campylobacter in chicken and chicken by-products retailed in Sapporo area, Hokkaido, Japan. *Food Control*, *18*(9), 1113-1120.

Sanderson, M. W., Sargeant, J. M., Shi, X., Nagaraja, T. G., Zurek, L., and Alam, M. J. (2006). Longitudinal emergence and distribution of *Escherichia coli* O157 genotypes in a beef feedlot. *Applied and Environmental Microbiology*, *72*(12), 7614-7619.

Sawant, A. A., Hegde, N. V., Straley, B. A., Donaldson, S. C., Love, B. C., Knabel, S. J., and Jayarao, B. M. (2007). Antimicrobial-resistant enteric bacteria from dairy cattle. *Applied and Environmental Microbiology*, *73*(1), 156-163.

Schwarz, S., and Chaslus-Dancla, E. (2001) Use of Antimicrobials in veterinary medicine and mechanisms of resistance. *Veterinary Research 32:* 201-225.

Sears, C. L., and Kaper, J. B. (1996). Enteric bacterial toxins: Mechanisms of action and linkage to intestinal secretion. *Microbiology and Molecular Biology Reviews, 60*(1): 167- 215.

Sekirov, I., Russell, S. L., Antunes, L. C. M., and Finlay, B. B. (2010). Gut microbiota in health and disease. *Physiological Reviews*, *90*(3), 859-904.

Shah, N., DuPont, H. L., and Ramsey, D. J. (2009). Global etiology of travelers' diarrhea: systematic review from 1973 to the present. *The American Journal of Tropical Medicine and Hygiene*, *80*(4), 609-614.

Son, R., Mutalib, S. A., Rusul, G., Ahmad, Z., Morigaki, T., Asai, N., Kim, Y. B., Okuda, J., and Nishibuchi, M., (1998). Detection of *Escherichia coli 0157:H7* in the beef marketed in Malaysia. *Applied Environmental Microbiology, 64,* 1153–1156.

Sousa, C. P. (2006). The versatile strategies of *Escherichia coli* pathotypes: a mini review. *Journal of Venomous Animals and Toxins Including Tropical Diseases*, *12*(3), 363-373.

Steelman S., Frimodt-Møller J., Petersen A. M., Struve C., Krogfelt K. A., Bingen E., Weill F. X., Lander E. S., Nusbaum C., Birren B. W., Hung D. T., and Hanage W. P. (2012). Genomic epidemiology of the *Escherichia coli* O104:H4 outbreaks in Europe, 2011. *Proceedings of the National Academy of Sciences. U. S. A. 109*: 3065–3070.

Sukhumungoon, P., Mittraparp-Arthorn, P., Pomwised, R., Charernjiratragul, W and Vuddhakul, V. (2011). High concentration of Shiga toxin 1-producing *Escherichia coli* isolated from Southern Thailand. *International Food Research Journal 18*: 683-688.

Sukhumungoon, P., Nakaguchi, Y., Ingviya, N., Pradutkanchana, J., Iwade, Y., Seto, K., Radu, S., Nishibuchi, M. and Vuddhakul, V. (2011). Investigation of stx2 eae+ *Escherichia coli* O157: H7 in beef imported from Malaysia to Thailand. *International Food Research Journal*, *18*(1), 381-386.

Summons, T. G. (1968). Animal feed additives, 1940-1966. *Agricultural History*, *42*(4), 305-313.

Swaminathan, B., Barrett, T. J., Hunter, S. B., Tauxe, R. V., and Force, C. P. T. (2001). PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. *Emerging Infectious Diseases*, *7*(3), 382.

Tanih, N. F., Sekwadi, E., Ndip, R. N., and Bessong, P. O. (2015). Detection of pathogenic *Escherichia coli* and Staphylococcus aureus from cattle and pigs slaughtered in Abattoirs in Vhembe District, South Africa. *The Scientific World Journal*, *2015*.

Tenover, F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. *American Journal of Infection Control, 34*(5): 3-10.

Teuber, M. (1999). Spread of antibiotic resistance with food-borne pathogens. *Cellular and Molecular Life Sciences, 56*(9): 755-763.

The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) 2006. Consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria fromfood animals, foods and humans in Denmark. Available at: http://www .danmap.org/pdfFiles/Danmap_2006.pdf. Accessed 28 August 2008.

Threlfall, E. J., Ward, L. R., Frost, J. A. and Willshaw, G. A. (2000). Review: The emergence and spread of antibiotic resistance in food-borne bacteria. *International Journal of Food Microbiology, 62*:1-5.

Tollefson, L., and Karp, B. E. (2004). Human health impact from antimicrobial use in food animals. *Medecine Et Maladies Infectieuses*, *34*(11), 514-521.

Tutenel, A.V., Pierard, D., Uradzinski, J., Jozwik, E., Pastuszczak, M., Van Hende, J., Uyttendaele, M., Debevere, J., Cheasty, T., Van Hoof, J. and De Zutter, L. (2002). Isolation and characterization of enterohaemorrhagic *Escherichia coli* O157: H7 from cattle in Belgium and Poland. *Epidemiology and Infection*, *129*(01), 41-47.

Uddin, M. A., Motazzim-ul-Haque, H. M., and Noor, R. (2011). Isolation and identification of pathogenic *Escherichia coli*, *Klebsiella spp*. and *Staphylococcus spp*. in raw milk samples collected from different areas of Dhaka city, Bangladesh. *Stamford Journal of Microbiology*, *1*(1), 19-23.

US Food and Drug Administration Center for Veterinary Medicine. Enrofloxacin for poultry. Available at: http://www.fda.gov/cvm/ FQWithdrawal.html. Accessed 15 September 2008.

US Government Accountability Office: Antibiotic Resistance: Agencies Have Made Little Progress Addressing Antibiotic Use in Animals. 2011. Available at: http://www.gao.gov/assets/330/323090.pdf. Accessed: 19 August 2013.

Vally, H., Hall, G., Dyda, A., Raupach, J., Knope, K., Combs, B., and Desmarchelier, P. (2012). Epidemiology of Shiga toxin producing *Escherichia coli* in Australia, 2000-2010. *BMC Public Health*, *12*(1), 1.

Van Donkersgoed, J., Berg, J., Potter, A., Hancock, D., Besser, T., Rice, D., LeJeune, J., and Klashinsky, S. (2001). Environmental sources and transmission of *Escherichia coli* O157 in feedlot cattle. *The Canadian Veterinary Journal, 42*, 714–720.

Viazis, S., and Diez-Gonzalez, F. (2011). Enterohemorrhagic *Escherichia coli*: the twentieth century's emerging foodborne pathogen: a review. *Advances in Agronomy*.

Viboud, G. I., Jouve, M. J., Binsztein, N., Vergara, M., Rivas, M., Quiroga, M., and Svennerholm, A. M. (1999). Prospective Cohort Study of Enterotoxigenic *Escherichia coli* Infections in Argentinean Children. *Journal of Clinical Microbiology*, *37*(9), 2829-2833.

Wani, S., Bhat, M., Samanta, I., Nishikawa, Y., and Buchh, A. (2003). Isolation and characterization of shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *Escherichia coli* (EPEC) from calves and lambs with diarrhoea in India. *Letters in Applied Microbiology, 37*(2): 121-126.

Wani, S., Watterworth, L., Topp, E., Schraft, H., and Leung, K. T. (2005). Multiplex PCR-DNA probe assay for the detection of pathogenic *Escherichia coli*. *Journal of Microbiological Methods, 60*(1): 93-105.

Wanke C. A., Schorling J. B., Barrett L. J., Desouza M. A., and Guerrant R. L. (1991). Potential role of adherence traits of *Escherichia coli* in persistent diarrhea in an urban Brazilian slum. *The Pediatric Infectious Disease Journal*, *10*(10), 746-751.

Watterworth, L., Topp, E., Schraft, H., and Leung, K. T. (2005). Multiplex PCR-DNA probe assay for the detection of pathogenic *Escherichia coli*. *Journal of Microbiological Methods, 60*(1): 93-105.

Weagant, S. D., and Bound, A. J. (2001). Evaluation of techniques for enrichment and isolation of *Escherichia coli* O157: H7 from artificially contaminated sprouts. *International Journal of Food Microbiology, 71*(1), 87e92.

Weintraub, A. (2007). Enteroaggregative *Escherichia coli*: epidemiology, virulence and detection. *Journal of Medical Microbiology*, *56*(1), 4-8.

Wennerås, C., and Erling, V. (2004). Prevalence of enterotoxigenic *Escherichia coli*-associated diarrhoea and carrier state in the developing world. *Journal of Health, Population and Nutrition*, 370-382.

White, D. G., Zhao, S. and Sudler, R. (2001). In: The road to resistance: Antibiotics as growth promoters for animals: The isolation of antibiotic resistant *Salmonella* from retail ground meats. *New England Journal of Medicine, 345*: 1147-54.

Willshaw, G. A., Thirlwell, J., Jones, A. P., Parry, S., Salmon, R. L., and Hickey, M. (1994). Vero cytotoxin-producing *Escherichia coli* O157 in beef burgers linked to an outbreak of diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome in Britain. *Letters in Applied Microbiology*, *19*(5), 304-307.

Wong, T. L., Hollis, L., Cornelius, A., Nicol, C., Cook, R. and Hudson, J. A. (2007). Prevalence, numbers, and subtypes of *Campylobacter jejuni* and *Campylobacter coli* in uncooked retail meat samples. *Journal of Food Protection 70*:566-573.

World Health Organisation, 2002, Use of antimicrobials outside human medicine and resultant Antimicrobial resistance in humans; Fact sheet January 2002 www.who.int/mediacentre/factsheets/fs268/en/

World Health Organisation, 2004. 1st Joint FAO/OIE/WHO Expert Workshop on Non-human Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment, Geneva, 1-5 Dec. 2003. http://www.who.int/foodsafety/publications/micro/nov2003/en/

Wright, D., Chapman, P., and Siddons, C. (1994). Immunomagnetic separation as a sensitive method for isolating *Escherichia coli* O157 from food samples. *Epidemiology and Infection, 113*(1), 31e40.

Yamamoto, S., Iwabuchi, E., Hasegawa, M., Esaki, H., Muramatsu, M., Hirayama, N. and Hirai, K. (2013). Prevalence and molecular epidemiological characterization of antimicrobial-resistant *Escherichia coli* isolates from Japanese black beef cattle. *Journal of Food Protection, 76*(3), pp 394–404.

Yoder, J. S., Cesario, S., Plotkin, V., Ma, X., Shannon, K. K., and Dworkin, M. S. (2006). Outbreak of enterotoxigenic *Escherichia coli* infection with an unusually long duration of illness. *Clinical Infectious Diseases*, *42*(11), 1513-1517.

Zaliha, I. and A.B. Rusli, (2004). A stydy on hygienic standard of food premises and microbiological quality of food in Kota Bharu. Working Paper. Universiti Sains Malaysia.

Zhang, Y., Laing, C., Steele, M., Ziebell, K., Johnson, R., Benson, A. K., Taboada, E. and Gannon, V. P. (2007). Genome evolution in major *Escherichia coli* O157: H7 lineages. *BMC Genomics, 8*(1), 1.