

**Republic of Iraq
Ministry of Higher Education
and Scientific Research
Thi-Qar University
College of Sciences
Department of Biology**



Isolation and Identification of Diarrheagenic *Escherichia coli* isolated from Raw Meat, Milk , Cheese and Detection of Some Virulence Genes by PCR in Thi-Qar

**A thesis
Submitted to College of Sciences – Thi-Qar University in
Partial Fulfillment of the Requirements for the Degree of
Master in Science of Biology**

**By
Athmar Dhahir Muttashar
B.SC Biology 2007**

**Supervised by

Asst. Prof. Dr. Rajee. T. Nasser**

2017 A.D

1438A.H

بسم الله الرحمن الرحيم

فتعالى الله الملك الحق ولا تعجل بالقرآن
من قبل أن يلقى اليك وحيه وقل ربي
زدني علما

صدق الله العلي العظيم

طه/الاية

(114)

Dedication

To

My country Iraq....

My beloved parents

**My brothers and sisters for lightening my
way**

All Iraqi people for all their sacrifices.

Athmar

Acknowledgment

In the name of the God the most merciful and most compassionate,
The peace and the mercy upon our Messenger prophet **Mohammed** and
his sanctified household. All thanks to God for granting me the strength,
and the patience with which this research had been accomplished.

It is a pleasure to express my deep appreciation to my supervisor Asst.
Prof. Dr. **Rajee T. Nasser** for his valuable guidance, assistance,
cooperation, and motivation throughout the course of preparing my
thesis, during the progress of the present work.

I would also like to thank Prof. Dr. **Mohammed A. Aouda** the Dean of
Science College, for his help and support all the time.

I am grateful to Dr. **Haider R. Malih** the Head of Biology department,
College of Science, Thi-Qar University, for his encouragement and care.

I would like to express my special thanks and respects to Prof. Dr. **Khalid
K. Al-Fartosi** for his help and support.

Very special thank to Dr. **Abbass Dekhail** (the Faculty of Veterinary
Medicine College ,Thi-Qar University) and Mr. **Mohammed Yossif** and
other staff of the Public Health Laboratory for their unlimited advice and
help to accomplish this work.

To all, Please accept my truthful thanks

Abstract:

The study was undertaken to isolate diarrheagenic *E. coli* from raw meat, milk and cheese, detection of virulence gene using Polymerase chain reaction (PCR) and investigate their antibiotic sensitivity pattern. Altogether 300 samples consisting of (100) raw meat, (100) raw milk, (100) cheese, were collected from milk vendors, retail shops located around Thi-Qar, under aseptic precautions. For the enrichment of the organism from the collected samples, MacConkey broth was used and inoculation was carried out on MacConkey agar and EMB agar. Later on, to confirm the isolates, various biochemical tests such as IMVC test, Urease test, Epi20 E were performed. Evaluation of antibiotic sensitivity pattern of *E. coli* was assessed by disk diffusion method. Finally the *E. coli* isolates were screened for the presence of virulence associated genes by PCR. A total of (18.33%) of *E. coli* was observed in the samples comprising of (25%) meat, (17%) milk, (13%) cheese. High Antibiotic sensitivity of 100% was recorded for imipenem, cephalothin, chloramphenicol, and netilmicin while a percentage of 81.8%, 72.27%, 87.27% and 56.36% were recorded for tetracycline, ciprofloxacin, gentamycin, and nalidixic acid respectively. On the other hand, Ampicillin, cefotaxime, and ceftriaxone show different behavior where the bacteria resist the action of those antibiotic by 45.4%, 67.27%, and 50.9%, respectively. In this study, out of 55 *E. coli* isolates, (12.7%) was positive for *rfbO157* gene, which consists of (16%) isolates for meat, (11.7%) isolates for milk and (7.6%) isolates for cheese. (32.72%) was positive for *eaeA* gene: which consists of (24%) isolates for meat, (29.41%) isolates for milk and (53.84%) isolates for cheese. (14.5%) was positive for *bfpA* gene: which consists of (8%) isolates for meat, (11.76%) isolates for milk and (30.76%) isolates for cheese. (12.72%) was positive for *ial* gene: which consists of (8%) for meat, (11.7%) for

milk and (23.07%) for cheese. (10.90%) was positive for *CVD432* gene : which consists of (8%) for meat , (11.7%) for milk and (15.3%) for cheese. (16.36%) was positive for *STLa* gene : which consists of (12%) for meat , (17.6%) for milk and (23.07%) for cheese.

In conclusion , the *E. coli* isolates show high frequency in meat samples compared with milk and cheese samples . Furthermore , the majority of tested *E. coli* isolates were sensitive to the most antibiotic agents.

LIST OF CONTENTS

NO	Subject	Pages number
	Abstract	I-II
	List of contents	III-VI
	List of tables	VII-IX
	List of figures	X
	List of Abbreviations	XI-XII
	Chapter One	
1.1	Introduction	1-5
	Chapter Two	
2.1	Literature Review	6
2.1.1	Historical back ground	6
2.1.2	Classification	10
2.1.3	Clinical groupings of human <i>E. coli</i> strains	11
2.1.4	Biochemistry of <i>E. coli</i>	12
2.1.5	Diarrheagenic <i>Escherichia coli</i>	13
2.1.5.1	Enterotoxigenic <i>E. coli</i> (ETEC)	13
2.1.5.2	Enteropathogenic <i>E. coli</i> (EPEC)	16
2.1.5.3	Enteraggregative <i>E. coli</i> (EAEC)	17
2.1.5.4	Enterohemorrhagic <i>E. coli</i> (EHEC)	20
2.1.5.5	Enteroinvasive <i>E. coli</i> (EIEC)	21
2.1.5.6	Diffusely adherent <i>E. coli</i> (DAEC)	22
2.1.6	Reservoirs	22
2.1.7	Transmission	22
2.1.7.1	<i>EHEC</i>	23
2.1.7.2	<i>EPEC</i>	23
2.1.7.3	<i>ETEC</i>	23
2.1.7.4	<i>EIEC</i>	23
2.1.7.5	<i>EAEC</i>	24
2.1.7.6	<i>DAEC</i>	24
2.1.8	Laboratory diagnosis	24
2.1.9	Diagnostic Tests	24
2.1.9.1	Detection of <i>ETEC</i>	24
2.1.9.2	Detection of <i>EPEC</i>	26
2.1.9.2.1	Phenotypic Tests	26
2.1.9.2.2	Genotypic Tests	27
2.1.9.3	Detection of <i>EHEC</i>	28

2.1.10	Virulence factors	28
2.1.10.1	Adhesion/colonization	28
2.1.11	Molecular methods	29
2.1.11.1	Polymerase Chain Reaction (PCR)	29
2.1.12	Control and prevention	30
	Chapter Three	
3	Materials and methods	32
3.1	Materials	32
3.1.1	Culture Media	32
3.1.2	Chemical and biochemical material	32
3.1.3	Instrument and Equipments	33
3.1.4	Antibiotics	34
3.1.5	Types of kits	35
3.1.5.1	Components of API 20E test	36
3.1.5.2	Components of DNA extraction Kit Geneaid (England)	36
3.1.5.3	Molecular Weight Marker	37
3.1.6	primers for detection of virulence factors of enteropathogenic <i>E. Coli</i>	37
3.2	Methods	38
3.2.1	preparation of culture media	38
3.2.1.1	MacConkey Agar Medium	38
3.2.1.2	MacConkey Broth	39
3.2.1.3	MacConky sorbitol Agar	39
3.2.1.4	preserve Media for microbe isolate	39
3.2.2	Samples collection	39
3.2.3	Preparation of Reagents and solution	40
3.2.3.1	Catalase	40
3.2.3.2	Oxidase	40
3.2.3.3	Methyl red	40
3.2.3.4	voges-proskauer	41
3.2.3.5	MacFarland standard	41
3.2.3.6	Normal saline solution	41
3.2.4	Tests that's used to identify bacterial isolates	41
3.2.4.1	Indole test	41
3.2.4.2	Methyl red test	41
3.2.4.3	Voges-Proskauer test	42
3.2.4.4	Citrate Utilization test	42

3.2.4.5	Urease test	42
3.2.4.6	Kliglers iron agar test	43
3.2.4.7	Carbohydrate Fermentation	43
3.2.5	Identification of the organism	43
3.2.5.1	gram staining	44
3.2.5.2	Colony morphology	44
3.2.5.3	Isolation of <i>E. coli</i> from samples	44
3.2.5.4	Cultural Properties on MacConkey Agar Plate	44
3.2.5.5	Cultural Properties on Blood Agar Plate	45
3.2.5.6	Screening of Suspected Isolates on EMB Plate	45
3.2.6	Confirmatory test System	45
3.2.6.1	API20 Multi Test System	45
3.2.7	Antimicrobial Susceptibility Test	45
	A-Preparation of Bacterial Inoculums'	46
	B-Streaking	46
	C-Application of Antibiotic Discs	46
3.2.8	Polymerase Chain Reaction Assay	47
3.2.8.1	Detection the virulence factors genes by PCR assay	47
3.2.8.1.1	DNA Extraction	47
3.2.8.2	Loading and Running DNA in Agarose Gel	48
3.2.8.3	Polymerase chain reaction	48
3.2.8.3.1	Amplification of <i>EPEC</i> gene (<i>eaeA</i> amplicon size 229)	48
3.2.8.3.2	Amplification of <i>EAEC</i> gene (CVD432)	49
3.2.8.3.3	PCR amplification of <i>STEC</i> (<i>O157</i> gene)	51
3.2.8.3.4	PCR amplification of <i>EPEC</i> (<i>bfpA</i> gene)	52
3.2.8.3.5	PCR amplification of <i>EIEC</i> (<i>ial</i> gene)	53
3.2.8.3.6	PCR amplification of <i>ETEC</i> (<i>STLa</i> gene)	55
	Chapter Four	
4.1	Isolation and identification of Diarrheagenic <i>Escherichia Coli</i>	57
4.1.1	Distribution food samples according to their type and number	57
4.1.2	Distribution of the food samples according to the geographic location	57

4.1.3	Identification of <i>E. coli</i> according to the growth on a selective media and the biochemical test	58
4.1.4	Identification of <i>E. coli</i> by using api-20E	59
4.2	Results of Screening by PCR for Virulence Factor	61
4.2.1	Detection of the Whole DNA	61
4.2.2	Detection of virulence factor diarrheagenic <i>E. coli</i> genes by polymerase chain reaction (PCR) analysis	62
4.2.2.1	.Detection <i>rfbO157</i> gene size (259 bp) encoded for <i>STEC</i>	62
4.2.2.2	Detection of <i>eaeA</i> gene (229 bp) size that encoded for <i>EHEC</i> group	64
4.2.2.3	Detection the <i>bfpA</i> gene size (450 bp) encoded for <i>EPEC</i> group	65
4.2.2.4	Detection the <i>ial</i> gene size(320) encoded for <i>EIEC</i> group	67
4.2.2.5	Detection of <i>CVD342</i> gene size (630) encoded for <i>EAEC</i> group	68
4.2.2.6	Detection of <i>STLa</i> gene size (170bp) encoded for <i>ETEC</i> group	70
4.3	Antibiotic susceptibility testing	72
Chapter Five		
5.1	Isolation and identification of <i>E. coli</i>	74
5.2	Detection of diarrheagenic <i>E. coli</i> virulence genes by PCR	76
5.3	Prevalence of antibiotic sensitivity of <i>E. coli</i>	82
	Conclusions and Recommendations	84-85
	References	86-130

LIST OF TABLE

NO	Subject	Page number
3-1	Selective and differential media used in the isolation and identification of diarrheagenic <i>E. coli</i>	32
3-2	The chemical and biological materials with their remarks	33
3-3	The instrument and the equipment that used in this study with their remarks	34
3-4	The antibiotics that used in this study and the dosage to each of them with their remarks	35
3-5	DNA marker with the description and source	37
3-6	The group of the strain ,DNA sequence, target gene, product size	37
3-7	Area of sampling, number and types of samples	40
3-8	PCR condition for <i>eaeA</i> gene	49
3-9	Reaction mixture (20μ) <i>eaeA</i> gene	49
3-10	The PCR condition for <i>CVD432</i> gene	50
3-11	The reaction mixture (20μ) for <i>CVD432</i> gene	51
3-12	The PCR condition for <i>O157</i> gene	52
3-13	Reaction mixture (20μ) for <i>O157</i> gene	52
3-14	PCR condition for <i>bfpA</i> gene	53
3-15	Reaction mixture (20μ) for <i>bfpA</i> gene	53

3-16	PCR condition for <i>ial</i> gene	54
3-17	Reaction mixture (20μ) for <i>ial</i> gene	54
3-18	PCR condition for <i>STLa</i> gene	55
3-19	Reaction mixture (20μ) for <i>STLa</i> gene	56
3-20	Reaction mixture (20 μ) for <i>STLa</i> gene	56
4-1	The distribution of the samples according to their type, the Number of samples, the percentage of the whole number	57
4-2	The distribution of the food samples according to the geographic Location, type of samples, number of samples, and their percentage	58
4-3	Number of samples, number of <i>E. coli</i> isolates, and their Percentage according to the biochemical and Api-20 E tests	61
4-4	The total isolates of <i>E. coli</i> , the positive number of isolates to <i>rfbO157</i> gene , and their percentage	63
4-5	The total isolates of <i>E. coli</i> , positive samples to <i>eaeA</i> gene, their Percentage, and the type of samples	64
4-6	The total isolates of <i>E. coli</i> , positive samples to <i>bfpA</i> gene, their Percentage, and the type of samples	66
4-7	The total isolates of <i>E. coli</i> , positive samples to <i>ial</i> gene, their Percentage , and the type of samples	67

4-8	The total isolates of <i>E. coli</i> , positive samples to <i>CVD432</i> gene, Their percentage, and the type of samples	69
4-9	The total isolates of <i>E. coli</i> , positive samples to <i>STLa</i> gene, Their percentage, and the type of samples	71
4-10	Antimicrobial drug sensitivity of <i>E. coli</i> isolates from meat, Milk, and cheese	73

LIST OF FIGURE

NO.	Subject	Page number
4.1	Identification of <i>E. coli</i> by using Api-20E system	60
4.2	Total DNA extraction from <i>E. coli</i> isolates	62
4.3	PCR products of (259bp) <i>rfbO157</i> gene on (1.5%) agarose_Gel for 45m and 70V	63
4.4	PCR products of (229 bp) <i>eaeA</i> gene on (1.5%) agarose_Gel for 45m and 70V	65
4.5	PCR products of (450 bp) <i>bfpA</i> gene on (1.5%) agarose_Gel for 45m and 70V	66
4.6	PCR products of (320 bp) <i>ial</i> gene on (1.5%) agarose_Gel for 45 m and 70V	68
4.7	PCR products of (630 bp) <i>CVD432</i> gene on (1.5%) agarose Gel for 45m and 70V	70
4.8	PCR products of (170 bp) <i>STLa</i> gene on (1.5%) agarose Gel for 45m and 70V	72

List of Abbreviations

CDC	Centers for Disease Control
WHO	World Health Organization
FAO	Food and Agriculture Organization
HUS	Hemolytic Uremic Syndrome
HACCP	Hazard Analysis and Critical Control points
HTST	High Temperature-Short Time
UHT	Ultra-Heat Treatments
LT	Heat-Labile
ST	Heat-Stable
CFAs	Colonization Factor Antigens
CT	Cholera Toxin
c AMP	Cyclic Adenosine Mono-phosphate
c GMP	Cyclic Guanidine Mono-phosphate
TTSS	Type Two Secretion System
Tir	Translocated intimin receptor
PAI	Pathogenicity Island
LEE	Locus of Enterocyte Effacement
A/E	Attaching and Effacing

Per	Plasmid encoded regulator gene
A/A	Aggregative Adherence pattern
SLTEC	Shiga Like Toxin producing <i>E. coli</i>
HC	Hemorrhagic Colitis
CHO	Chinese Hamster Ovary
HEP-2	Human Epithelial cells
LPS	Lipo- PolySaccharides
<i>bfpA</i>	bundle forming pilus
Psi	Pounds per square inch

Chapter One

Introduction

1.1 Introduction

Statistics indicate that meat as a food group is the most important food group responsible for infections and food poisoning in western countries where meat consumption is high, and it can be said that 60-70 percent of total cases attributable to meat. The importance of this ratio is clear if we know it is in the United States, for example, statistics from the Centers for Disease Control (CDC) indicate that about 76-81 million people suffer from food borne diseases. And on a global level, WHO estimates that at least 10% of the world's population suffer from these diseases every year (*Scotter et al., 2000 ; Peter et al., 2000*).

Raw milk consumers have existed in various parts of the world. World milk production reached 724 million tons in 2010 as per Food and Agriculture Organization, resulting in trade and massive consumption of various milk products (FAO; Food and Agriculture Organization, 2011). Raw milk is consumed directly by a large population in rural areas ,Milk is an excellent medium for the growth of numerous microbes which produced consequential spoilage of the milk and various milk products or infections in consumers (*Oliver et al ., 2005*). Because of the specific production, it is impossible to avoid contamination of milk with microorganisms ,therefore the microbial content of milk is a major feature in determining its quality (*Torkar, K. G. and Teger S. G., 2008*). The existence of food borne pathogens in raw milk may increases the threat of ingestion and transmission of food borne pathogens and ingestion of harmful toxins (*Srinu et al., 2012*).

Huge numbers of microbes can get access to milk and various milk products including *E. coli* which is an indicator of fecal contamination constituting a public health hazard. The most important causes of food borne diseases are Shiga toxin-producing *E. coli* (*STEC*) among the other seropathotypes of *E. coli*

(Kaufmann M et al.,2006) . *STEC* produce various complications including diarrhea, hemolytic uremic syndrome (HUS) and hemorrhagic colitis (HC) (Brett KN et al.,2003).

The detection and enumeration of pathogens in food and on surfaces that come into contact with food are an important component of any integrated program to ensure the safety of foods throughout the food supply chain. Both government authorities and food companies use microbiological analysis to monitor the state of contamination at all times and analyze its trends so as to detect emerging risks. Microbiological analysis is also an essential tool for carrying out tests in accordance with the microbiological criteria established for each food type, as well as being essential for evaluating the actions of different management strategies based on the Hazard Analysis and Critical Control Points (HACCP) system (Stannard., 1997; Jasson et al., 2010) . The implementation of preventive systems such as the HACCP has greatly improved food safety, but it will not be fully effective until better methods of analysis are developed. These new detection methods are the necessary technologies that will substantially improve our food safety once integrated in the HACCP (Bhunia., 2008) . Microbiological analysis of foods is based on the detection of microorganisms by visual, biochemical, immunological, or genetic means, either before enrichment (quantitative or enumerative methods) or after enrichment (qualitative methods, also known as presence/absence tests) (Jantzen et al., 2006a) . traditional culture methods for detecting microorganisms in food are based on the incorporation of the food sample into a nutrient medium in which the microorganisms can multiply, thus providing visual confirmation of their growth. These conventional test methods are simple, easily adaptable, very practical, and generally inexpensive (Feng et al., 2007) . Although not lacking in sensitivity, they can be laborious and depend on the growth of the microorganisms in different culture media (pre-enrichment, selective

enrichment, selective plating, identification), which may require several days before results are known (*Betts and Blackburn ., 2009*) . Products that are minimally processed have an inherently short shelf life, which prevents the use of many of these conventional methods. Therefore, extensive research has been carried out over the years to reduce assay time through the use of alternative methods for detecting food borne microorganisms and reduce the amount of manual labor by automating methods whenever possible (*Jasson et al., 2010*).

Infectious diarrhea is one of the world's leading causes of morbidity and mortality, resulting in about two million deaths per year (*World health organization., 1995 ; Alikhani et al., 2006*) . Studies showed that 1 – 5 % of food-infections were related to the consumption of milk and dairy products, and 53% of cases of food-borne infections caused by *EPEC* (*De buyser ML et al., 2001*).

Numerous studies performed in different countries have reported diarrheagenic *Escherichia coli* (DEC) pathotypes as being the most frequent and important among bacterial pathogens associated with AID in developing countries. However, the frequencies of these pathogens vary with geographic region and depend on the socioeconomic/sanitary conditions achieved (*Black et al., 2010; O'Ryan et al., 2010*).

Diarrheagenic *Escherichia coli* has been reported to be responsible for 34% of diarrheal episodes in Bangladesh (*ICDDR., B 2002*). *Escherichia coli* is the predominant nonpathogenic facultative anaerobic member of the human intestinal micro flora. Some *Escherichia coli* strains, however have developed the ability to cause disease of the gastrointestinal, urinary and central nervous systems in the human host. *Escherichia coli* has been implicated as an agent of diarrheal disease since the 1920s (*Nguyen et al., 2005*). There are now at least six types of diarrheagenic strains of *Escherichia coli* on

the basis of distinct epidemiology and clinical feature, special virulence determinants and association with certain serotypes: Enterotoxogenic *Escherichia coli* (ETEC), Enteropathogenic *Escherichia coli* (EPEC), Enterohemorrhagic *Escherichia coli* (EHEC), Enteraggregative *Escherichia coli* (EAEC), Enteroinvasive *Escherichia coli* (EIEC) and Diffusely adhering *Escherichia coli* (DAEC). Of these EPEC, ETEC, EIEC, EHEC and EAEC are clearly associated with different types of enteritis, while the another pathotype is candidate as potential pathogens but their association with diarrhea has not been clearly assessed and further studies are required to confirm their etiological role in diarrheal diseases (Rappelli et al., 2005). Due to lack of facilities diarrheagenic *Escherichia coli* cannot be detected in the routine diagnostic microbiology laboratory in developing countries, which is important in understanding the disease spectrum, tracing the sources of infection and the burden of the disease. Such identification would also assist the clinician to dispense appropriate management (Nessa et al., 2007). Identification of diarrheagenic *Escherichia coli* cannot be done only by culture and biochemical tests, since they are indistinguishable from the non pathogenic *Escherichia coli* commonly found in human feces. Moreover, specific serotyping is not always correlated with pathogenicity. The discrimination of different diarrheagenic *Escherichia coli* strains requires DNA target based screening by molecular techniques (Rappelli et al., 2005). PCR, one of the molecular based detection methods, commonly used for rapid and reliable diagnosis, which has a high sensitivity and specificity. To detect different diarrheagenic *Escherichia coli* strains, it is necessary to run several individual PCRs with different primer pairs. In order to simplify diagnosis, various multiplex PCR methods have been developed for the simultaneous detection of several specific genes of different diarrheagenic *Escherichia coli* strains. This method showed high sensitivity and

high specificity for identification of human diarrheagenic *Escherichia coli* (Toma *et al.*, 2003).

1.2 Aim of the study

The study aimed to :

Detection of some virulence genes in diarrheagenic *E. coli* isolated from raw meat , and dairy milk through :

1. Detection susceptibility of *Escherichia coli* to some antimicrobial agents .
2. Detection the presence of (*eaeA*, *Stla*, *rfbO157*, *ial*, *CVD342*, *bfpA*) genes in Diarrheagenic *Escherichia coli*.

Chapter Two

Literature Review

2.1 Literature Review

2.1.1 Historical background

Food borne pathogens are the leading cause of illness, and death in developing countries, costing billions of dollars in medical care, and medical and social costs. Changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors (*Wei et al., 2006*).

Contaminated raw meat is one of the main sources of food borne illnesses. The risk of the transmission of zoonotic infections is also associated with contaminated meat. International food management agencies, especially the World Health Organization (WHO), the Food and Agriculture Organization and the International Hazard Analysis Critical Control Point (HACCP) Alliance have already provided guidelines to member countries about safe handling procedures such as HACCP and Good Manufacturing Practices (GMPs) (*Nafisa et al., 2010*).

In the other hands milk is the complete food composed of higher solid contents such as lipids, proteins, milk sugar (lactose) and minerals. It is rich in vitamins A and B and is an important source of dietary Calcium and other minerals. Milk is much important food source for its chemical composition and nutritive properties. All types of milks are more or less similar in composition and nutritive properties irrespective of their source like cow, buffalo, goat and camel etc. The major constituents of milk are higher in buffalo milk than in cow, goat, camel and human milks (*Sheehan, W.J. and Phipatanakul, W. 2009*).

The milk is the ideal growth medium for several types of microorganisms (*Haridy, M.S.A.,. 1992*), The bacteriological quality of milk is the important

factor to be considered for the consumption of milk and other dairy products (Ahmad *et al.*, 2008).

The raw milk is usually colonized by pathogens like *Campylobacter jejuni*, enterohemorrhagic *E. coli*. The pathogens may enter the good quality milk when they are mixed with mastitis milk from the live stock. The pathogen *E. coli* is a common inhabitant of intestines of animals and gain access to the milk and the dairy products. Pathogen enters the milk through the mastitis infection of the cattle (Roca-Fernández, A.I. 2011).

The family *Enterobacteriaceae* comprises a large group of gram-negative non-spore-forming bacteria typically 1-5 μm in length. They are facultative anaerobes and with the exception of *Saccharobacter fermentans* and some strains of *Yersinia* and *Erwinia*, they share the ability to reduce nitrate to nitrite (Joosten *et al.*, 2008).

These bacteria are generally motile by peritrichous flagella except for *Shigella* and *Tatumella* and some other non-motile members of this family. A common feature of the *Enterobacteriaceae*, which helps to differentiate them from other closely related bacteria, is the lack of cytochrome C oxidase, although there are exceptions such as *Plesiomonas* spp (Gurtler *et al.*, 2005; Lehner and Stephan, 2004; Friedemann, 2007).

Enterobacteriaceae are catalase positive with the exception of *Shigella dysenteriae* 1 and *Xenorhabdus* species. *Enterobacteriaceae* ferment a variety of carbohydrates, but their ability to produce acid and gas from the fermentation of D-glucose is one characteristic that remains an important diagnostic property and is commonly used as a basis for their detection and enumeration (Anon., 2001 a & b), The name *Enterobacteriaceae* was first proposed by Rahn (1937).

The type genus is *Escherichia*. *Enterobacteriaceae* comprise a large group of genetically and biochemically related bacteria. Phylogenetic studies place them in the phylum *Proteobacteria*, Class *Gammaproteobacteria* and Order *Enterobacteriales* (Brenner et al., 2005).

Until the early 1960s bacterial classification was largely based on phenotypic characteristics and culture-based observations. The introduction of genetic methods, such as DNA-DNA hybridization and guanine plus cytosine (G+C) determination revolutionized bacterial taxonomy and classification. More recently analysis of 16S rRNA gene sequences has been used to elucidate further the genetic relationships between members of the *Enterobacteriaceae* and their similarity to other closely related bacteria. Consequently, there have been recent changes to the classification of members of this family. Indeed, the number of genera and species of *Enterobacteriaceae* increased from 12 genera and 36 species in 1974 to at least 34 genera, 149 species and 21 subspecies in 2006 (Baylis., 2006).

Enterobacteriaceae can cause food spoilage in a broad range of food products, namely milk, dairy products, meats, poultry, fish, sea foods, fruit, vegetables and other foods. The growth and metabolic activity of *Enterobacteriaceae* in food can result in off-flavors, odors, color defects and other organoleptic deviations. These changes may arise from enzymatic breakdown of proteins or lipids, production of volatile components and gas production (Cousin, 1982; Jay, 2000).

In milk and other dairy products, some strains of *Enterobacteriaceae* can impart bitterness by producing enzymes that break down casein. The thermo-stability of some lipases and proteinases suggests that high temperature-short time (HTST) and ultra-heat (UHT) treatments will not inactivate these enzymes. Besides

flavor defects, some *Enterobacteriaceae*, can produce a reddish pigment, whilst other bacteria may cause other color defects (ICMSF., 1998).

During the early ripening stage of fresh cheese production *Enterobacteriaceae* can also cause a spoilage termed “early blowing” which can result in holes within the product, or blown packs, caused by the production of gas from the fermentation of lactose (ICMSF., 1998).

Escherichia coli are genetically heterogeneous group of bacteria whose members are typically non pathogens that are a part of the normal microflora of the intestinal tract of humans and animals. However, certain subsets of this bacterial species have acquired genes that enable them to cause intestinal or extra intestinal disease (Bacon *et al.*, 2000; Abdella *et al.*, 2009).

Escherichia. coli that cause enteric disease have been divided into pathotypes, based on their virulence factors and mechanisms by which they cause disease. One of these pathotypes, called Shiga toxin-producing *E. coli* (STEC), refers to those strains of *E. coli* that produce at least 1 member of a class of potent cytotoxins called Shiga toxin. The STEC are also called verotoxin producing *E. coli* (Gill *et al.*, 1996 ; Gyles, 2007).

Sporadic cases and outbreaks of human diseases caused by STEC have been linked to ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water. Infections can also be acquired by direct contact with animals and by person to person spread. The organism is destroyed in pasteurization process, but insufficient heat treatment of ground meat and raw milk forms a potential infection risk (Rahimi *et al.*, 2012).

Escherichia coli were first described in 1885 by Theodor Escherich. Escherich, a Bavarian paediatrician, had performed studies on the intestinal flora of infants and had discovered a normal microbial inhabitant in healthy individuals, which

he named *Bacterium coli commune*. In 1919, the bacterium was renamed in his honour to *Escherichia coli* (Kaper., 2005).

Escherichia coli are ubiquitous intestinal bacterial flora of animals and humans. Although comprising a small proportion of the total fecal flora they are the predominant facultative anaerobe in the human colon and presumably exist there symbiotically (Wei *et al.*, 2006). Infants and post parturient animals are normally colonized shortly after parturition, acquiring their mothers' intestinal flora. Pathogenic strains or clones of *E. coli* exist or have developed with the ability to cause a wide variety of diseases in humans (Battisti *et al.*, 2006). During the past twenty years, a clonal group of *E. coli* has emerged or been recognized that possess a unique disease causing virulence factor armament, asymptomatically colonize food producing ruminants, and are capable of causing significant morbidity and mortality in humans (Johnsen *et al.*, 2001).

2.1.2 Classification

Escherichia coli is a gram negative, facultative anaerobic, non spore forming rod, which belongs to the *Enterbacteriaceae* family (Law, 2000). *E. coli* is a Gram negative bacterium in the Phylum Proteobacteria, class Gamma *proteobacteria*, order *Enterobacteriales*, family *Enterobacteriaceae* and genus *Escherichia*. In the family *Enterobacteriaceae* most important genera are *E. coli*, *Shigella*, *Salmonella*, *Yersinia* which have some common characteristic such as Gram negative rods, non spore forming facultative anaerobic, ferment glucose, simple growth requirements, most are motile with peritrichous flagella and many produce fimbriae (pili), capsules, or both. *E. coli* are one of coliform organisms (Quinn *et al.*, 2002).

Differences between strains of *E. coli* lie in the combination of different antigens they possess. There are three types of antigens: the somatic

lipopolysaccharide antigen (O), the flagellar antigens (H), and the capsular antigens (K) (Quinn *et al.*, 2002). There are approximately 174 O antigens, 56 H antigens, and 103 antigens that have been identified. There are several strains of *E. coli* that have been isolated. The enteric *E. coli* are divided on the basis of virulence properties into *Enterotoxigenic (ETEC)*, *Enteropathogenic (EPEC)*, *Enteroinvasive (EIEC)*, *Verotoxigenic (VTEC)*, *Enterohemorrhagic (EHEC)* and *Enterobioaggregative (EaggEC)* (Frenzen *et al.*, 2005).

2.1.3 Clinical groupings of human *E. coli* strains

E. coli strains of significance to humans can be classified according to genetic and clinical criteria into three groups: commensal strains, pathogenic intestinal (enteric or diarrheagenic) strains, and pathogenic extraintestinal strains (Russo *et al.*, 2000). Using the *E. coli* reference strains of (Ochman *et al.*, 1984; Goullet *et al.*, 1989) found that the reference strains formed six phylogenetic groups based on the electrophoretic polymorphism of esterases and other enzymes. These groups were designated A, BI, B2, C, D, and E. The majority of the normal facultative fecal bacterial strains found in healthy humans, mammals, and birds are commensal *E. coli* strains. The commensal strains are generally benign, do not cause intestinal tract disease, and can be beneficial to the host (Neill *et al.*, 1994; Russo *et al.*, 2003). However, commensal strains may cause illness if the host is compromised immunologically or medically (Picard *et al.*, 1999; Russo *et al.*, 2003). Generally, human commensal *E. coli* strains derive from phylogenetic groups A and BI and typically lack the specialized virulence determinants found in pathogenic strains that cause intestinal or extraintestinal diseases (Picard *et al.*, 1999; Russo *et al.*, 2000).

Intestinal pathogenic strains of *E. coli* are seldom found in the fecal flora of healthy individuals and are rarely a cause of extraintestinal disease. These obligate pathogens induce colitis or gastroenteritis if contaminated food or water

is ingested (Guerrant et al., 1995; Russo et al., 2000). The diarrhea-inducing strains include the enterotoxigenic, enterohemorrhagic, enteroinvasive, enteropathogenic, enteroaggregative, diffusely adherent, and cell-detaching *E. coli* pathotypes (Fratamico et al., 2006; Guerrant et al., 1995; Nataro et al., 1998).

2.1.4 Biochemistry of *E. coli*

E. coli is the type species of the genus *Escherichia*, which contains mostly motile gram-negative bacilli within the family *Enterobacteriaceae* and the tribe *Escherichia* (Bettelheim, K. A. 1994; Edwards et al., 1972). *E. coli* can be recovered easily on general or selective media at 37°C under aerobic conditions. *E. coli* are most often recovered on MacConkey or eosin methylene-blue agar, which selectively grow members of the *Enterobacteriaceae* and permit differentiation of enteric organisms on the basis of morphology (Balows et al., 1991). *Enterobacteriaceae* are usually identified via biochemical reactions. These tests can be performed in individual culture tubes or by using test “strips” which are commercially available. Either method produces satisfactory results. For epidemiologic or clinical purposes, *E. coli* strains are often selected from agar plates after presumptive visual identification. However, this method should be used only with caution, because only about 90% of *E. coli* strains are lactose positive; some diarrheagenic *E. coli* strains, including many of the *EIEC* strains, are typically lactose negative. The indole test, positive in 99% of *E. coli* strains, is the single best test for differentiation from other members of the *Enterobacteriaceae* (Banatvala et al., 1996).

2.1.5 Diarrheagenic *Escherichia coli*

The pathogenic *E. coli* species comprise a very versatile group with numerous virulence determinants (virulence factors) including adhesions, invasions, toxins and secretion systems that allow them to act as causative agents in both human and veterinary medicine (Kaper *et al.*, 2004; Nataro & Kaper., 1998). In human, these pathogens are responsible for three main types of clinical infections: (i) enteric or diarrheal diseases, (ii) urinary tract infections, and (iii) meningitis/septicemia. Based on their distinct virulence properties and clinical symptoms of the host, pathogenic *E. coli* strains are divided into numerous categories or pathotypes: Diarrheagenic *Escherichia coli* (DEC); Uropathogenic *Escherichia coli* (UPEC); Neonatal meningitis/sepsis associated *Escherichia coli* (NMEC) (Dawson *et al.*, 1999; Kaper *et al.*, 2004). Diarrheagenic *Escherichia coli* (DEC) belong to one of the most frequent, interesting and widespread versatile pathogenic groups of bacteria that cause severe disease in human (Campos *et al.*, 2004; Nataro & Karper., 1998). DEC has been divided into six well characterized subgroups or classes [enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), verocytotoxin producing *E. coli* (VTEC) or enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC)] based on clinical manifestations, phenotypic traits, specific virulence properties, and pathogenesis (Kaper *et al.*, 2004; Nataro & Karper., 1998).

2.1.5.1 Enterotoxigenic *E. coli* (ETEC)

Enterotoxigenic E. coli is one of the most studied pathotypes of DEC. Most studies have demonstrated the association of ETEC with diarrhea among infants less than five years of age (Aguero *et al.*, 1985; Shaheen *et al.* 2004). Most of the illness, both in terms of number of cases and severity of symptoms, occur in

infants after weaning. Moreover, epidemiological studies have implicated contaminated food and water as the most common vehicles of *ETEC* infection.

Thus, sampling of both food and water sources from endemic areas have demonstrated the spread of *ETEC* bacteria in the community (*Black et al., 1982*).

A high infectious dose (ranging 10⁶ to 10¹⁰ CFU) of *ETEC* has been demonstrated in human (*Levine et al., 1979; Nataro & Karper., 1998*), and a large number of serogroups of *ETEC* have been associated with diarrhea (*Stenutz et al., 2006*).

ETEC strains cause cholera-like watery diarrhea through the elaboration and action of LT (heat-labile) and/or ST (heat-stable) enterotoxins. The ability of *ETEC* strains to produce diarrheal illness by either or both of these enterotoxins is what defines an *ETEC* (*Nataro & Karper., 1998*). However, to cause diarrhea, by LT and/or ST, the *ETEC* must adhere and colonize the intestinal mucosa. This is achieved by attaching with one or more colonization factor antigens (CFAs), which are antigenically diverse and usually are encoded by plasmids (*Nataro & Karper 1998; Smith et al., 1983*).

LTs of *ETEC* are oligomeric toxins that are functionally and structurally related to the cholera toxin (CT) expressed by *Vibrio cholerae*, serogroups O1 and O139 (*Sixma et al., 1993*). There are two types of LTs, LT-I and LT-II that are commonly found in human and animal isolates. However, the term LT refers to LT-I, which is associated with disease in both human and animals, while LT-II is expressed only in animals, but it is rarely associated to disease (*Nataro & Karper., 1998; Smith et al., 1983*). LT-I is constituted by ~80% amino acid identity with CT that consists of a single A subunit and five identical B subunits. The A subunit is responsible for the enzymatic activity, and the B subunits are responsible for the toxin binding to the cell surface ganglioside. After

endocytosis the A subunit stimulates a series of intracellular processes leading to increased level of cyclic adenosine monophosphate (cAMP), resulting in an increased phosphorylation of chloride channels, and hence a reduced absorption of NaCl. This increased extracellular ions content results in osmotic diarrhea. Besides, there is an increased secretion of ions in the villi crypts (Nataro & Karper., 1998).

STs are small peptides including two unrelated classes, STa and STb, which differ in structure and mechanism of action. Genes for both classes *estA* and *estB* have been found either on plasmids or on transposons. Only toxin of the STa class has been associated with human and animal diseases. It has been established that the STa receptor is located on the apical surface of enterocytes and that binding to the receptors leads to increased intracellular cyclic guanine monophosphate (cGMP) levels, which affects the electrolytic balance in a similar manner as LT (Kaper *et al.*, 2004; Nataro & Karper., 1998).

In contrast, STb is primarily associated with diarrhea in piglets; however, some human *ETEC* isolates expressing STb have been reported (Nataro & Karper., 1998; Schulz *et al.*, 1990).

STb can elevate cytosolic Ca^{+2} concentrations, stimulating both the release of prostaglandin E2 and serotonin, which lead to increased ion secretion (Kaper *et al.*, 2004; Nataro *et al.*, 1998).

The clinical features of *ETEC* diarrhea are constant with the pathogenic mechanisms of its enterotoxins, and characterized by watery diarrhea without blood, mucus or pus, fever and vomiting (DuPont *et al.*, 2009).

The illness is typically abrupt, but can vary from mild, brief, and self-limiting to a severe disease similar to in *Vibrio cholerae* infection (Levine *et al.*, 1977)

Several studies have showed that the percentage of *ETEC* infection varies from 10 to 30% and the clinical presentation, as with the other DEC varies among different geographical areas (*Mangia et al., 1993*).

2.1.5.2 Enteropathogenic *E.coli* (*EPEC*)

EPEC was the first pathotype of *DEC* described, and associated to the infant diarrheal diseases worldwide (Nataro & Karper., 1998). The characteristic intestinal histopathology attaching and effacing (A/E) lesions - of *EPEC* are associated to striking cytoskeletal changes in the epithelial cell. This ability to induce-attaching and effacing lesions is encoded by genes for the adherence factor intimin (*eae*), a type II secretion system (TTSS) that includes the *esc* genes and the translocated intimin receptor (Tir) (Nataro & Karper 1998).

These genes are located on a 35-Kb (kilobase) pathogenicity island (PAI), called the locus of enterocyte effacement (LEE), which is present in all *EPEC* and *EHEC* (McDaniel et al., 1995).

The A/E mechanism induce microvilli destruction, intimate adherence of bacteria to the intestinal epithelium, pedestal formation, and aggregation of polarized actin and other elements of the cytoskeleton at the site of bacterial attachment (McDaniel et al., 1995; Nataro & Karper., 1998).

In addition, an *EPEC* virulence factor outside the LEE has also been described (Trabulsi et al., 2002), which is located on a plasmid encoding a type pilus called bundle forming pilus (*bfp*). Besides, on another plasmid there is a plasmid encoded regulator gene (*per*) which regulates several chromosomal and plasmid genes necessary for the pathogenesis of A/E lesions (Levine et al., 1985).

Beside the virulence factors described above, there are other potential virulence factors such as *EAST1* (enteroaggregative *E. coli* heat-stable enterotoxin1),

originally identified in Enteroaggregative *E. coli* strains, but its significance in pathogenesis is uncertain (Nataro & Kaper., 1998). Based on these properties, two variants of *EPEC* typical and atypical *EPEC* have been reported (Hien et al., 2007) and their prevalence seems to be different between developing and developed countries. The typical *EPEC* is a cause of diarrhea in developing countries, while atypical *EPEC* seems to be associated with diarrhea in developed countries (Afset et al.,2003).

The most special feature of the epidemiology of disease due to *EPEC* infection is the remarkable age distribution. *EPEC* infection is primarily a disease of infants younger than 2 years of age. *EPEC* infection, as with other *DEC* pathotypes, takes place via the fecal-oral route with contaminated food and water. The infectious dose among infants is not known, but is presumed to be much lower than 10⁸ CFU/microorganism (Nataro.& Kaper.,1998).

The clinical features of *EPEC* infection are characterized by watery diarrhea, vomiting and low-grade of fever. As compared to the developed countries, *EPEC* infection plays a more important role in developing countries, where it has been found as one of the foremost causes of diarrhea(Cravioto et al.,1988).

EPEC may also cause diarrhea under other circumstances, such as nosocomial outbreaks.

2.1.5.3 Enteroaggregative *E. coli* (EAEC)

EAEC was first described in 1985, recognized by its distinctive adherence to HEp-2 cells in an aggregative, stacked brick-like pattern (Nataro et al., 1998;Pereira et al.,2008). This adherence pattern, distinguishable from the adherence patterns manifested by *EPEC* and *DAEC*, was first significantly associated with diarrhea among Chilean children in 1987 (Nataro.et.al.,1987). *EAEC* is defined as an *E. coli* pathotype that does not secrete heat-labile or heat-

Stable enterotoxins but adheres to HEp-2 cells and other epithelial cells in an aggregative adherence (A/A) pattern, the genes for which are encoded on plasmids (Nataro et al., 1998).

Many epidemiological studies have used the A/A pattern and the plasmid-encoded probe *CVD432* or simply the A/A probe to identify *EAEC* (Baudry et al., 1990). Moreover, a transcription activator known as *AggR*, the gene which regulates the A/A genes has been described as the major *EAEC* virulence regulator (Nataro., 2005).

Recently, some epidemiological studies have suggested that *CVD342*-positive strains, which are predicted to carry the *AggR* regulators, are the true *EAEC* pathogens termed typical *EAEC* (Harrington et al., 2006). However, A/A probe negative isolates share virulence factors with A/A probe positive isolates, a finding which indicates that additional factors are involved in the A/A phenotype in these *EAEC* strains (Bouzari et al., 2001). The pathogenesis of *EAEC* is complex as the strains are relatively heterogeneous. However three major features of the *EAEC* infections have been suggested as basic strategy of *EAEC*:

(i) abundant adherence to the intestinal mucosa (colonization), (ii) elaboration and secretion of enterotoxins and cytotoxins, and (iii) induction of mucosal inflammation (Harrington et al., 2006; Nataro et al., 1998). Furthermore, several studies have reported the presence of fecal lactoferrin and pro-inflammatory cytokines, notably interleukin (IL)-8 (Greenberg et al., 2002), as well as the presence of a putative virulence factors, such as yersinia-bactin lectin (Basu et al., 2004), in patients infected with *EAEC*. Since its discovery, an increasing number of studies have associated *EAEC* with diarrhea in a variety of settings. These include endemic diarrhea of infants in both industrialized and developing countries (Elias et al., 1999), persistent diarrhea among acquired

immunodeficiency syndrome patients and travelers diarrhea (Jenkins et al., 2006).

The implication of *EAEC* in diarrheal outbreaks confirmed the fact that at least some strains exhibiting the A/A phenotype were true human pathogens, yet not in all studies were *EAEC* associated with diarrheal illness (Nataro et al., 1995).

Pathogenesis studies of *EAEC* experienced a step-forward in 1994, when the first *EAEC* volunteer study was published (Nataro et al., 1995). Although not all *EAEC* strains have been associated to diarrheal disease, the most commonly reported symptoms associated with *EAEC* infection are watery diarrhea with or without blood and mucus, abdominal pain, nausea, vomiting, and low-grade fever (Adachi et al., 2002). Besides, *EAEC* can cause both an acute and a chronic (>14 days) diarrheal illness in different parts of the world. A growing number of studies from both developing and developed countries have supported the association of *EAEC* with persistent diarrhea in young children and adults (Araujo et al., 2007).

It has also been reported that *EAEC* may be a leading cause of outbreaks of acute diarrheal disease affecting newborns and children (Cohen et al., 2005). *EAEC* have been reported as the second most common bacterial pathogen isolated in US adult travelers to developing countries and deployed US military personnel (Adachi et al., 2002).

In another study on adult US travelers to Mexico that evaluated the serologic response to the *EAEC* anti-aggregative protein dispersin, 48% of the travelers developed increases in antibody levels over time; the majority of patients, though, remained asymptomatic (Huang et al., 2008). These findings may possibly suggest that *EAEC* infection shows a variety of clinical presentations and support the idea of a great heterogeneity of *EAEC* as a virulent pathogen (Nataro et al., 1995).

2.1.5.4 Enterohemorrhagic *E. coli* (EHEC)

EHEC is an etiological agent of diarrhea with life-threatening complications. *EHEC* belongs to a group of *E. coli* called *VTEC* (verotoxigenic *E. coli* or Verocytotoxin-producing *E. coli*) or *STEC* (Shiga toxin-producing *E. coli*), formerly *SLTEC* (Shiga-like toxin-producing *E. coli*). *EHEC* colonize the intestinal mucosa inducing a characteristic attaching and effacing (A/E) lesion, which is also present in *EPEC* and *EAEC*. The classic intestinal histopathology characteristic of *EHEC* infection includes hemorrhage and edema in the lamina propria, which results in bloody diarrhea, hemorrhagic colitis, necrosis and intestinal perforation. The major virulence factor, and a defining characteristic of *EHEC*, is the Shiga toxin (*Stx*), a potent cytotoxin that leads to cell death and will aggravate the symptoms in patients infected (Nataro.&Kaper.,1998). Most studies have reported *EHEC* strains as important pathogens causing diarrhea, with association to Hemorrhagic Colitis (HC) and Hemorrhagic Uremic Syndrome (HUS), which are due to the interaction of Shiga toxin (*Stx*) with endothelial cells (Brown et al.,1989).

The term enterohemorrhagic *E. coli* (*EHEC*) was originally created to denote strains that cause HC and HUS, express *Stx*, cause A/E lesions on epithelial cells, and possess a plasmid (Levine., 1987). Thus, *EHEC* denotes a subset of *STEC* and includes a clinical connotation that is not implied with *STEC*. Whereas not all *STEC* strains are believed to be pathogens, all *EHEC* strains by the above definition are considered pathogens (Levine., 1987).

The *Stx* family contains two major, immunologically non-cross-reactive, groups called *Stx1* and *Stx2*. A single *EHEC* strain may express *Stx1* only, *Stx2* only, or both toxins or even multiple forms of *Stx2*. *Stx1* from *EHEC* is identical to Shiga toxin from *Shigella dysenteriae* type I (Kaper et al., 2004).

EHEC is an emerging pathogen that has stimulated worldwide interest in several large food borne outbreaks. A wide variety of sources have been implicated in the *EHEC* transmission, including beef, unpasteurized milk, fruit juice, and contaminated drinking water (Kaper et al., 2004). *EHEC* belongs to different serotypes or serogroups, which are useful for diagnostic and epidemiological studies. The most notorious serogroups associated with *EHEC* is *O157:H7*, which has caused several large outbreaks of the disease, mainly in North America, Europe, and Japan (Ezawa et al., 2004).

2.1.5.5 Enteroinvasive *E. coli* (*EIEC*)

Like most enteropathogens, these bacteria may also be important pathogens in developing countries where sanitation and hygiene levels have deteriorated. *EIEC* strains are biochemically, genetically, and pathogenically closely related to *Shigella spp.*, but produce less severe diarrheal disease, compared to the dysentery caused by *Shigella dysenteriae* type 1 (Nataro & Karper., 1998).

The precise pathogenetic scheme of *EIEC* has yet to be elucidated. However, pathogenesis studies of *EIEC* suggest that its pathogenic features are virtually those of *Shigella spp.* (Sansonetti., 1992). *EIEC* invades the colonic epithelial cell, thereby inducing an inflammation and mucosal ulceration, leading to the release of blood and mucus in the stool (Hart et al., 1993), similar to bacillary dysentery. Although *EIEC* is the prototype of invasive bacteria, that are unable to penetrate enterocytes via their luminal aspect. Instead, it passes through the M cells, which are antigen-sampling cells that are a major constituent of the specialized epithelium overlying the lymphoid follicles in the small and large intestine (Sansonetti., 1992).

The ability of *EIEC* to penetrate, survive, and multiply within the colonic enterocytes is part of rearrangements of the cell cytoskeleton, leading to disturbance and engulfment of the bacterium within a vacuole, a process which

is encoded by a cluster of genes carried on a large plasmid . Afterwards, bacteria move through the cytoplasm and extend into adjacent epithelial cells without being exposed to the external surroundings. Infection results in a shigellosis-like syndrome in which patients exhibit abdominal pain, nausea, vomiting, fatigue, mucus, and bloody stools, but many cases show signs of watery diarrhea that is indistinguishable from that due to infection by other *E. coli* pathotypes (Nataro & Karper., 1998).

2.1.5.6 Diffusely adherent *E. coli* (DAEC)

DAEC is a category of DEC that produces the diffuse adherence on Hep-2 cells (Nataro et al., 1998) similar to those *EAEC* strains. However, little is known about the pathogenesis of *DAEC*, but cells with a surface with fimbriae that mediate diffuse adherence have been cloned and characterized(Kaper et al., 2004).

Few epidemiological and clinical studies have been carried out to adequately describe the epidemiology and clinical aspect of diarrhea caused by *DAEC*. It was suggested that *DAEC* may cause disease in immunologically nave or malnourished infants. Furthermore, *DAEC* has been associated to diarrhea in infants older than 1 year of age (Scaletsky et al.,2002).

2.1.6 Reservoirs

Reservoirs of most diarrheagenic *E. coli* strains are humans, animals and contaminated food or water (Cole.,2006).

2.1.7 Transmission

Transmission of most diarrheagenic *E. coli* strains occurs from consumption of food or water contaminated with human or animal feces (Kaper et al., 2004; Kassenborg et al., 2004). For some pathotypes, fewer than1000 colony forming

units can cause disease; therefore, person-to person transmission from infected symptomatic people or asymptomatic carriers can be an important mechanism for secondary spread (Belongia., 1993; Russo., 2006).

2.1.7.1 *EHEC*

Major modes of transmission include consumption of contaminated foods (especially undercooked ground beef), exposure to contaminate recreational or drinking water, or contact with farm and petting zoo animals (Kassenborg., 2004). Person-to-person transmission most often occurs in schools, long-term care institutions, families, and day-care centers (Belongia ., 1993; Bopp., 2003).

2.1.7.2 *EPEC*

The major mode of transmission is person-to-person contact. Although outbreaks among infants in industrialized countries have largely disappeared, atypical *EPEC* have caused large outbreaks of diarrheal disease among children and adults in industrialized countries (Nguyen ., 2006). Nosocomial outbreaks have been reported and have been associated with person-to-person transmission as well as transmission via fomites and contaminated formula or weaning foods (Nataro., 1998).

2.1.7.3 *ETEC*

Transmission is primarily via consumption of fecally contaminated food or water (Qadri., 2005).

2.1.7.4 *EIEC*

Several food-borne outbreaks have been identified (Gordillo., 1992). Person-to-person spread also can occur.

2.1.7.5 *EAEC*

Several nosocomial outbreaks have been identified but the mechanisms of transmission were not clear. Food-borne transmission has been suggested (Nataro., 1998).

2.1.7.6 *DAEC*

Modes of transmission have not been clarified. Risk factors for infection include: i. Consumption of contaminated food, especially undercooked ground beef . ii. Drinking or swimming in contaminated water (Kassenborg.,2004).

2.1.8 Laboratory Diagnosis

Diarrheagenic *E. coli* is diagnosed by serotyping from stool samples, enzyme immunoassay, or by other molecular methods (Lopez-Saucedo., 2003).

2.1.9 Diagnostic Tests

These pathotypes are identified by detection of their respective virulence-associated factors (toxins, adherence, or invasiveness). Techniques for testing include bioassays (ex, cell culture), immunologic assays (ex, immunoblotting or EIA), and DNA assays (ex, PCR, colony blots). Methods for identification of *ETEC*, *EPEC*, *EIEC*, *DAEC*, *EHEC* and *EAEC* generally are available only in reference laboratories or research settings. Diagnosis of diarrheagenic *E coli* is difficult for most clinical laboratories, because of the difficulty in differentiating illness-causing organisms from the usual *E. coli* strains present in stool (Voetsch., 2004).

2.1.9.1 Detection of *ETEC*

Detection of *ETEC* has long relied on detection of the enterotoxins LT and/or ST. ST was initially detected in a rabbit ligated ileal loop assay (Evans et al.,

1973), but the expense and lack of standardization caused this test to be replaced by the suckling-mouse assay (Gianella ., 1976), which became the standard test for the presence of STa for many years. The suckling mouse assay entails the measurement of intestinal fluid in infant mice after percutaneous injection of culture supernatants.

Several immunoassays have been developed for detection of ST, including a radioimmunoassay and an enzyme-linked immunosorbent assay (ELISA). Both of these tests correlate well with results of the suckling-mouse assay and require substantially less expertise (Cryan., 1990).

The traditional bioassay for detection of LT involves the use of cell culture, either the Y1 adrenal cell assay or the Chinese hamster ovary (CHO) cell assay. In the Y1 assay, *ETEC* culture supernatants are added to Y1 cells and the cells are examined for rounding. In the CHO cell assay, LT will cause elongation of the CHO cells (Donta et al., 1974). Immunologic assays are easier to implement in clinical laboratories and include the traditional Biken test as well as newer immunologic methods such as ELISA, latex agglutination and two commercially available tests, the reversed passive latex agglutination test and the staphylococcal coagglutination test. Both of the commercially available tests are reliable and easy to perform (Honda et al., 1981).

ETEC strains were among the first pathogenic microorganisms for which molecular diagnostic techniques were developed. As early as 1982, DNA probes were found to be useful in the detection of LT- and ST-encoding genes in stool and environmental samples. Since that time, several advances in *ETEC* detection have been made, but genetic techniques continue to attract the most attention and use. It should be stressed that there is no perfect test for *ETEC*: detection of colonization factors is impractical because of their great number and heterogeneity; detection of LT and ST defines an *ETEC* isolate, yet many such

isolates will express colonization factors specific for animals and thus lack human pathogenicity (Moseley et al., 1981).

Several PCR assays for *ETEC* are quite sensitive and specific when used directly on clinical samples or on isolated bacterial colonies. A useful adaptation of PCR is the multiplex PCR assay, in which several PCR primers are combined with the aim of detecting one of several different diarrheagenic *E. coli* pathotypes in a single reaction. After multiplex PCR, various reaction products can usually be differentiated by product size, but a second detection step (e.g., non-isotopic probe hybridization) is generally performed to identify the respective PCR products definitively (Lang et al., 1995).

2.1.9.2 Detection of *EPEC*

There are two approaches to the detection of *EPEC* in the laboratory: phenotypic and genotypic. The phenotypic approach requires the use of cell cultures and fluorescence microscopy, and the genotypic method requires the use of DNA hybridization or PCR.

2.1.9.2.1 Phenotypic tests

The A/E (attaching and effacing) phenotype can be identified by using cultured HEp-2 or HeLa cells and the mushroom toxin phalloidin conjugated to rhodamine (available from Sigma, Molecular Probes,) as described by (Knutton et al., 2002).

The A/E phenotype can also be identified by electron microscopic examination of intestinal biopsy specimens or of cultured epithelial cells incubated with *EPEC*. There are a number of phenotypic tests to determine the other crucial characteristic of *EPEC*, the lack of *Stx* expression. An excellent phenotypic marker for the presence of the *EAF* (*EPEC* adhesive factor) plasmid is localized adherence on HEp-2 or HeLa cells. An ELISA for the detection of *EAF*-positive

EPEC, based on an antiserum raised against an *EAF* plasmid containing strain and absorbed against a plasmid-cured strain (Albert et al., 1991). There are no readily available immunoassays to detect the A/E pattern.

2.1.9.2.2 Genotypic tests

DNA probes and PCR primers have been developed and evaluated for the three major characteristics of *EPEC*: A/E, *EAF* plasmid, and lack of Shiga toxin. Typical *EPEC* strains would possess the *eae* gene for A/E and the *EAF* probe or *bfp* sequences, indicating the presence of the *EAF* plasmid. Atypical *EPEC* strains would possess the *eae* gene only without the *EAF* plasmid.

(a) *eae* gene: Possession of *eae* sequences correlates with possession of the 35-kb LEE pathogenicity island encoding A/E (McDaniel et al., 1995). No exceptions to this generalization have been reported, and so there is no need to test for other sequences in the LEE such as *espB* and *sep* unless specific strain differences are sought for molecular epidemiology purposes (Ebel et al., 1996).

A variety of *eae* primers for detecting this gene by PCR have been tested. As reported by (Gannon et al., 1997), primer pairs for the conserved 59 region amplified fragments from all *eae*-positive strains whereas primers from the 39 end were specific for certain serotype.

(b) *EAF* plasmid: The 1-kb *EAF* fragment probe originally described by (Nataro et al., 1998) is from a region of the *EAF* plasmid with an unknown function. This probe may be used with non radioactive labeling techniques. A sensitive and specific oligonucleotide probe consisting of 21 bases of the *EAF* fragment was developed by (Jerse et al., 1991), where a PCR primer pair to amplify a 397-bp region of the *EAF* probe developed by (Franke et al., 1989). The cloning of the *bfpA* gene allowed the development of DNA probes for specific plasmid encoded virulence factors. (Giron et al., 1997) reported an 850-bp *bfpA*

fragment probe that was slightly more sensitive than the *EAF* probe; among *EPEC* strains exhibiting localized adherence to HEp-2 cells, the *bfpA* and *EAF* probes hybridized with 99 and 96% of the strains, respectively.

2.1.9.3 Detection of *EHEC*

There are three major categories of methods used to diagnose *EHEC* infections. These are (i) isolation of *E. coli* *O157* strains from stool samples, (ii) detection of *Stx* producing organisms or fecal *Stx*, and (iii) detection of elevated antibody levels to *O157* LPS or other *EHEC* antigens in serum. Detailed protocols for many of these methods have been reviewed by Smith and Scotland (Smith and Scotland., 1993). There are no common biochemical characteristics that are associated with the great majority of *EHEC* serotypes. However, there are some biochemical characteristics of *E. coli* *O157:H7* that have been exploited in the isolation and identification of this serotype. An important characteristic is that *O157:H7* strains do not ferment D-sorbitol rapidly, in contrast to about 75 to 94% of other *E. coli* strains. *E. coli* *O157* strains also do not ferment rhamnose on agar plates, whereas 60% of non-sorbitol-fermenting *E. coli* belonging to other serogroups ferment rhamnose (Smith and Scotland., 1993).

2.1.10 Virulence Factors

2.1.10.1 Adhesion/colonization

Pathogenic *E. coli* strains possess specific adherence factors that allow them to colonize sites that *E. coli* does not normally inhabit, such as the small intestine and the urethra. Most frequently these adhesins form distinct morphological structures called fimbriae (also called pili) or fibrillae, which can belong to one of several different classes. Fimbriae are rod-like structures of 5–10 nm diameters that are distinct from flagella. Fibrillae are 2–4 nm in diameter and are either long and wiry or curly and flexible (Cassels and Wolf., 1995). The Afa

adhesins that are produced by many diarrhoeagenic and uropathogenic *E. coli* are described as afimbrial adhesins, but in fact seem to have a fine fibrillar structure that is difficult to visualize (Keller et al., 2002). Adhesins of pathogenic *E. coli* can also include outer-membrane proteins, such as intimin of *UPEC* and *EHEC*, or other non-fimbrial proteins. Some surface structures trigger signal transduction pathways or cytoskeletal rearrangements that can lead to disease. For example, the members of the Dr family of adhesins that are expressed by *DAEC* and *UPEC* bind to the Decay Accelerating Factor (*DAF* also known as CD55), which results in activation of phosphatidylinositol 3-kinase (PI-3-kinase) and cell-surface expression of the major histocompatibility complex (MHC) class I-related molecule MICA (Tieng et al., 2002). The IcsA protein of *EIEC* nucleates actin filaments at one pole of the bacterium, which allows it to move within the cytoplasm and into adjacent epithelial cells on a „tail“ of polymerized actin (Goldberg et al., 1995). Even surface structures that are present on commensal *E. coli* strains can induce signaling cascades if the organism encounters the appropriate receptor. The LPS of *E. coli* and other gram-negative bacteria binds to Toll-like receptor 4 (TLR4), triggering a potent cytokine cascade that can lead to septic shock and death (Tapping et al., 2000). Flagellin, the main component of flagella, can bind to TLR5, thereby activating interleukin (IL)-8 expressions and an inflammatory response (Hayashi et al., 2001).

2.1.11 Molecular methods:

2.1.11.1 Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a method used for the in vitro enzymatic synthesis of specific DNA sequences by Taq and other thermo resistant DNA polymerases. PCR uses oligonucleotide primers that are usually 20–30 nucleotides in length and whose sequence is homologous to the ends of the

genomic DNA region to be amplified. The method is performed in repeated cycles, so that the products of one cycle serve as the DNA template for the next cycle, doubling the number of target DNA copies in each cycle (Hill., 1996) .

The rapid increase in the number of copies of the target sequence that can be achieved with PCR-based methods makes them ideal candidates for the development of faster microbiological detection systems. Many PCR tests have been validated and commercialized to make PCR a standard tool used by food microbiology laboratories to detect pathogens in foods (Jasson et al., 2010 ; AOAC International., 2011) .

Conventional PCR relies on amplification of the target gene(s) in a thermo cycler, separation of PCR products by gel electrophoresis, followed by visualization and analysis of the resulting electrophoretic patterns, a process that can take a number of hours. The specificity can be subsequently confirmed by sequencing the amplified fragment. PCR can be superior to culture for detecting the main pathogens in food samples (Abubakar et al., 2007).

PCR itself requires only about 30–90 min, but all methods for detecting foodborne pathogens using PCR require pre-enrichment times that may vary from 6–8 to 48 h. Aside from that, if a positive result for PCR is reached, this must be confirmed using cultures (Rodriguez-Lazaro and Hernandez., 2006 ; Navas et al., 2006 ; Jasson et al., 2010) .

2.1.12 Control and prevention

Diarrhea caused by various diarrheagenic *E. coli* normally are self-limiting and do not require antibiotic treatment. For infants and children who are at high risk for dehydration, fluid replacement would be given in the case of fluid loss caused by *ETEC*, *EPEC* and *EAEC* (King et al., 2003).

Although antibiotics have been very useful in treating *E. coli* infections in the past, the increase in antibiotic resistance in *E. coli* make treatment of *E. coli*

infections complicated. For example, the emergence of fluoroquinolones resistant and extended-spectrum beta-lactamase producing strains have caused great concern due to limited therapeutic options for infections with those *E. coli* strains (Fadda et al., 2005).

Frequent hand washing, especially before eating or preparing food, and good hygiene are important in preventing transmission from animals and their environment. Hand washing facilities should be available in petting zoos and other areas where the public may contact livestock, and eating and drinking should be discouraged at these sites. To protect children and other household members, people who work with animals should keep their work clothing, including shoes, away from the main living areas and launder these items separately (Werber et al., 2008).

Chapter Three

Materials and Methods

3. Materials and Methods

3.1 Materials

3.1.1 Culture Media

Table (3-1) selective and differential media used in the isolation and identification of diarrheagenic *Escherichia coli*

Media	Manufacturer/origin
MacConky Agar	DCM/Netherlands
Peptone Water	DCM/Netherlands
Eosin-methylene blue Agar	DCM/Netherlands
Simmon's citrate Agar	Oxoid/England
Kligler Iron Agar	Oxoid/England
Nutrient Broth	DCM/Netherlands
Methyl red vogasproskeur broth	Oxoid/England
MacConky broth	DCM/Netherlands
Brain Heart infusion Broth	Himedia/India
Brain Heart infusion Agar	Oxoid/England
Blood Agar Base	Oxoid/England
Triple Sugar iron Agar	Himedia/india
Urea base Agar	Oxoid/England
Nutrient Agar	Himedia/india

3.1.2 Chemical and biochemical material

The chemicals and biological materials with their remarks that were used in this study are listed in table(3-2) :

Table (3-2) the chemical and biological materials with their remarks

NO.	Chemical	Company	Country
1	Agarose gel	Promega	USA
2	Absolute Ethanol	Sigma	U.S.A
3	Glycerol(C ₃ H ₈ O ₃)	BDH	England
4	Kovacs reagent	BDH	UK
5	Ethidium bromide	Promega	U.S.A
6	Ladder	Bioneer	Korea
7	Cider Oil		Switzerland
8	Ethylene-diamine-tetra-acetic acid EDTA	BDH	UK
9	Gram stain	Himedia	India
10	Hydrogen peroxide(H ₂ O ₂)	Fluka	Germany
11	Methyl red	BDH	UK
12	Nuclease free water	Promega	USA
13	Potassiumhydroxide (KOH)	Oxoid	UK
14	Sodium hydroxide (NaOH)	BDH	UK
15	Trise-base	Fluka	Germany
16	Urea solution	Mast Diagnostic	UK
17	α -naphthol (C ₁₀ H ₈ O)	BDH	UK

3.1.3 Instrument and Equipments

The instrument and the equipments with their remarks that were used in this study are listed in table (3-3):

Table (3-3): The instrument and the equipment that used in this study with their remarks

NO.	Equipment	Company	Country
1	Autoclave	Gemmy	Taiwan
2	Electric oven	Sartorius	Germany
3	Incubator	Memert	Germany
4	Electrophoresis unit	Bioneer	Korea
5	High speed cold centrifuge	Hettich	Germany
6	Water Bath	Memert	Germany
7	Power Electric	ATTA	Japan
8	Distillator(water distiller)	Biorad	U.S.A
9	Vortex Mixer	FANEM	USA
10	Hotplate Magnetic Stirrer		Japan
11	Microscope	Olympus	Japan
12	Laminar-Flow	Gallnkamp	England
13	Micropipettes	Haiou	China
14	Refrigerator	Concord	Lebanon
16	Sensitive Balance	Korn pfp&Sohn mbh	Germany
17	PCR Tubes		England
18	Calibrated loop 0.01	Himedia	India
19	Thermocycler	Himedia	India
20	Uv light spectrophotometer		

3.1.4 Antibiotics

The antibiotics with their remarks that were used in this study are listed in table (3-4).

Table (3-4): The antibiotics that used in this study and the dosage to each of them with their remarks

NO.	Antibiotics	Company	Country	Concentration gμ	symbol
1	Ampicillin	Bioanalyse	Turkey	10	AMP
2	Cefotaxim	Bioanalyse	Turkey	30	CTX
3	Ceftriaxone	Bioanalyse	Turkey	30	CRO
4	Cephalothin	Bioanalyse	Turkey	30	CEP
5	Chloroamphicol	Bioanalyse	Turkey	30	C
6	Ciprofloxacin	Bioanalyse	Turkey	5	CIP
7	Gentamicin	Bioanalyse	Turkey	10	CN
8	Imipenem	Bioanalyse	Turkey	10	IMP
9	Nalidixic acid	Bioanalyse	Turkey	30	NA
10	Netilmicin	Bioanalyse	Turkey	30	NET
11	Tetracyclin	Bioanalyse	Turkey	30	TE

3.1.5 Types of kits

Table (3-5) : The type of kits with their company and origin

Kit	Company	Origin
API 20 E test	BioMerieux	France
DNA extraction kit	Geneaid	England
Green Master mix	Bioneer	Korea

3.1.5.1 Components of API 20E test

1-Saline suspension(as per manufacturer's directions).

2-IncubationBox.

3-Mineraloil dropper.

4-Chemicalreagents:

a-ferric Chloride for tryptophan deaminase (TDA).

b-Kovacs reagent for indole test.

c-Voges-Proskauer reagent for VP test.

this reagent consisted of two solution(α -naphthol and 40% KOH).

3.1.5.2 Components of DNA extraction Kit Geneaid (England)

The kit includes the following solution :

Name	Volume
GB buffer	40ml
GT buffer	30ml
Wash buffer (Add Ethanol)	25ml(100ml)
W1 buffer	45ml
GD column	100 pcs
Elution buffer	30ml
2ml collection tube	200 pcs

the kit contents were stored at 20-25°C.

3.1.5.3 Molecular Weight Marker

Table (3-6) DNA marker with the description and source

DNA Marker	Description	Source
100 bp DNA ladder	100 bp DNA ladder is specially designed for determining the size of double strand DNA from 100 to 2,000 base pairs. The DNA Ladder consists of 13 double strand DNA fragment ranging in sizes from 100 to 1,000 bp in 100 bp increments and additional fragments of 1,200,1,600,2,000 bp.	Bioneer (korea)

3.1.6 primers for detection of virulence factors of enteropathogenic *E. coli*

Table (3-7) The group of primer sequence, target gene, product size and reference:

Reference strain	DNA Sequences(5'-3')	Target gene	Product size(bp)	Reference
EPEC	TGATAAGCTGCAGTCGAATCC CTGAACCAGATCGTAACGGC	<i>eaeA</i>	229	Svenungsson et al.,2000
	CACCGTTACCGCAGGTGTGA GTTGCCGCTTCAGCAGGAGT	<i>bfpA</i>	450	

EIEC	CTGGTAGGTATGGTGAGG CCAGGCCAACAATTATTTCC	<i>ial</i>	320	Aranda,fagundes- neto,&scaletsky.2004
EAEC	CTGGCGAAAGACTGTATCAT CAATGTATAGAAATCCGCTGTT	<i>CVD432</i>	630	Paton and Paton (1998)
STEC	CGGACATCCATGTGATATGG TTGCCTATGTACAGCTAATCC	<i>rfbO157</i>	259	Moyo, 2007; Khairun, 2007; Brandal, 2007; Nguyen, 2005; Rajendran, 2010; Franzolin, 2005
ETEC	TCTTTCCCCTCTTTTAGTCAGTC CCGCACAGGCAGGATTAC	<i>STLa</i>	170	

3.2 Methods

3.2.1 preparation of culture media

All used media were prepared according to the directions of the manufacturing companies, and were dissolved in D.W ,then sterilized by autoclave 121 C° for 15 min. under 15 psi pressure. after sterilizing leave cooling to (50-55)C°, blood agar base was supplemented with 5%-7% of human blood.

3.2.1.1 MacConkey Agar Medium

MacConkey agar is a selective and differential culture medium used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting Gram-negative bacteria. As *E. coli* can ferment lactose it gives pink color colony on MacConkey agar.

3.2.1.2 MacConkey Broth

MacConkey broth is selective medium used to for the presumptive identification of coliforms and for cultivating Gram-negative ,lactose fermenting bacilli from a variety of samples like water ,milk, and food.

3.2.1.3 MacConky sorbitol Agar

MacConkey sorbitol agar is used for isolation and identification of entero pathogenic *Escherichia coli* strains associated with infant diarrhea.

3.2.1.4 preserve Media for microbe isolate

Attended the preservation media to save the bacterial isolates by added 15% of glycerol to 85 ml of brain heart infusion broth, and distributed in small tubes with a tight lid and sterilized by autoclave, leave to cooled in room temperature then inoculated with pure bacterial colonies that growth on MacConky agar by using loop. tubes saved at -20C°,after incubated for 24 hours at 37C°.this method was used for long preservation period(8-12 months) for short period preservation(4 months) the tubes that contain brain heart agar slant inoculated with bacterial colonies, then incubated at 37C° for 24 hours then stored at 4C° for while it use. The microbe isolates have been maintained every month during the study by culturing on new culture media (Ausubel et al.,1987).

3.2.2 Samples collection

A total of 300samples were collected from various shops, local bazaar around Thi-Qar (Shatrah-Rifai-Suq- Alsheokh- Nasr) in the period between January 2016 to July 2016.the samples transported to the laboratory and then cultured on MacConkey broth.

Table (3-8) Area of sampling, number and types of samples

Place	Total number of samples	Types of Samples (Number of Samples
Shatrah	103	Meat(53),cheese(25), milk(25).
Rifai	71	Meat(30),cheese(21), milk(20).
Suqalsheokh	68	Meat(25),cheese(18),milk(25).
Nasr	58	Meat(20),cheese(18),milk(20).
Total	300	

3.2.3 Preparation of Reagents and solution

3.2.3.1 Catalase

Catalase reagent has prepared by mixing 1ml of concentrated hydrogen peroxide(30%) with 9ml of distilled water to get concentration (3%)hydrogen peroxide ,then preserved in fridge in a black bottle then used for detection the ability of the microbe to produce catalase enzyme(*Collee et al.,1996*).

3.2.3.2 Oxidase

Oxidase reagent has prepared freshly by dissolved 1gm of Tetramethyl p-phenyl diamine-dihydrochloride material in 90ml of distilled water and then volume completed to 100 ml in black bottle. This reagent used to detection production oxidase enzyme by the microbe(*koneman et al.,1992*).

3.2.3.3 Methyl red

It was prepared by dissolving 0.1gm of methyl red powder in 300ml of 96% ethanol and then the volume completed to 500ml by added 200ml of distill water, this reagent used as indicator for methyl red test.

3.2.3.4 Voges-Proskauer

Prepared of this reagent consist of two parts:

part one from dissolving 5gm of α -naphthol in 100ml of absolute ethanol 96%.

part two from dissolving 40gm of 40%KOH in 100ml d. w and the volume completed to 100ml.

3.2.3.5 MacFarland standard (0.5)

The solution prepared as the standard guidelines that described in(Baron&finegold.,1994).

3.2.3.6 Normal saline solution

Normal saline prepared as stated in (*Forbes et al,2002*) in order to use in dilution execution by dissolving 0.85gm of NaCl in 90ml D.W and then the volume completed to 100ml with D.W, the normal saline solution autoclaved at 121C^o for 15 minutes and stored at 4C^o until use.

3.2.4 Tests that's used to identify bacterial isolates

3.2.4.1 Indole test

This test used to detection the presence of indole ,which is one of the metabolism outputs of tryptophan amino acid as a result of the bacterial having enzyme tryptophanase ,the peptone water inoculated with fresh or a young culture and incubated at 37°C for 24-48 hours, 5 drops of Kovacs reagent were added to each tube and positive result led to red color while negative result yellow color observed immediately (Koneman et al.,1992).

3.2.4.2 Methyl red test

This test used to detection the bacterial capacity to produce big amounts of lactic acids or formic as a result of glucose metabolism, fresh culture inoculated into

3ml of MR-VP broth and then incubated overnight at 37°C. five drops of methyl red solution were added, mixed and the culture was examined. The result read immediately

A positive test was bright red while yellow indicated negative reaction (Koenman et al.,1992).

3.2.4.3 Voges-Proskauer test

This test used to detection the bacterial capacity to produce acetyl methyl carbinol(acetone),fresh culture inoculated into MR-VP broth and then incubated overnight at 37°C ,one ml of 40%KOH solution and three ml of 5% solution of α -naphthol were added to each tube. positive result led to appearance red color in 5 minutes(Koenman et al .,1992).

3.2.4.4 Citrate Utilization test

Simmons citrate slant were inoculated with fresh culture of bacteria and incubated at 37°C for 24-48 hours, the result is considered positive when the color of the media transform from the green to the blue as a result of the consumption of citrate.(Koneman et al.,1992).

3.2.4.5 Urease test

The surface of urea agar slant was streaked with fresh or ayoung culture of bacteria and incubated at 37°C .the result was read after 6h,24h,48h and 72h.changing color of urea medium to purple-pink is led to positive result of test. (Koneman et al.,1992).

3.2.4.6 Kliglers iron agar test

With a straight inoculation needle an inoculum was stabbed into butt of tube and streaked over surface of the agar slant and then incubated at 37°C for 24h. so the results were read as follows :

Slant/butt	color
Alkaline/Acid	Red/Yellow
Acid/Acid	Yellow/Yellow
Alkaline/Alkaline	Red/Red
H ₂ S production	Black precipitate

3.2.4.7 Carbohydrate Fermentation

Carbohydrate fermentation is done to determine the fermentation capability of the organism. It is done under anaerobic condition in a fermentation broth tube containing Durham tube for detection of gas production. Specific carbohydrate serves as substrate and phenol red as pH indicator. The critical nature of fermentation reaction and the indicator make it an imperative that all cultures have to be observed within 48 hours. Upon incubation carbohydrates that have been fermented with the production of acid and gas will cause the phenol red to turn yellow.

3.2.5 Identification of the organism

All isolates were subjected to gram staining for initial identification of organism according to their gram reaction, colony morphology and finally by biochemical test. gram negative bacteria were identified by motility test, indol production,

citrate utilization test, methyl red test, voges-proskauer test, urease production and reaction in KIA media.(Holt et al.,1994).

3.2.5.1 gram staining

Smear from the culture will establish the Gram reaction and cellular morphology (coccus or rod). Typical *E. coli* appears Gram negative rod.

3.2.5.2 Colony morphology

MacConkey agar :produce smooth pink colony
Blood agar: Rounded colonies of 1.4 mm diameter with or without haemolysis.

3.2.5.3 Isolation of *E. coli* from Samples

Samples were processed to isolate the *E. coli* as per the standard Bacteriological Analytical Manual (BAM), U.S. Food and Drug Administration (USFDA) method (Kumar et al., 2008). Twenty five gram of selected raw milk ,cheese and meat were measured and finely chopped aseptically. This was added to 225 ml of enrichment broth and homogenized and incubated at 37oC for 16-18 h. Then 1 ml of enriched broth was subjected to 10-fold dilution series from 10-1 to 10-4 in 9 ml of PBS. From each 10-1 to 10-3 dilution tubes, 0.1 ml of suspension was spread plated in duplicate onto MacConkey agar plates. The plates were then incubated at 37oC for 16-18 h, according to method of. (Bollton et al., 2001).

3.2.5.4 Cultural Properties on MacConkey Agar Plate

Morphological characteristics of colonies (size, shape, elevation, form, pigmentation and opacity) developed after incubation on MacConkey agar plate were carefully studied and recorded. Five to ten suspected *E. coli* colonies were picked up from the plate and sub-cultured onto fresh MacConkey plate to get pure culture. *Escherichia Coli* produce smooth pink colony on MacConkey agar.

3.2.5.5 Cultural Properties on Blood Agar Plate

Rounded colonies of 1.4 mm diameter with or without haemolysis.

3.2.5.6 Screening of Suspected Isolates on EMB Plate

Suspected *E. coli* isolates from the fresh MacConkey plate were streaked onto eosin methylene blue (EMB) agar plate for presumptive confirmation as *E. coli*. EMB agar medium contains lactose and the dyes eosin and methylene blue that permit differentiation between enteric lactose fermenters and non-fermenter as well as identification of the colon bacillus *E. coli*. The *E. coli* colonies are black colonies with a metallic green sheen caused by the large quantities of acid that is produced and that precipitates out the dyes onto the growth's surface (Cappuccino and Sherman., 2011).

3.2.6 Confirmatory test System

3.2.6.1 API20 Multi Test System

All isolates identified through the biochemical test were they confirmed by API 20E test. API 20E test used according to manufacturers protocol (Bio Merieux), to increase the reliability of the diagnostic process of the isolates to the level type which consist 20 biochemical test for all isolates. Wells of the biochemical test inoculated with overnight 0.5 McFarland bacterial suspension and they inoculated at temperature 37C for one day. The results were read after addition of all reagent, as 7-digit number that identified by API 20E analytical index.

3.2.7 Antimicrobial Susceptibility Test

using the standard method of Bauer & Kerby to testing the bacterial susceptibility to the antimicrobial (Vandepitte *et al.*, 1991).

A-Preparation of Bacterial Inoculums'

Three-Five pure isolated colonies of fresh culture has been suspended in 5ml of tryptic soy broth and incubated at 37 °C for 24h. the turbidity that produce from growth culture has been adjusted with sterile broth to give optical density comparable to the 0.5 McFarland standards(equivalent to 1.5×10^8 cell/ml).

B-Streaking

1.Cotton swab (sterile) have been dipped into the adjusted suspension and then rotated several times firmly on the inside wall of tube above the broth level to remove excess inoculums from the cotton swab.

2.All surface of a Mueller-Hinton agar plate has been streaked with the dipping cotton swab. The streaking surface of a Mueller-Hinton agar plate has been repeated two more times and the plate was rotated about 60° each time to ensure an even distribution of inoculums .AS a other step the rim of the agar was swabbed.

3.The Petri dishes were allowed to dry for time between 15-20 min at room temperature before the application of the antibiotic discs.

C-Application of Antibiotic Discs

1.The antibiotic discs have been spreading or dispensed onto the surface of the inoculated agar plate. Every disc has been pressed gently to ensure that will be complete contact with agar surface through helping sterilized forceps.

2.The plates of agar have been inverted and placed in an incubator at 37°C for 18-24h.

3.The diameter of growth inhibition zones on plates have been measured via using transparent ruler.

4.The results that produce were compared with the tables of the CLSI(2014).

3.2.8 Polymerase Chain Reaction Assay

3.2.8.1 Detection the virulence factors genes by PCR assay

3.2.8.1.1 DNA Extraction

1. Fresh culture (1ml) was Added to 1.5ml micro centrifuge tube.
- 2- Culture was Centrifuged for 1 minute at 14,000-16,000 x g. then discard the supernatant.
- 3.Added 180µl of GT buffer by micropipette, then re-suspend the cell pellet by vortex.
- 4.Added 20µl of proteinase K(make sure distilled water added).
- 5.Incubate at 60°C for 10 minutes ,during incubation invert the tube every 3minutes.
- 6.Added 200µl of GB buffer to the sample and mix by vortex for 10 seconds.
- 7.Incubate at 70°C for 10 minutes to ensure the sample lysate is clear, during incubation invert the tube every 3 minutes .At this time, pre-heat the required Elution Buffer (200µl per sample) to 70°C (for step DNA Elution).
- 8-Added 5µl of RNase A(50 mg/ml) to the clear lysate then shake vigorously and after that incubate at room temperature for 5 minutes.
- 9.Added 200µl of absolute ethanol to the sample lysate and mix immediately by shaking vigorously.
- 10.Place GD column in a 2 ml collection tube .Transfer mixture (including any insoluble precipitate) to GD column then centrifuge at 14,000-16,000 x g for 2 minutes. Discard the 2ml collection tube containing the flow-through then place the GD column in a new 2ml collection tube.
- 11.Added 400µl of W1Buffer to the GD column and then centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through .place the GD column back in the 2ml collection tube.

12. Added 600 μ l Wash buffer (make sure ethanol was added) to the GD column, centrifuge at 14,000-16,000 $\times$ g for 30 second then discard the flow-through. place the GD column back in 2ml collection tube.
13. centrifuge at 14,000-16,000 $\times$ g for 3 minutes to dry the column matrix.
14. transfer the dried GD column to a clean 1.5 micro centrifuge tube. then added pre-heated Elution Buffer into center of the column matrix. let stand for at least 3 minutes to allow Elution buffer to be completely absorbed.
15. Centrifuge at 14,000-16,000 $\times$ g for 30 seconds to elute the purified DNA.
16. Stored the DNA at 2-8°C.

3.2.8.2 Loading and Running DNA in Agarose Gel

DNA samples was transferred 7 μ l from it to Eppendorf tube, 3 μ l of loading buffer were added to tube and mixture was loaded into the wells in agarose gel. Electric current was allowed at 100 volt for 60 minutes. UV transilluminater were used for the observation of the DNA bands.

3.2.8.3 Polymerase chain reaction

3.2.8.3.1 Amplification of *EPEC* gene (*eaeA* amplicon size 229)

PCR amplification of *eaeA* gene of *Escherichia Coli*. PCR by using amplification was performed by using Accu power PCR premix (Bioneer Korea).

eaeA gene studied according to (Rappelli et al., 2001). Genomic DNA was amplified by using the primers.

The PCR condition amplification was performed using the thermal cycler program as following. PCR condition and reaction are displayed in table (3.9) and (3.10).

Table (3-9) PCR condition for *eaeA* gene

Stage	Step	Temperature	Time	No. of Cycles
First	Denaturation 1	95C°	3 minutes	1
Second	Denaturation 2	94C°	30 second	35
	Annealing	55C°	30 second	
	Extension 1	72C°	30 second	
Third	Final extension	72C°	5 minute	1

Table (3-10) Reaction mixture of (20µl) *eaeA* gene

DNA template	5µl
Master mix	5µl
Primer forward	1µl
Primer reverse	1µl
DW	8 µl

3.2.8.3.2 Amplification of *EAEC* gene (*CVD432*)

PCR amplified of *CVD432* gene of *Escherichia coli*. PCR by using amplification was performed by using Accu power PCR premix (Bioneer Korea). 5 µl template DNA, 2µl primers (1 µl forward and 1 µl reverse Primer) each 1µl contain 10 p mole of primer and final volume was reach to 20 µl by adding 8 µl.

The PCR condition amplification was performed using the thermal cycler program as following: primary denaturation 94 for 3 min then for 36 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 1 min.

The *CVD432* gene was studied according to (Aranda,Fagundes-Neto,&Scaletsky.2004). Genomic DNA was amplified by using the primers. PCR condition and reaction are displayed in table (3-11) and (3-12).

Table (3-11) The PCR condition for *CVD432* gene

Stage	Step	Option	Temperature	Time	No. of cycle
First	1	Denaturation 1	94°C	3 m	1
Second	1	Denaturation 2	94°C	1 m	35
	2	Annealing	57°C	1 m	
	3	Extension 1	72°C	1 m	
Third	1	Final extension	72°C	10 minutes	1

Table (3-12) The reaction mixture (20 μ l) for *CVD432* gene

DNA templates	5 μ l
Master mix	5 μ l
F primer	1 μ l
R primer	1 μ l
DW	8 μ l

3.2.8.3.3 PCR amplification of *STEC(rfbO157)* gene)

PCR amplification of *O157* gene of *Escherichia Coli* by using amplification was performed by using Accu power premix (Bioneer Korea). 5 μ l template DNA, 2 μ l primers (1 μ l forward and 1 μ l reverse Primer) each 1 μ primer contain 10 p mole and final volume was reach to 20 μ l by adding 8 μ of deionized water.

O157 gene was studied according to (Paton and Paton 1998). Genomic DNA was amplified by using the primers. PCR reaction and condition are displayed in tables (3-13) and (3-14).

Table (3-13) The PCR Condition for rfbO157 gene

Stage	Step	Temperature	Time	No. of Cycle
First	Denaturation 1	94°C	2 minutes	1
Second	Denaturation 2	95°C	30 sec.	35
	Annealing	61°C	1 min.	
	Extension 1	72°C	1 min	
Third	Final extension	72°C	10 min	1

Table (3-14) Reaction mixture (20µl) for O157 gene

DNA Template	5µl
Master mix	5µl
Primer forward	1µl
Primer reverse	1µl
DW	8µl

3.2.8.3.4 PCR amplification of *EPEC* (*bfpA* gene)

PCR amplification of *bfpA* gene of *Escherichia coli* by using amplification was performed by using Accu power PCR premix (Bioneer Korea). 5 µl template DNA, 2 µl primers (1 µl forward and 1µl reverse Primer) each µl primer contain 10 p mole and final volume was reach to 20 µl by adding 8 µ of deionized water.

bfpA gene was studied according to (Amisano et al., 2001). Genomic DNA was amplified by using the primers. PCR reaction and condition are displayed in tables (3-15) and (3-16).

Table (3-15) :PCR condition for *bfpA* gene

Stage	Step	Temperature	Time	No .of cycle
First	Denaturation 1	95°C	5 minute	1
Second	Denaturation 2	94°C	30 sec.	30
	Annealing	57°C	1 minute	
	Extension 1	72°C	1 minute	
Third	Final extension	72°C	7 minute	1

Table (3-16) Reaction mixture of (20μ) for *bfpA* gene

DNA Template	5μl
Mastermix	5μl
Primer forward	1μl
Primer reverse	1μl
DW	8μl

3.2.8.3.5 PCR amplification of *EIEC* (*ial* gene)

PCR amplification of *ial* gene of *Escherichia Coli* by using amplification was performed by using Accu power PCR premix (Bioneer Korea). 5 μl template

DNA, 2 μ l primers (1 μ l forward and 1 μ l reverse Primer) each μ l primer contain 10 p mole and final volume was reach to 20 μ l by adding 8 μ l of deionized water.

ial gene was studied according to (Svenungsson *et al.*, 2000) .Genomic DNA was amplified by using the primers. PCR reaction and condition are displayed in tables (3-17) and (3-18).

Table (3-17) PCR condition for *ial* gene

Stage	Step	Temperature	Time	No. of cycle
First	Denaturation 1	94°C	3 minute	1
Second	Denaturation 2	94°C	45 sec	36
	Annealing	61°C	1 minute	
	Extension 1	72°C	1 minute	
Third	Final extension	72°C	5 minute	1

Table (3-18) Reaction mixture of (20 μ) for *ial* gene

DNA Template	5 μ l
Master mix	5 μ l
Primer forward	1 μ l
Primer reverse	1 μ l
DW	8 μ l

3.2.8.3.6 PCR amplification of *ETEC* (*STLa* gene)

PCR amplification of *STLa* gene of *Escherichia coli* by using amplification was performed by using Accu power PCR premix (Bioneer Korea). 5 µl templet DNA, 2 µl primers (1 µl forward and 1µl reverse Primer) each µl primer contain 10 p mole and final volume was reach to 20 µl by adding 8 µ of deionized water.

STLa gene was studied according to (Moyo., 2007; Khairun., 2007; Brandal., 2007; Nguyen., 2005; Rajendran., 2010; Franzolin., 2005) .Genomic DNA was amplified by using the primers. PCR reaction and condition are displayed in tables (3-19) and (3-20).

Table (3-19) :PCR condition for *STLa* gene

Stage	Step	Temperature	Time	No. of cycle
First	Denaturation 1	95°C	5 minute	1
Second	Denaturation 2	95°C	1 minute	30
	Annealing	54°C	1 minute	
	Extension 1	72°C	1 minute	
Third	Final extension	72°C	8 minute	1

Table (3-20): reaction mixture for *STLa* gene

DNA Template	5µl
Master mix	5µl
Primer forward	1µl
Primer reverse	1µl
DW	8µl

Chapter Four

Results

4.1 Isolation and identification of Diarrheagenic *Escherichia Coli*

4.1.1 Distribution food samples according to their type and number

The total number of food samples was 300 sample ,they were collected through the period of more than 7 months from various shops ,local pazzars and street shops, they were randomly selected and examined for detection of gram negative bacteria .These food samples were 100(33.33%) samples collected from raw meat , 100(33.33%) samples collected from raw milk and 100(33.33%) samples collected from cheese ; As shown in the table (4-1), that explains distribution the type of the food samples ,The number of samples and the percentage from the whole samples.

Table (4-1) The distribution of the samples according to their type :

type of samples	Number of samples	(%)
Raw meat	100	33.33
Raw milk	100	33.33
Local cheese	100	33.33
Total	300	100%

4.1.2 Distribution of the food samples according to the geographic location

The food samples distributed to groups by location-derived thereof include Shatrah 103(34.33%) samples which involved 53 (16.20%) raw meat , raw milk 25(7.65%) and cheese 25(7.65%) ; Rifai 71 (21.7%) samples including 30(9.17%) raw meat samples , raw milk samples are 20(6.11%) and cheese samples are 21 (6.43%) ; Suq-alsheokh samples are 68 (20.7%) including

25(7.65%) raw meat samples /raw milk samples are 25 (7.65%) /cheese samples are 18 (5.50%); Nasr 58(17.7%) samples including 20 (6.11%) raw meat samples , raw milk samples are 20 (6.11%) and cheese samples are 18 (5.50%) The samples distribution can shown in table (4.2).

Table (4.2) The distribution of the food samples according to the geographic location, type of samples , number of samples, and their percentage

location	Type of sample	No. of sample	%
Shatrah	Raw meat	53	16.20
	Raw milk	25	7.65
	Cheese	25	7.65
Rifaii	Raw meat	30	9.17
	Raw milk	20	6.11
	Cheese	21	6.43
Suq-alsheok	Raw meat	25	7.65
	Raw milk	25	7.65
	Cheese	18	5.50
Nasr	Raw meat	20	6.11
	Raw milk	20	6.11
	Cheese	18	5.50
Total		300	100

4.1.3 Identification of *E. coli* according to the growth on a selective media and the biochemical test

The gram negative bacteria isolated were identified according to their morphological characteristics and strength as well as their ability to ferment lactose sugar on MacConkey agar . *E. coli* colonies showed pink color as a result

lactose fermented , dry, medium size, convex and regular .Negative for oxidase test and grow as a colonies with green metallic sheene on eosin methylene blue media. for microscopic examination, all isolates have appeared negative for the colored of gram stain and bacillary .it has been relyed on biochemical ,microscopic and agricultural tests to identification the isolates and confirm the initial diagnosis. The results show that 85 (25.99%) isolated positive for gram negative bacteria and from these value 55 (64.7%) isolates positive to *Escherichia coli* growth ;while 242 (74%) samples were negative for the microbial growth or no growth .

4.1.4 Identification of *E. coli* by using api-20E

To confirm the diagnosis of *E. coli* isolates, we used api-20E kits which characterized by easy and fast diagnosis to confirm the results of the biochemical tests .figure (4-1) shows the api-20E test. The isolates distribution after the identification by about 25(25.0%), as positive cases for growth of *E. coli* from 100 meat samples. out of 100 milk samples were collected ;17(17.0%)were positive for *E. coli*, and 100 samples of cheese were collected;13(13.0%) from this value as positive cases of *E. coli* growth as shown in table (4-2).

Table (4-3) Number of samples , number of *E. coli* isolates , and their percentage according to the biochemical and Api-20E tests.

Source of samples	No. of cases	No. of positive cases	%
Meat	100	25	25.0
Milk	100	17	17.0
Cheese	100	13	13.0
Total	300	55	55

4.3 Results of Screening by PCR for Virulence Factor

4.3.1 Detection of the Whole DNA

The purified DNA was detected by electrophoresis (80 V) in 1% agarose gel with ethidium bromide. Methylene blue (loading dye) stain was added to the DNA sample and visualizes the DNA by the UV light as found in (Figure 4-2).

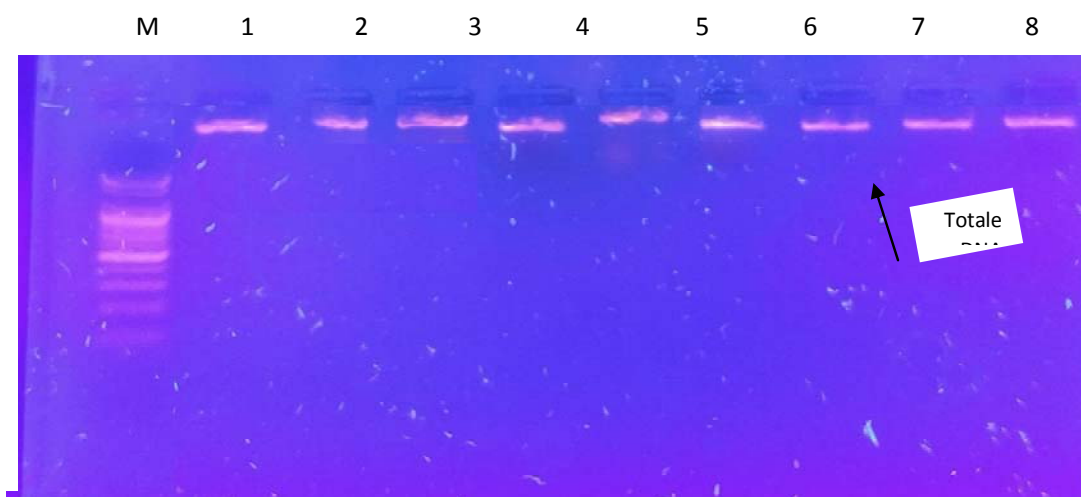


Figure (4-2) Total DNA extraction from *Escherichia coli* isolates

4.3.2 Detection of virulence factor diarrheagenic *E. coli* genes by polymerase chain reaction (PCR) analysis

Screening for virulence factor genes among the isolates of *Escherichia coli* included in this study was done by polymerase chain reaction (PCR). The amplification products were identified as virulence factor from their size in Et-Br-stained agarose gels.

4.3.2.1 Detection *rfbO157* gene size (259 bp) encoded for *STEC*

rfbO157 was gene encoding for the *rfbO157* somatic *STEC* group, testing was conducted to replicate DNA chain by using the primer for the *rfbO157* gene size (259 bp) which is located in the chromosomal region, this gene was confirmed diagnosis *E. coli* belonging to *STEC* group . After making PCR reaction and electrophoresis for products on agarose gel at concentration (1.5%) and visualized by UV light appeared bundle with molecular size 259 base pairs .This gene was identified in about 7/55(12.7%)of *E. coli* isolates. While 48/55(87.27%) isolates were negative to this gene ,as shown in figure (4-3). These values included meat 4(16%), milk 2(11.7%), cheese 1(7.6%) as shown in

table (4-4). The results appear a high percentage in the meat sample and a low percentage in the cheese samples.

Table (4-4):The total isolates of *Escherichia coli* ,the positive number of isolates to *rfbO157* gene , and their percentage.

Source of isolates	<i>E. coli</i> isolates	<i>rfbO157</i> gene positive	%
Meat	25	4	16
Milk	17	2	11.7
Cheese	13	1	7.6
Total	55	7	

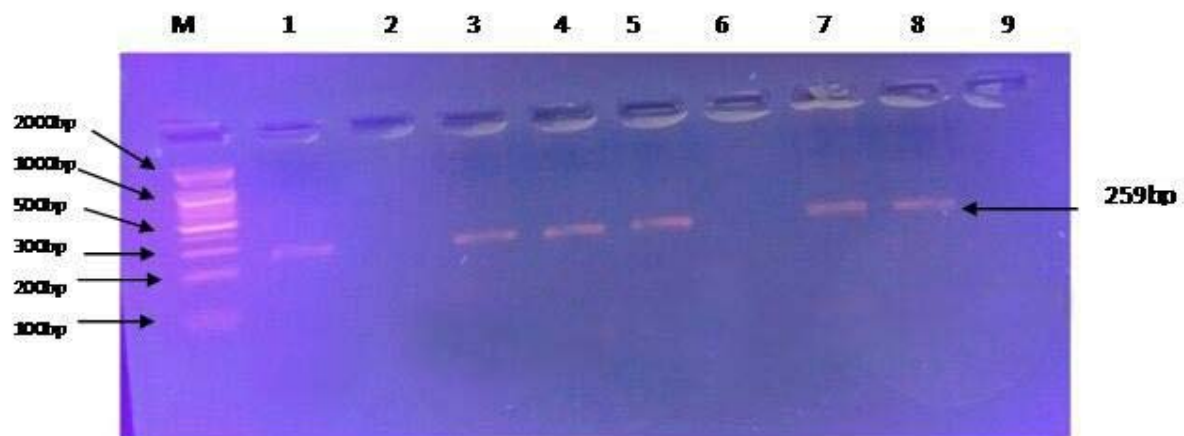


Figure (4-3): PCR products of (259bp) *rfbO157* gene on (1.5%) agarose gel for 45 minute and 70 V. Lane M: DNA ladder100 bp. Lanes 1,3,4,5,7 and 8: positive results to *rfbO157*gene. Lane 2and 9: negative result . lane 6 control negative.

4.3.2.2 Detection of *eaeA* gene (229 bp) size that encoded for *EPEC* group

In this study by using polymerase chain reaction PCR with the primer of *eaeA* gene (229 base pairs) it was appeared that 18 isolates of *E. coli* possess *eaeA* gene, which include meat 6(24%) samples, milk 5(29.41%) samples, cheese 7(53.84%) samples as shown in table (4-5) and Figure (4-4). It is clear from the results that the high percentage appears in milk product while the low percentage appears in meat.

Table (4-5): Total isolates of *E. coli* ,positive sample to *eaeA* gene , and the type of samples.

Source of isolate	<i>E. coli</i> isolates	<i>eaeA</i> gene positive	%
Meat	25	6	24
Milk	17	5	29.4
Cheese	13	7	53.8
Total	55	18	

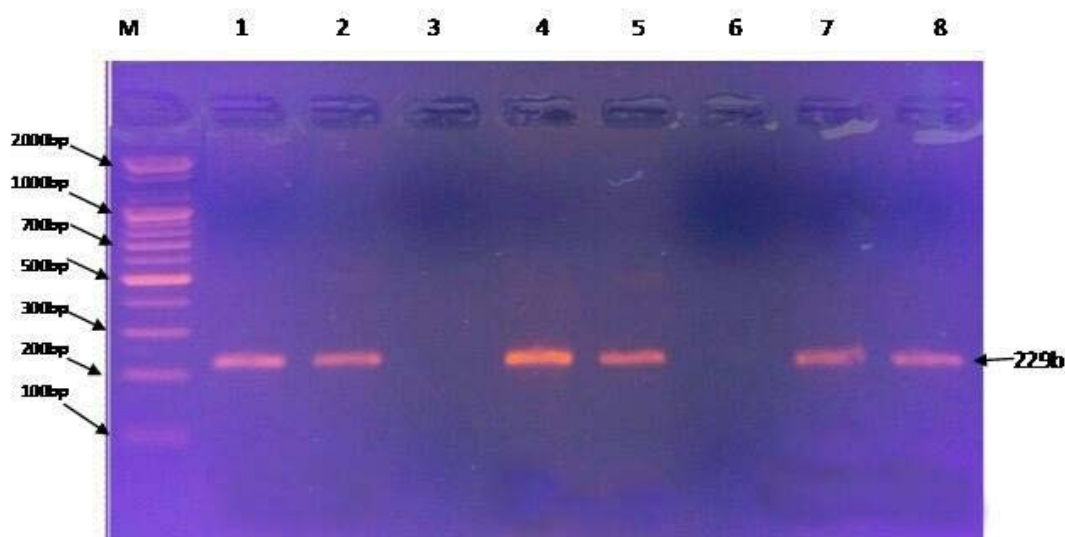


Figure (4-4): PCR products of (229bp) *eaeA* gene on (1.5%) agarose gel for 45 minute and 70 V. Lane M: DNA ladder100 bp. Lanes 1,2,4,5,7 and 8: positive results to *eaeA* gene. Lane 3and 9: negative result . lane 6 control negative.

4.3.2.3 Detection the *bfpA* gene size (450 bp) encoded for *EPEC* group

bfpA primer(450bp) using in PCR reaction to detect the presence of this gene which retired to enteropathgenic *E. coli* . gel electrophoresis appears the bundle of this gene at about 8 isolates of *E. coli* these include meat 2(8%) isolates, milk 2(11.76%) isolates and cheese 4(30.76%) isolates; as it clear from the results cheese posses the high value while milk posses the low value of the gene as shown in table (4.6) and figure (4.5).

Table(4.6): Total isolates of *E. coli* ,positive sample to *bfpA* gene , and the type of samples.

Source of isolates	<i>E. coli</i> isolates	<i>bfpA</i> gene positive	%
Meat	25	2	8
Milk	17	2	11.76
Cheese	13	4	30.76
Total	55	8	

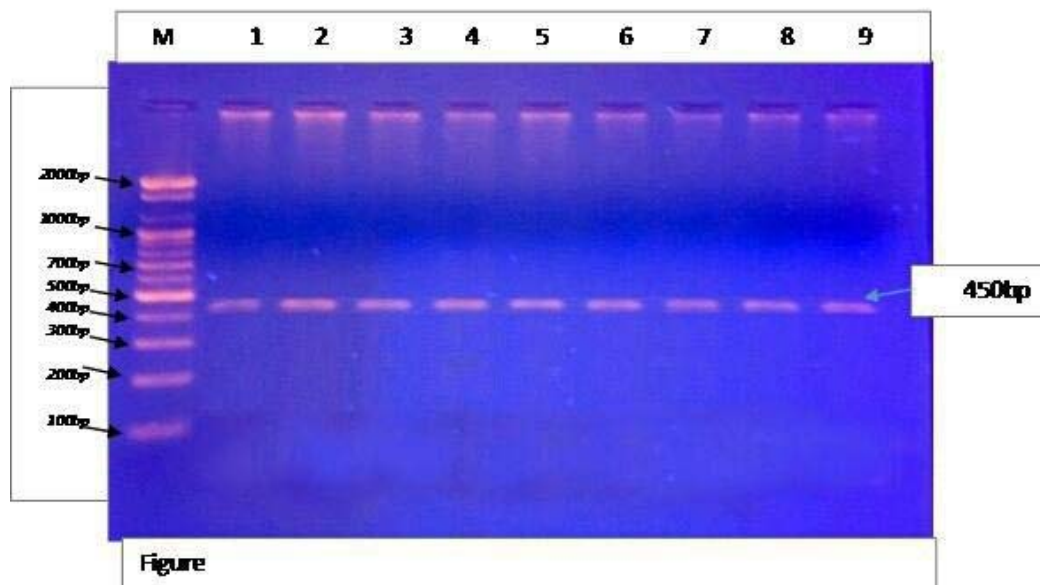


Figure (4-5): PCR products of (450bp) *bfpA* gene on (1.5%) agarose gel for 45 minute and 70 V. Lane M: DNA ladder100 bp. Lanes 1,2,3,4,5,7 and 8): positive results to *bfpA* gene. Lane 9: negative result . lane 6 control negative.

4-3-2-4 Detection the *ial* gene size(320) encoded for *EIEC* group

To find out whether this gene really exists, it was used *ial* primer (320 bp) and the picture of gel electrophoresis appears that 7 isolates of *E. coli* have *ial* gene these include meat 2(8%) isolates, milk 2(11.7%) isolates, and cheese 3(23.07%) isolates as shown in table (4-7) and figure (4-6).

Table(4-7): Total isolates of *E. coli* ,positive sample to *ial* gene , and the type of samples.

Source of isolates	<i>E. coli</i> isolate number	<i>ial</i> gene positive	%
Meat	25	2	8
Milk	17	2	11.7
Cheese	13	3	23.07
Total	55	7	

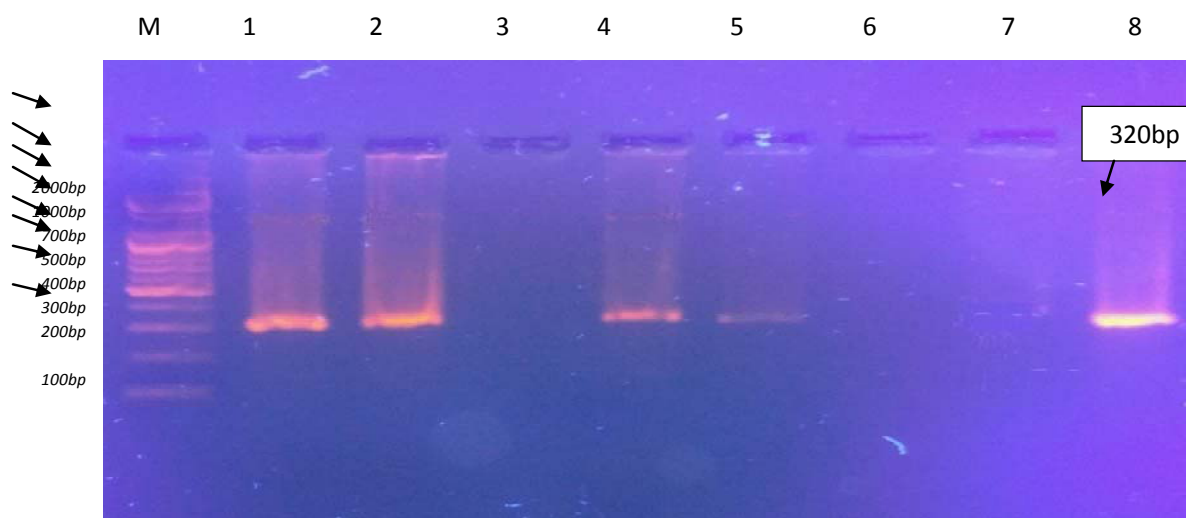


Figure (4-6): PCR products of (320bp) *ial* gene on (1.5%) agarose gel for 45 minute and 70 V. Lane M: DNA ladder 100 bp. Lanes 1,2,4,5 and 8): positive results to *ial* gene. Lane 7 and 9: negative result. lane 6 control negative.

4.3.2.5 Detection of *CVD342* gene size (630) encoded for *EAEC* group

In this study, it was used the primer of *CVD432* size (630) in the PCR reaction where the results of gel electrophoresis showed that 6 isolates of *E. coli* have *CVD342* gene these include meat 2 (8%) isolates, milk 2 (11.7%) isolates, and cheese 2 (15.3%) isolates. The high percentage retired for cheese and milk while the low percentage retired to meat as shown in table (4-8) and figure (4-7).

Table(4-8): Total isolates of *E. coli* ,positive sample to *CVD432* gene , and the type of samples.

Source of isolate	<i>E. coli</i> isolate number	<i>CVD342</i> gene positive	%
Meat	25	2	8
Milk	17	2	11.7
Cheese	13	2	15.3
Total	55	6	

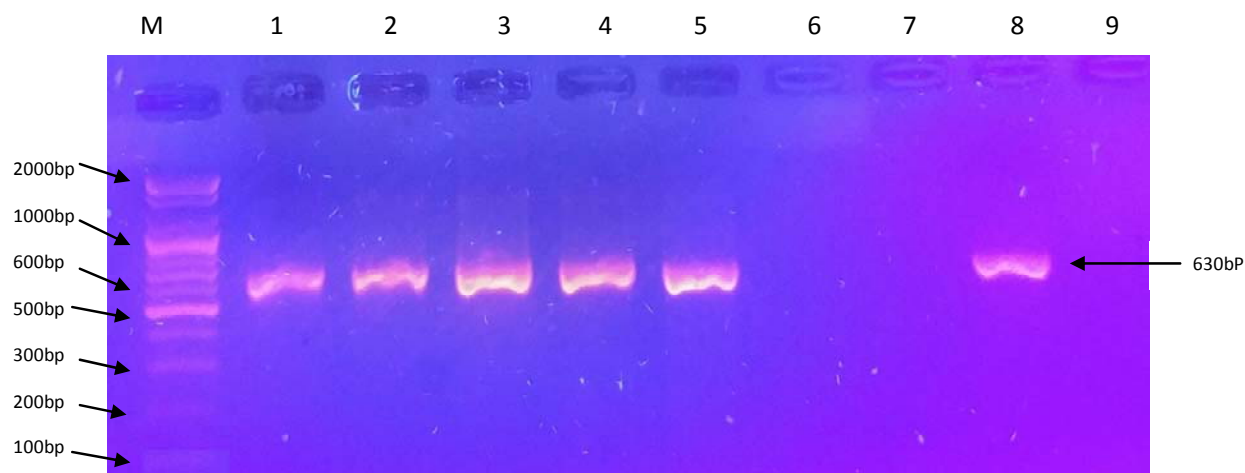


Figure (4-7): PCR products of (630bp) *CVD342* -gene on (1.5%) agarose gel for 45 minute and 70 V. Lane M: DNA ladder100 bp. Lanes 1,2,3,4,5 and 8): positive results to *CVD342* gene. Lane 7and 9: negative result . lane 6 control negative.

4.3.2.6 Detection of *STLa* gene size (170bp) encoded for *ETEC* group

It was used the polymerase chain reaction PCR to the DNA chain with the primer of *STLa* in the size (170 bp) and after the gel electrophoresis and visualized by UV light, it was appear contain 9 isolates of *E. coli* have *STLa* gene these include meat 3(12%) isolates, milk 3 (17.6%) isolates, and cheese 3 (23.07%) isolates as shown in table (4-9) and figure (4-8).

Table(4-9): Total isolates of *E. coli* ,positive sample to *STLa* gene , and the type of samples.

Source of isolates	<i>E. coli</i> isolate number	<i>STLa</i> gene positive	%
Meat	25	3	12
Milk	17	3	17.6
Cheese	13	3	23.07
Total	55	9	

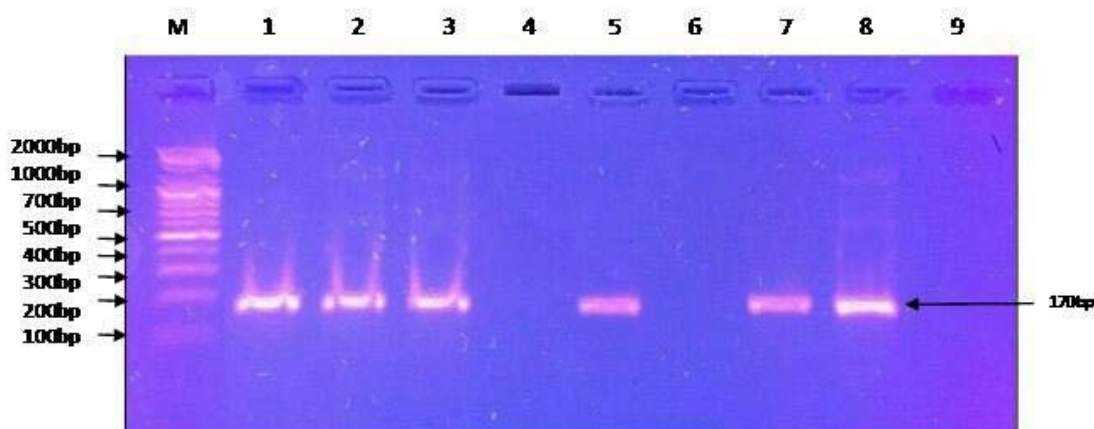


Figure (4-8): PCR products of (170bp) *STLa* gene on (1.5%) agarose gel for 45 minute and 70 V. Lane M: DNA ladder100 bp. Lanes 1,2,3,5,7 and 8): positive results to *STLa* gene. Lane 4and 9: negative result . lane 6 control negative.

4.4 Antibiotic susceptibility testing

Antimicrobial susceptibility testing was based on the disk diffusion method as recommended by the Clinical and Laboratory Standard Institute (CLSI) guidelines(2014),this susceptibility of all *E. coli* isolates was performed on Muller Hinton agar by modified Bauer and Kirby disc to detect whether the bacteria was resistant ,intermediate or sensitive to the different antibiotics used against negative isolates like family *Enterobacteriaceae* such as *E. coli* .

All *Escherichia coli* isolates (n=55) were screened against eleven antimicrobial agents belonging to several structurally diverse classes .Results show that isolates varied in their resistance and sensitivity to the antibiotics. Overall isolates of *Escherichia coli* in this study were highly susceptible to carbapenems such as imipenem (100%) , aminoglycoside such as netilmicin (100%) and gentamycin (100%) , chloramphenicols such as chloramphenicol (100%) , tetracyclins such as tetracycline (81.8%) , quinolones

such as ciprofloxacin (72.72%), while it showed low sensitive to nalidixic acid (56.36%) , and cephalosporins such as cephalothin (100%), while the isolates showed high resistant to cefotaxime (45.45%) and ceftriaxone (66.6%) , so that in the case of β -lactams such as ampicillin, the resistant percentage was (67.27%) as show in table (4.10).

Table (4.10): Antimicrobial drug sensitivity of *E. coli* isolates from meat , milk , and cheese.

Antibiotics	Code	<i>E. coli</i> isolates (Number =55)					
		Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
Ampicillin	AM	10	18.18	8	14.54	37	67.27
Cefotaxime	CTX	15	27.27	5	9.09	25	45.45
Ceftriaxone	CRO	20	36.36	7	12.72	28	66.6
Cephalothin	CEP	55	100	-	-	-	-
Ciprofloxacin	CIP	40	72.72	5	9.09	10	18.18
Chloramphenicol	C	55	100	-	-	-	-
Gentamicin	CN	48	87.27	4	7.27	3	5.45
Imepinim	IPE	55	100	-	-	-	-
Nalidixic acid	NA	31	56.36	12	21.81	12	21.81
Netilmicin	NET	55	100	-	-	-	-
Tetracyclin	TE	45	81.8	3	5.45	7	12.72

Chapter Five

Discussions

5.1 Isolation and identification of *E. coli*

E. coli is the most common species of facultative anaerobe found in the gastrointestinal tract of both human and animals and the most commonly encountered pathogen in the enterobacteriaceae family, therefore the presence of such organism in foods is indicative of fecal pollution (Soomro *et al.*, 2002; Benkerroum *et al.*, 2004).

The results presented that *E. coli* was isolated within a percentage of (17%) from milk, Nearly similar results were reported by (Rajeev and Amit., 2010) who found that out of all milk samples examined the highest contamination was recorded from the milk collected from vendors (26%) followed by dairy farms (20%), while (Ali AA, Abdelgadir., 2011; Gwida and El-Gohary., 2013) isolated *E. coli* from raw milk in a percentage of 63% and 41.2% respectively. Lower results were recorded by (Kivaria *et al.*, 2006) who detected *E. coli* in (6.3%) of the examined raw milk samples.

Although global importance of *E. coli* as a causative agent for diarrheal illness has decreased markedly over the past 50 years following the implementation of improved sanitary practices, it is still the major cause of illness in under developed nations (Ryser., 1998).

The higher incidence of *E. coli* in market milk than bulk farm milk may be due to that bulk farm milk is mainly transported directly to the dairy plant for processing while market milk is usually collected from small farms or farmers, therefore, it will be liable to cross contamination by different ways as mixed fresh clean milk with unclean milk by hands of workers, containers of transportation or contaminated water used for cleaning utensils could be source for contamination (Murphy and Boor., 2000).

Prevalence of *E. coli* reported (13%) in cheese, the similar result (12.9%) in cottage cheese was reported by (Singh and Prakash, A. 2008) and (16.6%) in

cheese was reported by (*Farzan et al.*, 2012). while it dissimilar to the result (29.2 %) in cheese which recorded by (*Fadel et al.*, 2009). Higher prevalence (96 %) in cheese was reported by (*Paneto et al.*, 2007). The presence of this pathogen in milk products proved to be variable and these variation may be due to geographical location ,season , farm size , number of animals on the farm , hygiene status, farm management practices ,variation in sampling , variation in types of samples evaluated and differences in detection methods (*Levine, 1987; Donnenberg and Kaper , 1992;Vallance and finlay ,2000;De Buyser et al., 2001; Jelacic et al .,2003*).

E. coli, which are normal flora of the human and animal intestine, have been identified as a leading cause of food borne illness all over the world. *E. coli* strains previously have been isolated from meat samples and also have been implicated in the contamination of vegetables such as lettuce (*Hilborn et al., 1999; Enabulele and Uraih., 2009*).

In this study, the incidence of *E. coli* was (25%) in raw meat. This was lower than that reported by other authors (*Al-Gallasa et al., 2002; Hussein and Sakuma., 2005; Temelli et al., 2012*); while it was higher than that reported by (*Abdul-Raouf et al., 1996; Heuvelink et al., 1998; 1999; Dontorou et al., 2003*). It disagree with the report of (*Balcha et al., 2014*) in which prevalence of *E. coli* was (62.5%), while in agreement with (*Mohammed et al., 2014*) who reported that the prevalence of *E. coli* in meat was (17.29%) . This result was also agree with the report of (*Taye et al., 2013*) in which the overall prevalence of carcass contamination with *E. coli* was (30.97%) . There are many factors affecting the differences in prevalence rates among studies; these differences are mainly related to samples and sampling (type, source/location and initial bacterial load), environmental and seasonal factors and the detection methodology used differences in hygienic status abattoir (source of samples) (*Temelli et al., 2012*).

Usually high incidence is attributing to behavior of slaughtering and manipulation of food before reaching the market .

4.2 Detection of diarrheagenic *E. coli* virulence genes by PCR

Diarrheagenic *E. coli* is known to cause not only infectious disease but also food-borne outbreaks . Diarrheagenic *E. coli* is mainly divided into five major categories , namely; enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), and enteroinvasive *E. coli* (EIEC) (Nataro and Kaper., 1998).

However, these five categories of DEC cannot be distinguished by colony formation on agar media. Thus it is necessary to carry out further test to detect each specific virulence factor.

In the present study , six genes, (CVD342 for EAEC, *STLa* for ETEC, *bfpA* for EPEC, *ial* for EIEC, *eaeA* for EHEC, O157 for STEC) have been studied by using polymerase chain reaction PCR assay (Czeczulin et al., 1999; Nataro and Kaper., 1998; Panchuk-Voloshina et al.,1999).

Dairy products were the samples that was most frequently contaminated by DEC bacteria followed by meat samples. It is therefore reasonable to speculate that the source of contamination could be the result fecal contamination of those products . For example, the cheese industry is artisanal , so producers regularly manipulate their products with their bare hands , or may be the same pair of gloves , from the beginning of the production process until the final products are shipped (Nataro and Kaper .,1998;Stell.et.al.,2012).

The first DES strain was Eneropathogenic *E. coli*(EPEC) strains are defined as intimin-containing diarrhoeagenic *E. coli* without *stx* genes(Kaper., 1996). EPEC strains may be either typical , producing bundle forming pili (encoded by the *bfpA* gene) that mediate localized adherence on cultured epithelial cells or

atypical , which are negative for these appendages but show localized like adherence, diffuse adherence or aggregative adherence patterns (*Trabulsi et al., 2002*) .

The *EPEC* pathotype was classified as typical if the strain possessed, the *E. coli* adherence plasmid (EAF) and the *bfpA* encoding the structural subunit of *BFP*. *E. coli* strains that do not possess the *EAF* plasmid or *bfpA* gene classified as atypical *EPEC* (*Trabulsi et al ., 2002*) .

The ratio of *EPEC* in our study was (40%) this is in agreement with another study in Iran,(*Alikhani et al., 2013*) that reported the most frequently identified *DEC* was *EPEC* (47.5%). While it disagree with (*Usein et al., 2009*) who showed that (9%) of the isolates carried *EPEC* isolates . The reason for different rates of identification of the *EPEC* may be due to health status in various areas (*Albert et al., 1995*) . The *tEPEC*(*eaeA+*, *bfpA+*) in our testing were (18.18%) , it is in agreement with (*Canizalez-Roman et al., 2013*)who showed that 9 strains (20%) were *tEPEC*. Whereas the prevalence of *aEPEC* (*eaeA+*/ *bfpA-*) was (63.63%),This ratio agreement with result in three Iranian Provinces, Tehran, Ilam and Mazandaran as a reservoir for intimin gene positive *E. coli* types showed that (40.5%) and (20.0%) of harbored *eaeA* gene respectively(*Alikhani MY et al., 2006*), While it disagree with (*Samira Badri et al.,2008*) in which prevalent of *aEPEC* (*eaeA+* without *bfpA*) was (11%), also the ratio of *aEPEC*(*bfpA+* without *eae*) was 4(18.18%) . The ratio of *aEPEC* also was higher than that reported in other studies (*Dhanashree and Mallya, 2008*; *Sharifi-Yazdi, 2011*), while it is in agreement with (*Cristina Rugeles et al., 2010*) who showed that *bfpA+* is in the ratio (10.7%). Until the 1990s, typical *EPEC* strains were more commonly isolated than atypical *EPEC* , Though recent studies have indicated that the prevalence of typical *EPEC* strains has decreased and that they are gradually being replaced by atypical *EPEC* (*Trabulsi et al., 2002*). This bacterial burden in food samples may be related to the

potential of atypical *EPEC* strains to cause human disease. For example , a recent paper by (*Estrada-Garcia et al ., 2009*) reported that the prevalence of atypical and typical *EPEC* strains isolated from cases of diarrhea in children from Mexico was (44.5%) and (10%) respectively. Similarly , studies in Peru (*Contreras et al., 2010*), Brazil (*Nunes Mdo et al.,2012*), Japan (*Yatsuyanagi et al., 2003*) and Australia (*Nguyen et al., 2006*) have reported an increase in diarrheal cases due to atypical *EPEC* , suggesting that atypical *EPEC* is an emerging diarrheagenic pathogen , not only in developing countries , but also in industrial countries.

The second *DES* strain that was detected was *EAEC* ;*EAEC* strains were also further classified as typical and atypical . The term typical or atypical *EAEC* refers to strains harboring or lacking the gene encoding the transcriptional regulator *AggR* , respectively some studies have demonstrated an association of typical *EAEC* with diarrhea (*Estrada-Garcia et al., 2009; Huang et al., 2006*). While the pathogenicity of atypical *EAEC* has not been fully elucidated , these strains have been associated with food-borne outbreaks (*Cobeljic et al., 1996*) and clearly have been associated with a massive outbreak of gastrointestinal illness in school children from Japan (*Itoh et al., 1997*), Brazil (*Regua-Mangia et al., 2009*), and other developing countries (*Bangar and Mamatha, 2008; Scavia.et.al.,2008*).

Our study reported (10.90%) of isolated having *CVD432*. Our finding is agree to those which reported by(*Aranda et al., 2004; Ifeanyi et al., 2015; Aslani et al., 2011*) in which they found that the rate of *EAEC* was 2%, 2% and 10.7%, respectively; while it is in disagreement with (*Adjéhi Dadie et al., 2014*) who showed that the rate of *EAEC* was (36%) . The *EAEC* pathovars would therefore be most frequently involved in human diarrhea in our environment. This observation not only confirms our previous results, but also shows the emerging nature of this pathovar, as reported by other authors (*Veilleux and*

Dubreuil, 2005; Franck et al., 2011). It is also known that EAEC play a special role in chronic diarrhea in people with HIV/AIDS infection (*Kelly et al., 2003; Gassama-Sow et al., 2004*). The high proportion of these pathovars in human strains studied may be due to the fact that food from animal origin is a reservoir for this gene(*Cohen et al., 2005*).

Another important *DEC* that was evaluated in this study was enterohemorrhagic *E. coli* , also known as Shiga-toxin producing *E. coli* (*STEC*). *EHEC* is a group of well-recognized pathogens that are responsible for serious human infectious such as hemorrhagic colitis and hemolytic-uremic syndrome (*Law, 2000; Nataro and Kaper, 1998*). The most important serotype of these group was *O157:H7*, This serotype is the most frequent *EHEC* that is implicated in food-borne outbreaks worldwide(*Mead and Griffin., 1998*).

Our study detected *rfbO157* in (12.72%) of food samples; only(12.12%) showed hybridization between *eae* and *rfbO157* genes . A study at an abattoir in northern England revealed a prevalence of 15.7% (752 out of 4800) *E. coli* *O157* in cattle prior to slaughter (*Chapman et al., 1997*). In a nationwide Dutch study , 10.6% (57 out of 540) of fecal samples from adult cattle collected at the major abattoir were positive for *E. coli* *O157* (*Heuvelink et al., 1998*) . Another nationwide study performed in Finland at 14 major abattoir revealed that 1.31% (19 out of 1448) were *E. coli* *O157*. Isolation rate of our study was higher than that of the reported studies conducted in European countries. This strain has been isolated from beef carcasses at a slaughter plant in Mexico 2.7% (7/258) (*Varela-Hernandez et al., 2007*). Moreover , between 2009 and 2010 six cases of disease produced by *E. coli* *O157:H7* reported by the Public Health Agency in Canada (PHAC), affected Canadian travelers whom had recently in Mexico; The implicated food was not detected (*PHAC., 2009, 2010*). The reason of this discrepancy might be due to the differences in the examined geographical regions in the studies according to the climate or different methodologies used

for the isolation of *E. coli* O157. The higher frequency may be due to differences in the composition of the gastrointestinal flora resulting from differences in diet (Heuvelink *et al.*, 1998), also the isolation rate of O157 varied through the monthly isolation it has been observed to be highest during the warm months in parallel to reported human infections (Griffin and Tauxe., 1991) in UK, (Chapman *et al.*, 1997) observed the highest isolation rate in spring and late summer, while in Finland the highest isolation rate was in July and September. (Bonardi *et al.*, 1999) observed high isolation rate of *E. coli* O157 during the warm months. As we saw in our result, the high percentage of gene found in the meat, this is because the minced meat is contaminated during slaughter and salting and mixed with other during chopping, it cannot distinguish the meat contaminated with bacteria in its shape, color, and odor therefore it is difficult to distinguish between contaminated and non contaminated meat (Stephen and Nduka., 2009). Also, may be confirmed that young animals are the most important reservoir of highly pathogenic *eae* STEC (Blanco *et al.*, 2001; Blanco *et al.*, 1996; Blanco *et al.*, 1997). Another DEC pathotypes group was ETEC, The ETEC was established as a causative pathogen of diarrhea in developing countries (Al-Gallas N *et al.*, 2007). Several countries in the world, including Bangladesh, Egypt and Mexico, have reported ETEC as the most common cause of diarrhea among all *E. coli* intestinal pathotypes (Gomez-Duarte *et al.*, 2013, Presterl *et al.*, 2003 & Moriel *et al.*, 2012).

In the present study, ETEC were detected in (16.36%) isolates. Our finding is dissimilar to that reported by (Pejman Abbasi *et al.*, 2013), in which they found that the rate of STLa was 50%, simultaneously. Our finding is similar to the results of the study by (Jafari *et al.*, 2008), in Tehran. ETEC strains are associated with two major clinical syndromes; weanling diarrhea among children in the developing world, and traveler's diarrhea. Traveler's diarrhea is

usually contracted from contaminated food and water (*Black., 1990; Mattila., 1994*). Several studies have shown the importance of *ETEC* in infections among the tourists visiting Asia , Africa and south America (*Adachi et al ., 2001; Daniels., 2006; Daniels et al.,2000*).

Despite the role of food as vehicle of *ETEC* in outbreaks and sporadic diarrheal cases worldwide , in the present study this category of diarrheagenic *E. coli* was determined in (16.36%) of potentially virulence isolates. The rate of isolation of the *ETEC* reported by(*Wolk et al., 1995*) and(*Viboud et al., 1999*) was 20.7% and 18.3%, respectively. In studies conducted by (*Hegde et al., 2012*) and (*Nguyen et al., 2005*) the rate of isolation of the *ETEC* was 3.5% and 2.2%, respectively . it is clear from the percentage of our study that gene value high in each cheese and milk samples ,This might be indication that *E. coli* strains associated with mammary gland infections (*Blum et al., 2008*). The last group of *DEC* was *EIEC* , *EIEC* was responsible for several outbreaks but there are few reports on routes of transmission and distribution of this bacterium in nature . water and cheese were described as potential sources (*Borian et al., 1959 – Valentini et al., 1992*) as well as the direct transmission through person to person contact (*Harris et al., 1985*) . In the 1970, a major outbreak of diarrhea was reported in the united states , which affected 387 patients the transmission vehicle was an imported cheese (*Marier R et al., 1973*). According to the food and drug control agency of the United States (Food and Drug Administration –FAD), Outbreaks caused by *EIEC* have been associated with milk and milk products and beef; however, any food or water contaminated with human feces of an individual patient can cause disease in other individuals (*FAD., 2009*) . In Brazil , there is a report of three samples isolated from water (*Valentini SR et al., 1992*) . Outbreaks involving two *EIEC* were recently reported in Europe , one in Italy in 2012 involving 109 cases and

the other one is in United Kingdom in 2014 involving 50 cases (*Michelocc V et al., 2016* , *Newitts et al., 2016*) .

Our findings reported from 55 isolates of *E. coli*, (12.72%) were *EIEC*. This result is disagree with the study reported by (*Hegde et al., 2012*) who reported a prevalence of *EIEC* in 1.5% isolates in India. In Calcutta the prevalence of *EIEC* was 16.3% (*Chaterjee et al., 1984*) , while in Thailand , China and other Asian countries, a prevalence of 4 to 7% has been seen (*Echeverria P et al., 1992- Taylor DN et al., 1986*). In Bolivia, the report showed a 2% prevalence (*Utsunomiya A et al., 1995*), The isolation of *EIEC* in Brazil has ranged from 0.5% to 15% depending on the population investigated (*Almeida MTG et al., 1998- Oliveira MG et al., 1989*). The data suggest that the presence of *EIEC* is related to socioeconomic conditions (*Toledo and Trabulsi , Toledo et al., 1990*).

We found that *ETEC* was the major (16.36%) *DEC* in our study which agree with (*Shaheen et al., 2009*) in Egypt who found that the major *DEC* was *ETEC* (60.4%).

5.3 Prevalence of antibiotic sensitivity of *E. coli*

In present investigation among 55 *E. coli* isolates from milk , cheese ,and meat the highest sensitivity was recorded for imipenem (100%) , cephalothine (100%), chloramphenicol (100%), netilmicin (100%) , gentamycine (87.27%), tetracycline (81.8%), ciprofloxacin (72.72%), and nalidixic acid (56.36%). This finding agrees with the work of (*Hiko et al., 2008; Osaili et al., 2013*). However, the study conducted in Saudi Arabia (*Naser and Wabel, 2007*), revealed that there was resistant strain to the drugs such as Tetracycline, Nalidixic acid, gentamycine, , Ciprofloxacin and Chloramphenicol. Also our results to those of previous reports associated with mastitis infectious (*Fazlani et al., 2011; Mshelia et al., 2014*) .This variation probably attributed to the expression of resistant gene code by the pathogen which associated with emerging and re

emerging aspects of the isolates with the regards of different agro ecology (Reuben and Owuna, 2013).

On the other side, the current study revealed that all isolates were highly resistant to Ampicillin (67.27%), cefotaxime (45.45%), and cefotriaxone (66.6%) . Similar findings were reported by many researchers (Mora *et al.*, 2005;Srinivasan *et al.*, 2007;Taye *et al.*, 2013). Also previous study showed resistance in isolates of *E. coli* to ampicillin from food of animals origin in Tunis (Jouini *et al.*, 2009) and isolates from raw meat in Iran (Momtaz *et al.*, 2013). However, resistance to Ciprofloxacin and Naldixic acid observed in our study (18.18% and 21.81% respectively) that agree with earlier reports of (Jouini *et al.*, 2009) who found 18% and 33% of naldixic acid and ciprofloxacin respectively in *E. coli* isolates . This might be due to the use of inappropriate antibiotics for treatment of diseases (Sharada *et al.*, 2010) and also excessive use of antimicrobials for therapeutic and prophylactic treatment (Majaliya *et al.*, 2010). On the other hand, resistance to gentamicin (5.45%) observed in our results disagree with the study of (Momtaz *et al.*, 2013) who found a higher resistance to gentamicin among *E. coli* isolates . The difference in antimicrobial resistance results observed in our study may be due to overuse of antimicrobial drugs in both human and animal medicine therefore it is thought to be the main cause of the increase in bacterial resistance (Szmolka *et al.*, 2013).

Conclusions and Recommendations

Conclusions and Recommendations

Conclusions

According to the results obtained in this study, the following conclusions maintain :

- 1- The frequency of *E. coli* isolation from meat was more than those isolated from milk and cheese.
- 2- The investigation including the use of molecular technique is necessary to allow accurate identification and epidemiological study of these pathogens.
- 3- The predominant virulence genes were ETEC and EPEC among diarrheagenic *E. coli* isolated in this study.
- 4- Shiga-toxigenic *Escherichia coli* isolated in this study was high in meat than in milk and cheese.
- 5- The vast majority of the tested *E. coli* isolates were sensitive to the most antibiotic used in this study.

Conclusions and Recommendations

Recommendations

- 1- Establish awareness classes for the public people, meat sellers and chefs about the dangers of food contamination and the measures that should be taken to avoid such contamination.
- 2- Improvement of method and hygienic of meat processing in the slaughterhouse and milk product processing .
- 3- Studying virulence genes of diarrheagenic *E. coli* extensively.
- 4- Awareness to the use of antibiotic as an animal food booster , causing mutant, resistant or virulent strains.

References

References



- ❖ A. Jouini, K.B. Slama, Y. Saenz, N. Klibi, D. Costa, L. Vinue, M. Zarazaga, A. Boudabous, C. Torres. Detection of multiple-antimicrobial resistance and characterization of the implicated genes in *Escherichia coli* isolates from foods of animal origin in Tunis. *J. Food Prot.* 72 (2009) 1082-1088.
- ❖ A. Szmolka, B. Nagy, Multidrug resistance commensal *Escherichia coli* in animals and its impact for public health, *Front. Microbial.* 4(2013) 4.
- ❖ Abdella, M., Siham, A., Suliman, Y. H. and Alian, A. Microbial contamination of sheep carcasses at EI Kadero slaughter house Khartoum state, Sudan. *Journal of Veterinary Science. Animal Husbandry*, (2009): 48:1-2.
- ❖ Abdul-Raouf, U.M., M.S. Ammar and L.R. Beuchat. Isolation of *Escherichia coli* O157: H7 from some Egyptian foods. *Int. J. Food Microbial.*, 1996. 29: 423-426.
- ❖ Abubakar I, Irvine L, Aldus CF, Wyatt GM, Fordham R, Schelenz S, Shepstone L, Howe A, Peck M, Hunter PR , A systematic review of the clinical, public health and cost-effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food. *Health Technol Assess* (2007) 11:1–216.
- ❖ Adachi, J.A., Jiang, Z.D., Mathewson, J.J., Verenkar, M.P., Thompson, S., Martinez-Sandoval, F., Steffen, R., Ericsson, C.D., Dupont, H.L.,. Enterohaggard *Escherichia coli* as a major etiologic agent in travelers' diarrhea in 3 regions of the world. *Clinical Infectious Diseases* ,(2001) 32, 1706-1709.
- ❖ Adachi, JA, Ericsson, CD, Jiang, Z D, Pallegar, SR and DuPont, HL, Natural history of enterohaggard and enterotoxigenic *Escherichia coli* infection among US travelers to Guadalajara, Mexico, *J infect dis*, vol. (2002) 185, pp. 1681-1683.
- ❖ Adjéhi Dadie, Nazaire Kouassi, Etienne Dako, Marcellin Dje and Mireille Dosso. Virulence, serotype and phylogenetic groups of diarrhoeagenic *Escherichia coli* isolated during digestive infections in Abidjan, Côte d'Ivoire . (2014)

References

- ❖ Afset, JE, Bergh, K and Bevanger, L, High prevalence of atypical enteropathogenic *Escherichia coli* (EPEC) in Norwegian children with diarrhea, J M Microbiol, vol. 2003,52, pp. 1015-1019.
- ❖ Ahmad, S., Gaucher, I., Rousseau, F., Beaucher, E., Piot, M., Grongnet, J.F. and Gaucheron, F. Effects of Acidification on Physicochemical Characteristics of Buffalo Milk: A Comparison with Cow's Milk. Food Chemistry, (2008) , 106, 11-17.
- ❖ Ahren CM, Gothefors L, Stoll BJ, Salek MA, Svennerholm AM. Comparison of methods for detection of colonization factor antigens on enterotoxigenic *Escherichia coli*. J Clin Microbiol 1986; 23: 586-591.
- ❖ Albert, MJ, Ansaruzzaman, M, Faruque, SM, Neogi , PK,B, Haider, K and Tzipori, S 1991, An ELISA for the detection of localized adherent classical enteropathogenic *Escherichia coli* serogroups, J Infect Dis, vol. 164, pp. 986-989.
- ❖ Albert MJ, Faruque SM, Faruque AS, et al. Controlled study of *Escherichia coli* diarrheal infections in Bangladeshi children. J Clin Microbiol. 1995;33(4):973-977.
- ❖ Al-Gallas N, Bahri O, Bouratbeen A, Ben Haasen A, Ben Aissa R. Etiology of acute diarrhea in children and adults in Tunis, Tunisia, with emphasis on diarrheagenic *Escherichia coli*: prevalence, phenotyping, and molecular epidemiology. Am J Trop Med Hyg. 2007;77(3):571–82.
- ❖ Al-Gallasa, N., R. Ben Aissa Ra, T. Attia Annabib, O. Bahric and A. Boudabousd, 2002. Isolation and characterization of shiga toxin-producing *Escherichia coli* from meat and dairy products. Food Microbial., 19: 389-398.
- ❖ Ali AA, Abdelgadir SW . Incidence of *Escherichia coli* in raw cow's milk in Khartoum state Br J dairy Sci 2011;2(1):23-6. 229- Gwida MM, EL-Gohary FA . Zoonotic bacterial pathogenic isolated from raw milk with special reference to *Escherichia coli* and *Staphylococcus aureus* in Dakahlia . Governorate , Egypt 2013;2(4):705.
- ❖ Alikhani MY, Hashemi SH, Aslani MM, Farajnia S. Prevalence and antibiotic resistance patterns of diarrheagenic *Escherichia coli* isolated from adolescents and adults in Hamedan, West Iran. Iran J Microbiol. 2013;5(1):42-47.

References

- ❖ Alikhani MY, Mirsalehian A, Aslani MM. Detection of typical and atypical enteropathogenic *Escherichia coli* (EPEC) in Iranian children with and without diarrhea. *J Med Microbiol*. 2006;55(Pt 9):1159-1163.
- ❖ Alizadeh AHM, Behrouz N, Salmazadeh S, Ranjbar M, Azimzadeh MH, Habibi E, et al. *Escherichia coli*, *Shigella*, and *Salmonella* species in acute diarrhoea in Hamadan, Islamic Republic of Iran. *East Mediterr Health J* 2007; 13: 243-249.
- ❖ Almeida MTG, Silva RM, Donaire LM, Moreira LE, Martinez MB. Enteropathogens associated with acute diarrheal disease in children. *J Pediatr*. 1998; 74: 291-298.
- ❖ Toledo MRF, Trabulsi LR. Frequency of enteroinvasive *Escherichia coli* in children with diarrhea and healthy controls in Sao Paulo, SP, Brazil. *Rev Microbiol*. 1990; 21: 1-4.
- ❖ Alteri CJ, Smith SN and Mobley HLT. Fitness of *Escherichia coli* during urinary tract infection requires gluconeogenesis and the TCA cycle. *PLoS Pathog*. 2009 5:e1000448.60-Wiles TJ, Kulesus RR and Mulvey MA. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. *Exp Mol Pathol*. 2008; 85:11-19.
- ❖ Amisano G, Fornasero S, Migliaretti G, Caramello S, Tarasco V, Savino F. Diarrheagenic *Escherichia coli* in acute gastroenteritis in infants in North-West Italy. *New Microbiol*. 2011;34:45–51.
- ❖ Anon . microbiology of food and animal feeding stuffs – horizontal method for the enumeration of β -glucuronidase positive *Escherichia coli*. Part 1, colony-count technique at 44°C using membranes and 5-bromo-4-chloro-3-indolyl- β -D-glucuronide. International Organisation for Standardisation, Geneva. (2001a). ISO 16649-1.
- ❖ Anon . microbiology of food and animal feeding stuffs – horizontal method for the enumeration of β -glucuronidase positive *Escherichia coli*. Part 2, colonycount technique at 44°C using 5-bromo-4-chloro-3-indolyl- β -D-glucuronide. International Organisation for Standardisation, Geneva. (2001b). ISO 16649-2 .
- ❖ AOAC INTERNATIONAL (2011) Performance Tested Methods sm Validated Methods. October 2011. Accessed 18 November 2011.

References

- ❖ Aranda KR, Fagundes-Neto U, Scaletsky IC. Evaluation of multiplex PCR for diagnosis of infection with diarrheagenic *Escherichia coli* and *Shigella* spp. *J Clin Microbiol*. 2004;42(12):5849-5853.
- ❖ Araujo, JM, Tabarelli, GF, Aranda, KU, Mendes, CM and Scaletsky, Typical enteroaggregative and atypical enteropathogenic types of *Escherichia coli* are the most prevalent diarrhea-associated pathotypes among Brazilian children, *J Clin Microb*, vol. I C 2007: 45, pp. 3396-3399.
- ❖ Aronon SS, Schechter R, Inglesby VT, Henderson DA, Bartlett JG, Ascher SM, Eitzen E, Fine AD, Hauer J, Layton M, Lillibridge S, Osterholm MV, O'Toole T, Parker G, Perl TM, Russell PK, Swerdlow DL, Tonat K. Botulinum toxin as a biological weapon. *J Am Med Assoc*. 2001; 285:1059-1069.
- ❖ Aslani MM, Alikhani MY, Zavari A, Yousefi R, Zamani AR. Characterization of aggregative (EAEC) clinical isolates and their antibiotic resistance. *Int J Infect Dis* 2011; 15: e136-e139.
- ❖ Aslani MM, Alikhani MY, Zavari A, Yousefi R, Zamani AR. Characterization of enteroaggregative *Escherichia coli* (EAEC) clinical isolates and their antibiotic resistance pattern. *Int J Infect Dis*. 2011;15(2):136-139. doi: 10.1016/j.ijid.2010.10.002.
- ❖ Ausubel, F. M.; Brent, R.; Kingston, R. E.; Moore, D. D.; Smith, J. A.; Seidman, J. D. & Struhl, K. *Current Protocols in Molecular Biology*. John Wiley and Sons, Inc. New York. 198-
*Collee, J. G.; Fraser, A. G.; Marmion, B. P. and Simmons, A. (1996). *Mackie and McCartney practical medical microbiology*. 14th ed. Churchill Livingstone. pp. (1987). 173-174.

References



- ❖ Bacon, R., Belk, K., Sofos, J., Clayton, R., Reagan, J. and Smith, G. Microbial Populations on Animal Hides and Beef Carcasses at different stages of slaughter in plants employing Multiple sequential interventions for Decontamination. *J.Food Prot.*, (2000): 63:1080–1086.
- ❖ Balcha, E., Kumar, A. and Tassew H. Evaluation of Safety of Beef sold in and around Mekelle with speicla reference to Enterohemorrhagic *Escherichia coli* O157:H7. *Global Veterinaria* ,(2014): 12(4): 569-572 .
- ❖ Balows, A., W. J. Hausler, K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy. *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C. 1991.
- ❖ Banatvala, N., A.R. Magnano, M.L. Cartter, T.J. Barrett, W.F. Bibb, L.L. Vasile, P. Msher, M. A. Lambert-Fair, J. H. Green, N. H. Bean, and R. V. Tauxe. Meat grinders and molecular epidemiology: two supermarket outbreaks of *Escherichia coli* O157:H7 infection .*J. Infect .Dis.* 1996: 173: 480–483 .
- ❖ Bandyopadhyay, S., Lodh, C., Rahaman, H., Bhattacharya, D., Bera, A. K., Ahmed, F. A., Mahanti, A., Samanta, I., Mondal, D. K., Sarkar, S., Dutta, T. K., Maity, S., Paul, V., Ghosh, M. K., Sarkar, M. and Baruah, K. K. (2011). Characterization of shiga toxin producing (STEC) and enteropathogenic *Escherichia coli* (EPEC) in raw yak (*Poephagus grunniens*) milk and milk products. [.Doi.org/10.1016/j.rvsc.2011.12.011.10/04/2013](https://doi.org/10.1016/j.rvsc.2011.12.011.10/04/2013) .
- ❖ Bangar, R., Mamatha, B., Identification of enteroaggregative *Escherichia coli* in infants with acute diarrhea based on biofilm production in Manipal, south India. *Indian Journal of Medical Sciences*.2008. 62, 8-12.
- ❖ Baron, E.J. & Finegold, S.M. *Microorganisms Encountered In Urinary Tract in Baily & Scott,s Diagnostic Microbiology*. (1994). (9th) ed. Mosby Company U.S.A.

References

- ❖ Basu, S, Ghosh, S, Ganguly, NK and Majumdar, A biologically active lectin of enteroaggregative *Escherichia coli*, Biochimie, vol. S 2004,86, pp. 657-666.
- ❖ Battisti, A., Lovari, S., Franco, A., Diegidio, A., Tozzoli, R., Caprioli, A. and Morabito, S. Prevalence of *Escherichia coli* O157 in Lambs at Slaughter in Rome, Central Italy. Epidemiol.Infect, (2006):134:415–419.
- ❖ Baudry B, Savarino SJ, Vial P, Kaper JB, Levine MM. A sensitive and specific DNA probe to identify enteroaggregative coli, a recently discovered diarrheal pathogen. J Infect Dis 1990; 161: 1249-1251.
- ❖ Bavaro, M. F. *E. coli* O157:H7 and other toxigenic strains: the curse of global food distribution. Current Gastroenterology Reports , (2012). 14(4) , 317-323.
- ❖ Baylis, C.L., Penn, C.W., Thielman, N.M. et al. *Escherichia coli* and *Shigella spp.* In Principles and Practice of Clinical Bacteriology. 2nd Edition. Gillespie, S.H. and Hawkey, P.M. (Eds). John Wiley & Sons Ltd., London: pp (2006). 347-365.
- ❖ Beddoe T, Paton AW, Le Nours J, Rossjohn J, Paton JC. Structure, biological functions of the AB5 toxins. Cell 2010; 35: 411-418.
- ❖ Belongia, EA, Osterholm, MT and Soler, JT, Transmission of *Escherichia coli* O157:H7 infection in Minnesota child day-care facilities, JAMA, vol. 1993,269, no. 7, pp. 883-888.
- ❖ Benkerroum N , Bouhal Y , EL-Attar A , Marhaben A . Occurrence of shiga toxin producing *E. coli* O157 : H7 in selected diary and meat products marketed in the city of Rabat , Morocco . J food prot 2004; 67 : 1234 – 7.
- ❖ Benner DJ, Fanning GR, Steigerwalt AG, Orskov I, Orskov F. Polynucleotide sequence relatedness among three groups of pathogenic *Escherichia coli* strains. J Bacteriol 1972; 109: 953-965.
- ❖ Benz I, Schmidt MA. AIDA-I, the adhesin involved in diffuse adherence of the diarrhoeagenic *Escherichia coli* strain 2787 (O126:H27), is synthesized via a precursor molecule. Mol Microbiol 1992; 6: 1539-1546.

References

- ❖ Benz I, Schmidt MA. Cloning and expression of an adhesin (AIDA-I) involved in diffuse adherence of enteropathogenic *Escherichia coli*. *Infect Immun* 1989; 57: 1506-1511.
- ❖ Bernier C, Gounon P, Le Bouguénec C. Identification of an aggregative adhesion fimbriae (AAF) type III encoding operon in enteroaggregative *Escherichia coli* as a sensitive probe for detecting the AAF-encoding operon family. *Infect Immun* 2002; 70: 4302-4311.
- ❖ Bettelheim, K. A. Biochemical characteristics of *Escherichia coli*, *Escherichia coli* indomestic animals and humans. CAB International, Wallingford, United Kingdom. 1994, p. 3–30 .In C .L. Gyles(ed.)
- ❖ Betts R, Blackburn CW . Detecting pathogens in food. In: Foodborne pathogens: hazards, risk analysis and control, 2nd edn. Edited by: Blackburn CW, McClure PJ. Woodhead Publishing, Oxford, UK. pp(2009). 17–65.
- ❖ Bhunia AK .Biosensors and bio-based methods for the separation and detection of foodborne pathogens. *Adv Food Nutr Res*(2009); 54:1–44.
- ❖ Bilge SS, Clausen CR, Lau W, Moseley SL. Molecular characterization of a fimbrial adhesin, F1845, mediating diffuse adherence of diarrhea-associated *Escherichia coli* to HEP-2 cells. *J Bacteriol* 1989; 171: 4281-4289.
- ❖ Black, RE, Cousens, S, Johnson, HL, Lawn, JE, Rudan, I, Bassani, DG and Jha, Global, regional, and national causes of child mortality in 2008: a systematic analysis, *Lancet*, vol P 2010. 375, pp. 1969-1987.
- ❖ Black, R.F., Epidemiology of travelers' diarrhea and relative importance of various pathogens. *Reviews of Infectious Diseases* 12(Suppl. 1), 1990; S73-S79.
- ❖ Blanco, J., M. Blanco, J. E. Blanco, A. Mora, M. P. Alonso, E. A. Gonzales, and M. I. Bernardez. Epidemiology of verocytotoxigenic *Escherichia coli* (VTEC) in ruminants, Verocytotoxigenic *Escherichia coli*. Food & Nutrition Press Inc., Trumbull, N.J. 2001 p. 113–148. In G. Duffy et al. (ed.)
- ❖ Blanco, M., J. E. Blanco, J. Blanco, A. Mora, C. Prado, M. P. Alonso, M. Mourinho, C. Madrid, C. Balsalobre, and A. Juárez.

References

- Distribution and characterization of faecal verotoxin-producing *Escherichia coli* (VTEC) isolated from healthy cattle. *Vet. Microbiol.* 1997; 54:309–319.
- ❖ Blanco, M., J. E. Blanco, J. Blanco, E. A. González, A. Mora, C. Prado, L. Fernández, M. Rio, J. Ramos, and M. P. Alonso. Prevalence and characteristics of *Escherichia coli* serotype O157:H7 and other verotoxin-producing *E. coli* in healthy cattle. *Epidemiol. Infect.* 1996. 117:251–257.
 - ❖ Boisen N, Ruiz-Perez F, Scheutz F, Krogfelt KA, Nataro JP. High prevalence of serine protease autotransporter cytotoxins among strains of Enterohaggative *Escherichia coli*. *Am J Trop Med Hyg* 2009; 80: 294-301.
 - ❖ Bolton, D.J.; Doherty A.M.; and Sheridan .Beef HACCP intervention and non intervention systems.int. *J. Microbiol.* J.J.(2001).66:119-129.
 - ❖ Bonardi, S., Maggi, E., Bottarelli, A., Pacciarini, M.L., Ansuini, A., Velini, G., Morabito, S., Caprioli, A., Isolation of verocytotoxin-producing *Escherichia coli* O157:H7 from a slaughter in Italy. *Veterinary Microbiology*; 1999.67, 203-211.
 - ❖ Bopp, CA, Brenner, FW and Fields, *Escherichia*, *Shigella* and *Salmonella*, in Murray, PR, Baron, EJ and Jorgensen, JH, *Manual of clinical microbiology*, 8th edition, Washington, pp. , PI 2003; 234-289 .
 - ❖ Borian A, Csizmazia F, Karvaly E, Mihalffy F, Redey B. Enterocolitis epidemic caused by water contaminated with *E. coli* O124 in Veszpren. *Orv Hetil.* 1959; 100: 1072-1074.
 - ❖ Bouzari S, Aslani MM, Oloomi M, Jafari A, Dashti A. Comparison of multiplex PCR with serogrouping and PCR-RFLP of *fliC* gene for the detection of enteropathogenic *Escherichia coli* (EPEC). *Braz J Infect Dis* 2011; 15: 365-369.
 - ❖ Bouzari S, Jafari A, Azizi A, Oloomi M, Nataro JP. Characterization of enterohaggative *Escherichia coli* isolates from Iranian children. *Am J Trop Med Hyg.* 2001; 65: 13-14.
 - ❖ Bouzari S, Jafari A, Farhodi-Moghaddam AA, Shokouhi F, Parsi M. Adherence of non-enteropathogenic *Escherichia coli* to HeLa cells. *J Med Microbiol* 1994; 40: 95-7.

References

- ❖ Bouzari S, Jafari A, Zarepour M. Distribution of virulence related genes among enteroaggregative *Escherichia coli* isolates: using multiplex PCR and hybridization. *Infect Genet Evol.* 2005; 5: 79-83.
- ❖ Bouzari S, Jafari MN, Shokouhi F, Parsi M, Jafari A. Virulence-related sequences and adherence patterns in strains of enteropathogenic *Escherichia coli*. *FEMS Microbiol Letts* 2000; 185: 89-93.
- ❖ Brandal, L.T., Lindstedt, B.A., Lena, A., Stavnes, T.L., Lassen, J., Kapperud, G. Octaplex PCR and fluorescence-based capillary electrophoresis for identification of human diarrheagenic *Escherichia coli* and *Shigella* ssp. *J. Microbiol. Methods.*, 2007; 68: 331–42.
- ❖ Brenner, D.J., Krieg, N.R. and Staley, J.R. *Bergey's Manual® of Systematic Bacteriology : The Proteobacteria, Part B: The Gamma proteobacteria*. New York, Springer. (Eds) (2005). Volume 2.
- ❖ Brett KN, Hornitzky MA, Bettelheim KA, Walker MJ, Djordjevic. Bovine non-O157 Shiga toxin 2- containing *Escherichia coli* isolates commonly possess stx2edl933 and/or stx2vhb subtypes. *J. Clin. Microbiol. SP* (2003) 41:2716 – 2722 .
- ❖ Brown, JE, Echeverria, P, Taylor, DN, Seriwatana, J and Newland, Determination by DNA hybridization of Shiga-like-toxin-producing *Escherichia coli* in children with diarrhea in Thailand, *J Clin Microbiol*, vol. J W 1989 27, pp. 291-294.
- ❖ Brzuszkiewicz E, Thurmer A, Schuldes J, Leimbach A, Liesegang H, Meyer F-D, et al. Genome sequence analyses of two isolates from the recent *Escherichia coli* outbreak in Germany reveal the emergence of a new pathotype: Entero-Aggregative-Haemorrhagic *Escherichia coli* (EAHEC). *Arch Microbiol* 2011; 193: 883-891.
- ❖ Brzuszkiewicz E, Briiggemann H, Liesegang H, et al. How to become a uropathogen: comparative genomic analysis of extraintestinal pathogenic *Escherichia coli* strains. *Proc Natl Acad Sci USA* 2006;103:12879-12884.

References



- ❖ CAC. Recommended International Code of Practice General Principles of Food Hygiene. CAC/RCP 1-1969. In Food hygiene: basic texts, fourth edition, pp. 2009; 1–33. Rome.
- ❖ Canizalez-Roman A, Gonzalez-Nuriez E, E. Vidal J., Flores-Villaserior H., Leon-Sicairos. Prevalence and antibiotic resistance profiles of diarrheagenic *Escherichia coli* strains isolated from food items in northwestern Mexico. N. 2013; 211-220.
- ❖ Caprioli A, Morabito S, Brugère H, Oswald E. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. Vet Res 2005; 36: 289-311.
- ❖ Cassels FJ and Wolf MK. Colonization factors of diarrheagenic *E. coli* and their intestinal receptors. J Ind Microbiol. 1995; 15:214-226.
- ❖ Chapman, P.A., Siddons, C.A., Gerdan Malo, A.T., Harkin, M.A., A 1-year study of *Escherichia coli* O157:H7 in cattle, sheep, pigs and poultry. Epidemiology and Infection. 1997;119, 245-250.
- ❖ Chatterjee BD, Sanyal SN. Is it all Shigellosis. Lancet. 1984; 2: 574.
- ❖ Chen HD, Frankel G. Enteropathogenic *Escherichia coli*: unraveling pathogenesis. FEMS Microbiol Rev 2005; 29: 83-98.
- ❖ Cobeljic, M., Miljkovic-Selimovic, B., Paunovic-Todosijevic, D., Velickovic, Z., Lepsanovic, Z., Zec, N., Savic, D., Ilic, R., Konstantinovic, S., Jovanovic, B., Kostic, V., Enteroaggregative *Escherichia coli* associated with an outbreak of diarrhea in a neonatal nursery ward. Epidemiology and Infection 1996;117, 11-16.
- ❖ Cohen, M.B., Nataro, J.P., Bernstein, D.I., Hawkins, J., Roberts, N., Staat, M.A., 2005. Prevalence of diarrheagenic *Escherichia coli* in acute childhood enteritis: a prospective controlled study. The journal of Pediatrics .2005; 146, 54-61.
- ❖ Cole, D, Drum, DJ and Stallknecht, . Free-living Canada geese and antimicrobial resistance, Emerg Infect Dis J, vol. DE 2005; 11 , no. 6, pp. 935-8.

References

- ❖ Contreras, C.A., Ochoa, T.J., Lacher, D.W., Debroy, C., Navarro, A., Talledo, M., Donnenberg, M.S., Ecker, L., Gil, A.I., Lanata, C.F., Cleary, T.G., Allelic variability of critical virulence genes (eae, bfpA and perA) in typical and atypical *Escherichia coli* in Peruvian children. *Journal of Medical Microbiology*.2010; 59, 25-31.
- ❖ Coombs BK, Gilmour MW, Goodman CD. The evolution of virulence in non-O157 Shiga toxin-producing *Escherichia coli*. *Front Microbiol* doi:10.3389/fmicb.2011.00090.
- ❖ Cousin, M.A. Presence and activity of psychrotrophic microorganisms in milk and dairy products: a review. *Journal of Food Protection*, (1982); 45: 172-207.
- ❖ Cravioto A, Gross RJ, Scotland SM, Rowe B. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. *Curr Microbiol* 1979; 3: 95-99.
- ❖ Cravioto, A, Reyes, RE, Fernandez, G, Hernandez, R and Lopez, D 1988, Prospective study of diarrhoeal disease in a cohort of rural Mexican children: incidence and isolated pathogens during the first two years of life, *Epidemiology and infection*, vol. 101, pp. D 1988; 123-134.
- ❖ Cristina Rugeles L., Jing Bai, Juliana Martinez A., Consuelo Vanegas M., Gomez-Duarte O.G., Molecular characterization of diarrheagenic *Escherichia coli* strains from stool samples and food products in Colombia. 2010; 117-120.
- ❖ Croxen MA and Finlay BB. Molecular mechanisms of *Escherichia coli* pathogenicity. *Nat Rev Microbiol*. 2010; 8:26-38. 53- Kaper JB, Nataro JP and Mobley HL. Pathogenic *Escherichia coli*. *Nature Rev Microbiol*. 2004; 2:123-140.
- ❖ Croxen MA, Finlay BB. Molecular mechanisms of *Escherichia coli* pathogenicity. *Nat Rev Microbiol* 2010; 8: 26-38.
- ❖ Cryan, Comparison of three assay systems for detection of enterotoxigenic *Escherichia coli* heat-stable enterotoxin, *J Clin Microbiol*, vol. 28 pp. B 1990;792-794.
- ❖ Czekulin JR, Balepur S, Hicks S, Hicks S, Henderson IR, Le Bouguénec C. Aggregative adherence fimbria II, a second fimbrial

References

- antigen mediating aggregative adherence in enteroaggregative *Escherichia coli*. *Infect Immun* 1997; 65: 4135-4145.
- ❖ Czczulin, J.R., Whittam, T.S., Henderson, I.R., Navarro-Garcia, F., Nataro, J.P., Phylogenetic analysis of enteroaggregative and diffusely adherent *Escherichia coli*. *Infect. Immune*. 1999; 67, 2692-2699.



- ❖ Daniels, N.A., Enterotoxigenic *Escherichia coli*: travelers' diarrhea comes home. *Clinical Infectious Diseases*. 2006;42, 335-336.295-
- Daniels, N.A., Neimann, J., Karpati, A., Parashar, U.D., Greene, K.D., Wells, J.G., Srivastava, A., Tauxe, R.V., Mintz, E.D., Quick, R., Traveler's diarrhea at sea: three outbreaks of waterborne enterotoxigenic *Escherichia coli* on cruise ships. *Journal of Infectious Diseases* 2000;181, 1491-1495.
- ❖ De Buyser , M.L., Dufour, B., Maire, M., Lafarge, V., Implication of milk and milk products in food-borne diseases in France and in different industerilised countries . *Int. J. Food microbial* . 2001; 67(1-2), 1-17.
- ❖ De SN, Bahatachary K, Sarkar JK. A study of the pathogenicity of strains of *Bacterium coli* from acute and chronic enteritis. *J Pathol Bacteriol* .1956; 71: 201-209.
- ❖ Deng, Wenbin and Poretz RD. Oligodendroglia in developmental neurotoxicity. *Neurotoxicology*. 2003;24:161-178.
- ❖ Deszo EL, Steenbergen SM, Freedberg DI and Vimr ER. *Escherichia coli* K1 polysialic acid O-acetyltransferase gene, neuO and the mechanism of capsule form variation involving a mobile contingency locus. *Proc Natl Acad Sci*. 2005; 102:5564-5569.
- ❖ Dhanashree, B. and P.S. Mallya,. Detection of Shiga-Toxigenic *Escherichia Coli* (STEC) in diarrhoeagenic stool and meat samples in Mangalore, India. *Indian J. Med. Res.*, 2008;128: 271-277.
- ❖ Dilkharin K. 2001. Apoptosis in the in vivo mammalian forebrain. *Neurobiol Dis*. 8:359-379.
- ❖ Dobrindt U, Hacker J ,Targeting virulence traits: potential strategies to combat extraintestinal pathogenic *E. coli* infections. *Curr Opin Microbiol*. (2008); 11: 409-413.

References

- ❖ Donnenberg, M.S., Kaper, J.B., Enteropathogenic *Escherichia coli* . Infect. Immun. 1992; 60(10), 3953-3961.
- 238- Vallance , B.A., Finlay, B.B., Exploitation of host cells by enteropathogenic *Escherichia coli* . Proc. Natl. Acad. Sci. 2000; 97(16), 8799-8806.
- ❖ Donta SE, Smith DM. Stimulation of steroidogenesis in tissue culture by enterotoxigenic *Escherichia coli* and its neutralization by specific antiserum. Infect Immun 1974; 9: 500-505.
- ❖ Donta, ST, Moon, HW and Whipp, SC, Detection of heat-labile *Escherichia coli* enterotoxin with the use of adrenal cells in tissue culture, Science , vol. 183, pp. 1974; 334-336.
- ❖ Dontorou, C., C. Papadopoulou, G. Filioussis, V. Economou and I. Apostolou et al., Isolation of *Escherichia coli* O157:H7 from foods in Greece. Int. J. Food Microbiol., 2003; 82: 273-279.
- ❖ Drasar, B.S. Some factors associated with geographical variations in the intestinal microflora. In: The normal microbial flora of man. (eds), Academic Press, London pp. 1974; 187-196.
- ❖ Drasar, B.S. and P.A. Barrow. Intestinal Microbiology. In: (eds), Amer. Soc. Microbiol. Press, Washington, DC pp. 1985; 19-40.
- ❖ DuPont, HL, Clinical practice: Bacterial diarrhea, N Engl J Med, vol. 361, pp. 2009; 1560-1569.



- ❖ Ebel, F, Deibel, AU, Kresse, CA, Guzman, A and Chakraborty, T, Temperature- and medium-dependent secretion of proteins by Shiga toxinproducing *Escherichia coli*, Infect Immun, vol. 64, pp. 1996; 4472-4479.
- ❖ Echeverria P, Seriwatana J, Leksomboon U, Tirapat U, Chaicumpa W, Rowe B. Identification by DNA hybridization of enterotoxigenic *Escherichia coli* in homes of children with diarrhea. Lancet 1; 1984: 63-66.
- ❖ Echeverria P, Sethabutr O, Serichantalergs O, Lexomboon U, Tamurak. Shigella and enteroinvasive *Escherichia coli* infections in households of children with dysentery in Bangkok. J Infect Dis. 1992; 165: 144-147.

References

- ❖ Edwards, P. R., and W. H. Ewing. Identification of Enterobacteriaceae. Burgess Publishing Co., Minneapolis, Minn. 1972; 85-96.
- ❖ Elias, WP, Suzart, S, Trabulsi, LR, Nataro, JP and Gomes, TA, Distribution of aggA and aafA gene sequences among Escherichia coli isolates with genotypic or phenotypic characteristics, or both, of enteroaggregative *E. coli*, J Med Microbiol, vol. 48, pp. 1999; 597-599.
- ❖ Enabulele, S.A. and N. Uraih,. Enterohaemorrhagic Escherichia coli 0157:H7 Prevalence in meat and vegetables sold in Benin City, Nigeria. Afr. J. Microbiol. Res., 2009; 3: 276-279.
- ❖ Estrada-Garcia, T., Lopez-Saucedo, C., Thompson-Bonilla, R., Abonce, M., Lopez-Hernandez, D., Santos, J.I., Rosado, J.L., Dupont, H.L., Long, K.Z.,. Association of diarrheagenic Escherichia coli Pathotypes with infection and diarrhea among Mexican children and association of atypical Enteropathogenic *E. coli* with acute diarrhea. Journal of Clinical Microbiology 2009; 47, 93-98.
- ❖ Evans, DG, Evans, DJ and Pierce, NF, Differences in the response of rabbit small intestine to heat-labile and heat-stable enterotoxins of Escherichia coli, Infect Immun, vol. 7, pp. 1973; 873-880.
- ❖ Evans DG, Evans Jr. DJ, Tjoa W. Hemagglutination of human group A erythrocytes by enterotoxigenic Escherichia coli isolated from adults with diarrhea: correlation with colonization factor. Infect Immun 1977; 18: 330-337.
- ❖ Ezawa, A, Gocho, F, Saitoh, M, Tamura, T, Kawata, K, Takahashi, T and Kikuchi, N, A three-year study of enterohemorrhagic *Escherichia coli* O157 on a farm in Japan, J Vet Med Sci ,vol. 66, pp. 2004; 779-784.



- ❖ Fadda, G., Nicoletti, G., Schito, C. and Tempera, G. Antimicrobial susceptibility patterns of contemporary pathogens from uncomplicated urinary tract infections isolated in a multicenter Italian survey: possible impact on guidelines. J Chemother (2005); 17:251-257.

References

- ❖ Fadel, H. M. and Ismail, J. Prevalence and Significance of *Staphylococcus aureus* and *Enterobacteriaceae* species in Selected Dairy Products and Handlers. *Int .J. Dairy science*. 4(3): (2009) ;100-108.
- ❖ FAO (Food and Agriculture Organization), *Food Outlook, Global Market Analysis* 17 October 2011, pp. 43–44.
- ❖ Farzan, R., Rahimi, E. and Momtaz, H. Virulence properties of Shiga Toxin-Producing *Escherichia coli* isolated from Iranian raw milk and dairy products. *Slov. Vet. Res.* (2012) ;49(4): 159-66.
- ❖ Feng P . Rapid methods for the detection of foodborne pathogens: current and next-generation technologies. In: *Food microbiology, fundamentals and frontiers*, 3rd edn. Edited by: Doyle MP, Beuchat LR. ASM Press, Washington, D.C. pp(2007); 911–934.
- ❖ Fleckenstein JM, Hardwidge PR, Munson GP, Rasko DA Sommerfelt H Steinsland H. Molecular mechanism of enterotoxigenic *Escherichia coli* infection. *Microbes Infect* 2010; 12: 89-98.
- ❖ Food and Drug Administration. Enteroinvasive *Escherichia coli* 9 EIEC), 2009. [http://www. Gov/food/Food Safety/Food borne Illness/Food borne Pathogens Natural Toxins/Bad Bug Book/ucm071298.htm](http://www.Gov/food/Food Safety/Food borne Illness/Food borne Pathogens Natural Toxins/Bad Bug Book/ucm071298.htm).
- ❖ Frank C, Werber D, Cramer JP, Askar M, Faber M, Heiden MA, Bernard H, Fruth A, Prager R, Spode A. Epidemic Profile of ShigaToxin- Producing *Escherichia coli* O104:H4 Outbreak in GermanyPreliminary Report. *N. Engl. J. Med.* (2011).
- ❖ Frankel, G, Giron, JA, Vlamossoi, J and Schoolnik, Multi-gene amplification: simultaneous detection of three virulence genes in diarrheal stool, *Mol Microbiol*, vol. 3, pp. 1989; 1729-1734.
- ❖ Frankel G, Riley L, Giron JA, Valmassoi J, Friedmann A, Strockbine N, Falkow S & Schoolnik GK. Detection of *Shigella* in feces using DNA amplification *Journal of Infectious Diseases*, (1990); 161: 12521256.
- ❖ Franzolin, M.R., Alves, R.C., Keller, R., Gomes, T.A., Beutin, L., Barreto, M.L., et al. Prevalence of diarrheagenic *Escherichia coli* in children with diarrhea in Salvador, Bahia, Brazil. *Mem do Instituto Oswaldo Cruz.*, 2005;100: 359–63.

References

- ❖ Fraser ME, Fujinaga M, Cherney MM, Melton-Celsa AR, Twiddy EM, O'Brien AD, et al. Structure of Shiga toxin type 2 (Stx2) from *Escherichia coli* O157:H7. *J Biol Chem* 2004; 279: 27511-27517.
- ❖ Fratamico PM, Smith JL. *Escherichia coli* infections. In Riemann H, Cliver D (eds.): *Foodborne Infections and Intoxications*, 3rd edition. New York: Elsevier, 2006:205-258.
- ❖ Friedemann, M. *Enterobacter sakazakii* in food and beverages (other than infant formula and milk powder). *International Journal of Food Microbiology*, (2007); 116: 1-10.
- 334- Joosten, H., Marugg, J., Stephan, R. et al. A rapid and reliable alternative to ISO 215281:2004 for detection of *Enterobacteriaceae*. *International Journal of Food Microbiology*, (2008);125: 344-346.



- ❖ G.D. Meshelia, G. Okpaje, Y.A.C. Voltaire, G.O. Egwu, Comparative studies on genital infections and antimicrobial susceptibility patterns of isolates from camels (*Camels dromedaries*) and cows (*Bos indicus*) in Maiduguri, north-eastern Nigeria, *Springer Plus* 3 (2014) 91 .
- ❖ Gannon, VP, DSouza, JS, Graham, T, King, RK, Rahn, K and Read, S, Use of the flagellar H7 gene as a target in multiple PCR assays, *J Med Microbiol*, vol. 57, pp. 1997; 123-34.
- ❖ Gannon VPJ, Rashed M, King RK & Thomas EJ. Detection and characterization of the *eae* gene of Shiga-like toxinproducing *Escherichia coli* using the polymerase chain reaction. *Journal of Clinical Microbiology*, (1993); 31: 1268-1274.
- ❖ Gassama-Sow A, Sow PS, Gueye M, Gueye-N'diaye A, Perret JL, M'boup S, Aidara-Kane A. Characterization of pathogenic *Escherichia coli* in human immunodeficiency virus-related diarrhoea in Senegal. *J. Infect. Dis.* (2004); 189 (1):75-7.
- ❖ Gianella, RA, Suckling mouse model for detection of heat-stable *Escherichia coli* enterotoxin: characteristics of the model, *Infect Immun* , vol. 14, pp . 1976; 95-99.

References

- ❖ Gill, C., Badoni, M. and Jones, T: Hygienic effects of trimming and washing operations in a beef-carcass-dressing process. *J. Food Prot.*, (1996); 59: 666–669.
- ❖ Giron, JA , Gloria, I, Viboud, V, Oscriba, G and Kaper, JP, Prevalence and Association of the Longus Pilus Structural Gene (IngA) with Colonization Factor Antigens, Enterotoxin Types, and Serotypes of Enterotoxigenic *Escherichia coli* , infect and immun, vol. 63, no. 10, pp. 1995; 4195-4198.
- ❖ Goldberg MB and Theriot JA. *Shigella flexneri* surface protein IcsA is sufficient to direct actin-based motility. *Proc Natl Acad Sci.* 1995; 92:6572-6576.
- ❖ Gomez-Duarte OG, Romero-Herazo YC, Paez-Canro CZ, EslavaSchmalbach JH, Arzuza O. Enterotoxigenic *Escherichia coli* associated with childhood diarrhoea in Colombia, South America. *J Inf Dev Ctries.* 2013;7(5):372–81.
- ❖ Gordillo, ME, Reeve, GR and Pappas, J, Molecular characterization of strains of enteroinvasive *Escherichia coli* O143, including isolates from a large outbreak in Houston, Texas, *J Clin Microbiol*, vol. 30, no. 4, pp. 1992; 889-93.
- ❖ Gould LH, Bopp C, Strockbine N, Atkinson R, Baselski V, Body B, et al. Recommendations for Diagnosis of Shiga Toxin-Producing *Escherichia coli* Infections by Clinical Laboratories. *Morb Mort Wkly Rep (MMWR)* 2009; 58: 1-14.
- ❖ Goulet P, Picard B. Comparative electrophoretic polymorphism of esterases and other enzymes in *Escherichia coli*. *J Gen Microbiol* 1989;135:135-143.
- ❖ Greenberg, DE, Jiang, ZD, Steffen, R, Verenker, MP and DuPont, HL, Analysis of incidence of infection with enterotoxigenic *Escherichia coli* in a prospective cohort study of infant diarrhea in Nicaragua, *J Clin Microb*, vol. 35, pp. 2002; 1404-1410.
- ❖ Griffin PM. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*, pp. 1995; 739–761. In *Infections of the gastrointestinal tract* (Blaser MJ, Smith PD, Ravdin JJ, Greenberg HB and Guerrant RL eds), Raven Press, New York, USA.

References

- ❖ Griffin, P.M., Tauxe, R.V., The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and associated hemolytic uremic syndrome. *Epidemiologic Reviews*;1991; 13, 60-98.
- ❖ Guerrant RL, Brunton LL, Schnaitman TC, Rebhun LI, Gilman AG. Cyclic adenosine monophosphate and alteration of Chinese hamster ovary cells morphology: a rapid, sensitive in vitro assay for the enterotoxins of *Vibrio cholerae* and *Escherichia coli*. *Infect Immun* 1974; 10: 320-327.
- ❖ Guerrant RL, Thielman NM. Types of *Escherichia coli* enteropathogens. In Blaser MJ, Smith PD, RavdinJI, Greenberg HB, Guerrant RL (eds.): *Infections of the Gastrointestinal Tract*. New York: Raven Press, 1995:687-707.
- ❖ Gunzburg ST, Tornieporth NG & Riley LW. Identification of enteropathogenic *Escherichia coli* by PCR-based detection of the bundle-forming pilus gene. *Journal of Clinical Microbiology*, (1995); 33: 1375-1377.
- ❖ Gurtler, J.B., Kornacki, J.L. and Beuchat, L.R. *Enterobacter sakazakii*: a coliform of increased concern to infant health. *International Journal of Food Microbiology*, (2005); 104: 1-34.
- ❖ Gwida MM, EL-Gohary FA. Zoonotic bacterial pathogenic isolated from raw milk with special reference to *Escherichia coli* and *Staphylococcus aureus* in Dakahlia . Governorate , Egypt 2013; 2(4): 705.
- ❖ Gyles CL. Shiga-producing *Escherichia coli*: an overview. *J Anim Sci* 2007; 85(E. Suppl.): E45-E62.
- ❖ Gyles, C.L.: Shiga toxin-producing *Escherichia coli*: overview *Anim Sci*,85:45-62. Hancock, D., Besser, T., Lejeune, J., Davis, M. and Rice, D. (2001): The control of VTEC in the animal reservoir. *Int J Food Microbiol*, .(2007); 66:71-78.



- ❖ H. Momtaz, F.S. Dehkordi, E. Rahimi, H. Ezadi, R. Arab, Incidence of Shiga-toxin producing *Escherichia coli* serogroups in ruminant's meat, *Meat Sci.* 95 (2013) 381-388.

References

- ❖ Haridy, M.S.A. Yeast Flora of Raw Milk in El-Minia City, Egypt. *Cryptogamie Mycologie*, (1992) ; 13, 321-326.
- ❖ Harrington, SM, Dudley, EG and Nataro, JP, Pathogenesis of enteroaggregative *Escherichia coli* infection, *FEMS Microbiol Lett*, vol. 254, pp. 2006; 12-18.
- ❖ Harris JR, Mariano J, Wells JG, Payne BJ, Donnell HD, Cohen ML. Person to person transmission in an outbreak of enteroinvasive *Escherichia coli*. *Amj Epidemiol*. 1985; 122: 245-252.
- ❖ Hart, CA, Batt, RM and Saunders, JR, Diarrhoea caused by *Escherichia coli*. *J Clin Microbiol*, vol. 75, pp. 1993; 404-419.
- ❖ Hartman AB, Venkatesan M, Oaks EV, Buysse JM. Sequence and molecular characterization of a multicopy invasion plasmid antigen gene, *ipaH*, of *Shigella flexneri*. *J Bacteriol* 1990; 172: 1905-1915.
- ❖ Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM and Aderem A. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature*. 2001; 410:1099-1103.
- ❖ Hegde A, Ballal M, Shenoy S. Detection of diarrheagenic *Escherichia coli* by multiplex PCR. *Indian J Med Microbiol*. 2012;30(3):279-284. doi:10.4103/0255-0857.99485.
- ❖ Hegde A, Ballal M, Shenoy S. Detection of diarrheagenic *Escherichia coli* by multiplex PCR. *Indian J Med Microbiol*. 2012;30(3):279-284.
- 316- Dhanashree, B. and P.S. Mallya,. Detection of Shiga-Toxigenic *Escherichia Coli* (STEC) in diarrhoeagenic stool and meat samples in Mangalore, India. *Indian J. Med. Res.*, 2008; 128: 271-277.
- ❖ Henderson IR, Czeczulin J, Eslava C, Noriega F, Nataro JP. Characterization of Pic, a secreted protease of *Shigella flexneri* and enteroaggregative *Escherichia coli*. *Infect Immun* 1999; 67: 5587-5596.
- ❖ Hernandez RT, Elias WP, Vieira MAM, Gomes TAT. An overview of atypical enteropathogenic *Escherichia coli*. *FEMS Microbiol Lett* 2009; 292: 137-149.

References

- ❖ Heuvelink, A.E., F.L.A.M. Van Den Biggelaar, J.T.M. Zwartkruis-Nahuis, R.G. Herbes and R. Huyben et al., 1998. Occurrence of Verocytotoxin-Producing *Escherichia coli* O157 on Dutch Dairy Farms. *J. Clin. Microbial.*, 36: 3480-3487.
- ❖ Heuvelink, A.E., J.T. Zwartkruis-Nahuis, R.R. Beumer and E. De Boer, 1999. Occurrence and survival of verocytotoxin-producing *Escherichia coli* O157 in meats obtained from retail outlets in The Netherlands. *J. Food Prot.*, 62: 1115-1122.
- ❖ Heuvelink, A.E., Van Den Biggelaar, F.L.A.M., De Boer, E., Herbes, R.G., Melchers, W.J.G., Huis, in 'T Veld, J.H.J., Monnens, L.A.H., Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 strains from Dutch, cattle and sheep. *Journal of Clinical Microbiology*.1998; 36, 878-882.
- ❖ Hien, BT, Trang, d, Cam, PD, Molbak, K and Dalsgaard, A, Diarrhoeagenic *Escherichia coli* and other causes of childhood diarrhoea: a casecontrol study in children living in a wastewater-use area in Hanoi, Vietnam, *J Clin Microb*, vol. 56, pp. 2007; 1086-1096.
- ❖ Hiko, A., Asrat, D. and Zewde,G.: Occurrence of *Escherichia coli* O157:H7 in retail raw meat products in Ethiopia. *J Infect Dev Ctries.* (2008); 2: 389-393.
- ❖ Hilborn, E.D., J.H. Mermin, P.A. Mshar, J.L. Hadler and A. Voetsch et al., A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. *Arch. Intern. Med.*, 1999; 159: 1758-1764.
- ❖ Hill DR, Beeching NJ. Travelers' diarrhea. *Curr Op Infect Dis* 2010; 23: 481-487 .
- ❖ Hill WE . The polymerase chain reaction: application for the detection of foodborne pathogens. *CRC Crit Rev Food Sci Nutrit* (1996) ; 36:123–173.
- ❖ Hill WE, Madden JM, McCardell BA, Shah DB, Jagow JA, Payne WL, et al. Food borne enterotoxigenic *Escherichia coli*: detection and enumeration by DNA colony hybridization. *Appl Environ Microbiol* 1983; 45: 1324-1330.
- ❖ Honda T, Akhtar Q, Glass RI, Kibriya AK. A simple assay to detect *Escherichia coli* producing heat labile enterotoxin: results of

References

- a field study of the Biken tests in Bangladesh. *Lancet* 1981; ii: 609-610.
- ❖ Honda, T, Taga, S, Takeda, Y and Miwatani, T, Modified Elek test for detection of heat-labile enterotoxin of enterotoxigenic *Escherichia coli*, *J Clin Microbiol*, vol. 13, pp. 1981; 1-5.
 - ❖ Horakova K, MlejnkovaH, Mlejnek P. Specific detection of *Escherichia coli* isolated from water samples using polymerase chain reaction targeting 4 genes: cytochrome bd complex, lactose permease, beta-D-glucuronidase, and beta galactosidase. *J Appl Microbiol* 2008; 105: 970-976.
 - ❖ Huang, DB and Dupont, HL, Enteraggregative *Escherichia coli*: an emerging pathogen in children, *Pediatr Infect Dis*, vol. 15, pp. 2004; 266-271.
 - ❖ Huang DB, Mohanty A, Dupont HL, Okhuysen PC, Chiang T. A review of an emerging enteric pathogen: enteroaggregative *Escherichia coli*. *J Med Microbiol* 2006; 55: 1303 1311.
 - ❖ Huang, D.B., Mohanty, A., Dupont, H.L., Okhuysen, P.C., Chiang, T., A review of an emerging enteric pathogen: enteroaggregative *Escherichia coli*. *Journal of Medical Microbiology* 2006; 55, 1303-1311.
 - ❖ Hussein, H.S. and T. Sakuma,. Invited review: Prevalence of Shiga toxin-producing *Escherichia coli* in dairy cattle and their products. *J. Dairy Sci.*, 2005; 88: 450-465.



- ❖ ICDDR, B, Trends in Aetiologies for Diarrhoeal Diseases, *Health and Science Bulletin*, vol. 1, no. 1 , pp. 2002; 12-15.
- ❖ ICMSF. Micro-organisms in Foods 6: Microbial Ecology of Food Commodities. London, Blackie Academic & Professional. 339- Jay, J.M. (2000). *Modern Food Microbiology*. Gaithersberg, Maryland, Aspen Publishers Inc. (1998).
- ❖ Ifeanyi CI, Ikeneche NF, Bassey BE , Al-Gallas N, Ben Aissa R, Boudabous A. Diarrheagenic *Escherichia coli* pathotypes isolated from children with diarrhea in the Federal Capital Territory Abuja, Nigeria. *J Infect Dev Ctries*. 2015;9(2):165-174.

References

- ❖ Isidean SD, Riddle MS, Savarino SJ, Porter CK. A systemic review of ETEC epidemiology focusing on colonization antigen factor and toxin expression. *Vaccine* 2011; 29: 6167-6178.
- ❖ Itoh, Y., Nagano, I., Kunishima, M., Ezaki, T., Laboratory investigation of enteroaggregative *Escherichia coli* O untypeable :H10 associated with a massive outbreak of gastrointestinal illness. *Journal of Clinical Microbiology*.1997; 35, 2546-2550.



- ❖ Jafari F, Garcia-Gil LJ, Salmanzadeh-Ahrabi S, Shokrzadeh L, Aslani MM, Pourhoseingholi MA, et al. Diagnosis and prevalence of enteropathogenic bacteria in children less than 5 years of age with acute diarrhea in Tehran children's hospitals. *J Infect* 2009b; 58: 21-27.
- ❖ Jafari F, Hamidian M, Rezadehbakhshi M, Doyle M, Salmanzadeh-Ahrabi S, Derakhshan F, et al. Prevalence and antimicrobial resistance of diarrheagenic *Escherichia coli* and *Shigella* species associated with acute diarrhea in Tehran, Iran. *Can J Infect Dis Med Microbiol* 2009a; 20: e56-62 .
- ❖ Jafari F, Shokrzadeh L, Hamidian M, Salmanzadeh-Ahrabi S, Zali MR. Acute diarrhea due to enteropathogenic bacteria in patients at hospitals in Tehran. *Jpn J Infect Dis*. 2008;61(4):269–73.
- ❖ Jafari F, Shokrzadeh L, Hamidian M, SalmanzadehAhrabi S, Zali MR. Acute diarrhea due to enteropathogenic bacteria in patients at hospitals in Tehran. *Jpn J Infect Dis* 2008; 61: 269-73.
- ❖ Jantzen MM, Navas J, Corujo A, Moreno R, Lopez V, Martinez-Suarez JV Review. Specific detection of *Listeria monocytogenes* in foods using commercial methods: from chromogenic media to real-time PCR. *Span J Agric Res*,(2006a); 4:235–247.
- ❖ Jasson V, Jacxsens L, Luning P, Rajkovic A, Uyttendaele M Review. Alternative microbial methods: An overview and selection criteria. *Food Microbiol* . (2010) ;27:710–730.
- ❖ Jelacic , J.K., Damrow , T., Chen , G.S., Jelacic, S., Bielaszewska, M., ciol, M., Carvalho, H.M., Melton-Celsa, A.R., O'Brien, A.D., Tarr, P.I., Shiga toxin-producing *Escherichia coli* in Montana:

References

- bacterial genotypes and clinical profiles . J. Infect. Dis. 2003;188, 719-729.
- ❖ Jenkins, C, Chart, H, Willshaw, GA, Cheasty, T and Smith, HR, Genotyping of enteroaggregative *Escherichia coli* and identification of target genes for the detection of both typical and atypical strains, Diagnostic Microbiology and Infectious Disease, vol. 55, pp. 2006; 13-19.
 - ❖ Jerse, AE, and Kaper, JB, The eae gene of enteropathogenic *Escherichia coli* encodes a 94-kilodalton membrane protein, the expression of which is influenced by the EAF plasmid, Infect Immun, vol. 59, pp. 1991; 4302-4309.
 - ❖ Johnsen, G., Wasteson, Y., Heir, E., Berget, I. and Herikstad, H.: *Escherichia coli* O157:H7 in Feces from Cattle, Sheep and Pigs in The Southwest Part of Norway During 1998 and 1999. Int. J. Food Microbiol., (2001); 65:193–200.
 - ❖ Johnson JR, Russo TA. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. Int J Med Microbiol 2005;295:383-404.
 - ❖ Johnson TJ, Nolan LK. Pathogenomics of the virulence plasmids of *Escherichia coli*. Microbiol Mol Biol Rev 2009; 73: 750-774.
 - ❖ Johnson JR, Murray AC, Gajewski A, et al. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrob Agents Chemother 2003a;47:2161-2168.



- ❖ Kaper, J.B., Defining EPEC. Review of Microbiology 1996; 27, 130-133.
- ❖ Kaper, J.B.: Pathogenic *Escherichia coli*. Int J Med Microbiol (2005); 295(6-7), 355-6.
- ❖ Karmali MA, Gannon V, Sargeant JM. Verotoxin-producing *Escherichia coli* (VTEC). Vet Microbiol 2010; 140: 360-370.
- ❖ Karmali MA, Mascarenhas M, Shen S, Ziebell K, Johnson S, Reid-Smith R, et al. Association of genomic O island 122 of *Escherichia coli* EDL 933 with Vero cytotoxin producing

References

- Escherichia coli sero pathotypes that are linked to epidemic and/or serious disease. *J Clin Microbiol.* 2003; 41: 4930-4940.
- ❖ Kassenborg, HD, Hedberg, CW and Hoekstra, M, Farm visits and undercooked hamburgers as major risk factors for sporadic Escherichia coli O157:H7 infection: data from a case-control study in 5 FoodNet sites, *J Clin Infect Dis*, vol. 5, pp. 2004; 38-45.
 - ❖ Katouli M, Shokouhi F, Farhoudi-Moghaddam AA, Amini S. Occurrence of colonization factor antigens I & II in enterotoxigenic Escherichia coli associated diarrhea in Iran & correlation with of disease. *Indian J Med Res* 1992; 95: 115-120.
 - ❖ Kauer P, Chakraborti A, Asea A. Enteroaggregative Escherichia coli: an emerging enteric food borne pathogen. *Interdiscip Perspect Infect Dis* doi; 10.1155/2010/254159 .
 - ❖ Kaufmann M, Zweifel C, Blanco M, Blanco JE, Blanco J, Beutin L, Stephan R . Escherichia coli O157 and nonO157 Shiga toxin-producing Escherichia coli in fecal samples of finished pigs at slaughter in Switzerland. *J. Food Prot.* (2006) ; 69:260-266 .
 - ❖ Keller R, Ordoñez JG, De Oliveira RR, Trabulsi LR, Baldwin TJand Knutton S. Afa, a diffuse adherence fibrillar adhesion associated with enteropathogenic Escherichia coli. *Infect Immun.* 2002; 70:2681-2689.
 - ❖ Kelly P, Hicks S, Oloya J, Mwansa J, Sikakwa L, Zulu I, Phillips A. Escherichia coli enterovirulent phenotypes in Zambians with AIDS-related diarrhoea. *Trans. Roy. Soc. Trop. Med. Hyg.* (2003); 97(5):573-576.
 - ❖ Khairun, N., Dilruba, A., Johirul, I., Lutful, K., Anowar, M.H. Usefulness of a Multiplex PCR for Detection of Diarrheagenic Escherichia coli in a Diagnostic Microbiology Laboratory Setting. *Bangladesh J. Med. Microbiol.*, 2007; 1: 38–42.
 - ❖ Kiernan MC, Isbister GK, Cindy S, Lin Y, Burke D and Bostock H. Acute Tetrodotoxin induced neurotoxicity after ingestion of puffer fish. *Annals of Neurology.* 2005; 57:339-348.
 - ❖ King, K., Glass, C., Bresee, J.S. and Duggan, C.: Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy. *MMWR Recomm Rep* (2003); 52:1-16.
 - ❖ Kivaria FM , Noordhuizen JPTM , Japaga AM. Evaluation of the hygienic quality and associated public health . hazards of raw milk

References

- marketed by smallholder dairy producers in the Dares Salaam region , Tanzania . Trop Anim Health prod 2006;38(3):185-94.
- ❖ Knutton S, Baldwin T, Williams PH, McNeish AS. Actin accumulation at sites of bacterial adhesion to tissue culture cells: basis of a new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli*. Infect Immun 1989; 57: 1290-1298.
 - ❖ Knutton S, Loyd DR, McNeish AS. Adhesion of enteropathogenic *Escherichia coli* to human intestinal enterocytes and cultured human intestinal mucosa. Infect Immun 1987; 55: 69-77.
 - ❖ Knutton, S, Shaw, R, Phillips, AD, Smith, HR, Willshaw, GA, Watson, P and Price, E, Phenotypic and genetic analysis of diarrhea-associated *Escherichia coli* isolated from children in the United Kingdom, J Pediatr Gastroenterol Nutr, vol. 33, pp. 2001; 32-40.
 - ❖ Koneman, E.W; Allen, S.D; Janda, W.M; Schreckenberger, P.C and Winn, W.C.J. Color Atlas and Textbook Of Diagnostic Microbiology. .(1992); (4th) ed. J.B.Lippincott Company. Philadelphia.
 - ❖ KONOWALCHUK J., SPEIRS J.I. & STAVRIC S. Vero response to a cytotoxin of *Escherichia coli*. Infect. Immun., 18, (1977); 775–779.
 - ❖ Kumar, R., Surendran, P. K. and Thampuran, N. Evaluation of culture, ELISA and PCR assays for the detection of *Salmonella* in seafood. Letters in Applied Microbiol. 46(2): (2008) ; 221-226.



- ❖ Labigne-Roussel AF, Lark D, Scoolink G, Falkow S. Cloning and expression of an afimbrial adhesin (Afa-I) responsible for P blood group-independent mannose-resistant hemagglutination from a pyelonephritic *Escherichia coli*. Infect Immun 1984; 46: 251-259.
- ❖ Lan R, Alles MC, Donohoe K, Martinez MB, Reeves PR. Molecular evolutionary relationship of enteroinvasive *Escherichia coli* and *Shigella* spp. Infect Immun 2004; 72: 5080-5088.

References

- ❖ Lang, AL, Tsai, YL, Mayer, CL and Palmer, CJ, Multiplex PCR for detection of the heat-labile toxin gene and Shiga-like toxin I and II genes in *Escherichia coli* isolated from natural waters, *Appl Environ Microbiol*, vol. 60, pp. 1994; 3145-3149.
- ❖ Law, D. : Virulence factors of *Escherichia coli* O157 and other shiga toxin-producing *E. coli*. *J. Appl. Microbiol.*, 88: (2000); 729-745.
- ❖ Law, D., Virulence factor of *Escherichia coli* O157 and other Shiga toxin-producing *E. coli* . *Journal of Applied Microbiology* 88, 2000; 729-745.
- ❖ Le Bouguénec C, Archambaud M, Labigne A. specific detection of the *pap*, *afa*, and *sfa* adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. *J Clin Microbiol* 1992; 30: 189-1193.
- ❖ Le Bouguénec C, Lalioui L, du Merle, L, Jouve M, Courcoux P, Bouzari S, et al. Characterization of AfaE adhesins produced by extraintestinal and intestinal human *Escherichia coli* isolates: PCR assays for detection of Afa adhesins that do or do not recognize Dr blood group antigens. *J Clin Microbiol* 2001; 39: 1738-1745.
- ❖ Le Bouguénec C, Servin AL. Diffusely adherent *Escherichia coli* strains expressing Afa/Dr adhesins (Afa/Dr DEAC): hitherto unrecognized pathogens. *FEMS Microbiol Lett* 2006; 256: 185-194.
- ❖ Lehner, A. and Stephan, R. Microbiological, epidemiological, and food safety aspects of *Enterobacter sakazakii*. *Journal of Food Protection*, 67: (2004); 2850-2857.
- ❖ Levine, MM, Caplan, ES, Waterman, D, Hornick, RB and Snyder, MJ, Diarrhea caused by *Escherichia coli* that produce only heat-stable enterotoxin, *Infect Immun*, vol. 17, pp. 1977; 78-82.
- ❖ Levine, M.M., *Escherichia coli* that cause diarrhea : enterotoxigenic , enteropathogenic , enteroinvasive , enterohemorrhagic ,and enteroadherent. *J. Infect. Dis.* 155(3), 1987; 377-389.
- ❖ Levine, MM, Nataro, JP, Karch, H, Baldini, MM, Kaper, JB and Black, RE, The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent on a

References

- plasmid encoding an enteroadhesiveness factor, J Infect Dis, vol. 152, pp. 1985; 550-559.
- ❖ Lim JY, Hong JB, Sheng H, Shringi S, Kaul R, Besser TE, et al. Phenotypic diversity of *Escherichia coli* O157:H7 strains associated with the plasmid O157. J Microbiol 2010b; 48: 347-357.
 - ❖ Lim JY, Yoon JW, Hovde CJ. A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. J Microbiol Biotechnol 2010a; 20: 1-10.
 - ❖ Lopez-Saucedo, C, Cerna, JF and Villegas-Sepulveda, Single multiplex polymerase chain reaction to detect diverse loci associated with diarrheagenic *Escherichia coli*, Emerg Infect Dis, vol. 9, no. 1, pp. 2003; 127-31.
 - ❖ Low AS, Holden N, Rosser T, Roe AJ, Constantinidou C, Hobman JL, et al. Analysis of fimbrial gene clusters and their expression in enterohaemorrhagic *Escherichia coli* O157:H7. Environ Microbiol 2006; 8: 1033-1047.



- ❖ Majalija, S., Francis, O., Sarah, W.G., Lubowa, M., Vudriko, P. and Nakamya, F.M. Antibiotics susceptibility profiles of fecal *Escherichia coli* isolates from Dip-Litter broilers chickens in Northern and Central Uganda. Department of veterinary parasitology and microbiology. Veterinary Research 3: (2010); 75-80.
- ❖ Mangia, AH, Duarte, AN, Duarte, R, Silva, LA, Bravo, VL and Leal, MC, Markers of inflammation in bacterial diarrhea among travelers, with a focus on entero-aggregative *Escherichia coli* pathogenicity, J Infect Dis, vol. 185, pp. 1993; 944-949.
- ❖ Manning SD, Motiwala AS, Springman AC, Qi W, Lacher DW, Ouellette LM, et al. Variation in virulence among clades of *Escherichia coli* O157:H7 associated with disease outbreaks. PNAS 2008; 105: 4868-4873.
- ❖ Marier R, Wells JC, Swanson RC, Callahan W, Mehlman V. An outbreak of enteropathogenic *E. coli* foodborne disease traced to imported cheese. Lancet. 1973; 302: 1376-1378.

References

- ❖ Mattila, L., Clinical features and duration of travelers' diarrhea in relation to its etiology. *Clinical Infectious Diseases* 19, 1994; 728-734.
- ❖ McDaniel, TK, Jarvis, KG, Donnenberg, MS and Kaper, JB, A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens, *J Infect Dis* , vol. 46, pp. 1995; 69-81.
- ❖ McDaniel TK, Jarvis KG, Donnenberg MS, Kaper JB. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. *PNAS* 1995; 92: 1664-1668.
- ❖ Mead, P.S., Griffin, P.M., *Escherichia coli* O157:H7. *Lancet* 352, 1998; 1207-1212.
- ❖ Micheloc V, Prosseda G, Maugliami A, et al. Characterization of an emergent clone of enteroinvasive *Escherichia coli* circulating in Europe. *Clin Microbial Infect.* 2016; 22(3): 287. E11-9.
- ❖ Mohammed, O., Shimelis, D., Admasu P. and Feyera, T.: Prevalence and Antimicrobial Susceptibility Pattern of *E. coli* isolates from raw meat samples obtained from Abattoirs in Dire Dawa City, Eastern Ethiopia. *International Journal of Microbiological Research* 5 (1): (2014); 35-39 .
- ❖ Mohd, R., Kotwal, S., Malik, M. A. and Prevalence, M. Genetic profile of virulence determinants and multidrug resistance of *Escherichia coli* isolates from foods of animal origin. *Vet World*, 6; 139-142.
- ❖ Monteiro BT, Campos LC, Sircili MP, Franzolin MR, Bevilacqua LF, Nataro JP, et al. The dispersin-encoding gene (*aap*) is not restricted to enteroaggregative *Escherichia coli*. *Diagn Microbiol Infect Dis* 2009; 65: 81-84.
- ❖ Monteiro-Neto V, Bando S, Moreira-Filho CA, Giron JA. Characterization of an outer membrane protein associated with haemagglutination and adhesive properties of enteroaggregative *Escherichia coli* 0111:H12. *Cell Microbiol* 2003; 5: 533-547.
- ❖ Mora A, Blanco M, Yamamoto D, Dahbi G, Blanco JE, López C, et al. HeLa-cell adherence patterns and actin aggregation of enteropathogenic *Escherichia coli* (EPEC) and Shiga-toxin-producing *E. coli* (STEC) strains carrying different *eae* and *tir* alleles. *Int Microbiol* 2009; 12: 243-251.

References

- ❖ Mora A, Herrera A, López C, Dahbi G, Mamani R, Pita JM, Alonso MP, Llovo J, Bernárdez MI, Blanco JE, Blanco M, Blanco J. Characteristics of the Shiga-toxin-producing enteroaggregative *Escherichia coli* O104:H4 German outbreak strain and of STEC strains isolated in Spain. *Intl Microbiol.* 14: 2011; 121-141.
- ❖ Mora, A., Blanco, J.E., Blanco,M., Alonso, M.P. and Dhabi, G.: Antimicrobial Resistance of Shiga Toxin (Verotoxin)-Producing *E. coli* O157:H7 and Non-O157 Strains Isolated from Humans, Cattle, Sheep and Food in Spain. *Res. Microbiol.* 156: (2005); 793-806.
- ❖ Moriel DG, Rosini R, Seib KL, Serino L, Pizza M, Rappuoli R. *Escherichia coli*: great diversity around a common core. *MBio.* 2012;3(3).
- ❖ Moseley, SL, Echeverria, P, Tirapat, C and Chaicumpa, W, Identification of enterotoxigenic *Escherichia*, *J Infect Dis*, vol. 18, pp. 1982; 44-49.
- ❖ Moyo, S.J., Maselle, S.Y., Matee, M.I., Langeland, N., Mylvaganam, H. Identification of diarrheagenic *Escherichia coli* isolated from infants and children in Dar es Salaam, Tanzania. *BMC Infect. Dis.*, 7: 2007; 92.
- ❖ Murphy SC, Boor KJ. Trouble –Shooting sources and causes of high bacterial counts in raw milk . *Dairy food Environ Sani* . 2000;606-11.



- ❖ Nafisa,H., Ali.I.,Amber, F., Adnan.K., Ameera.Y., Khan.G and Shahana, U.: Kazmi1Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan2Department of Pathology, Jinnah Medical and Dental College, Karachi, Pakistan.*J Infect Dev Ctries.* 4(6): 2010; 382-388.
- ❖ Nagy B, Fekete PZS. Enterotoxigenic *Escherichia coli* in veterinary medicine. *Int J Med Microbiol* 2005; 295: 443-454.
- ❖ Nanu, E., Latha, C., Sunil, B., Thomas M. and Menon, K. V. Quality assurance and public health safety of raw milk at the production point. *Am. J. Food Technol.* 2: (2007) ; 145-152.

References

- ❖ Naser, A. and Wabel, A.: Antibiotic susceptibility of *E. coli*O157:H7 isolated from beef burger. Bull. Pharm. Sci., 30: (2007); 131-134.
- ❖ Nataro JP, Baldini MM, Kaper JB, Black RE, Bravo N, Levine MM. Detection of an adherence factor of enteropathogenic *Escherichia coli* with a DNA probe. J Infect Dis 1985; 152: 560-565.
- ❖ Nataro, JP, Deng, Y, Cookson, S, Guers, LD, Levine, MM and Tacket, CO, Heterogeneity of enteroaggregative *Escherichia coli* virulence demonstrated in volunteers, J Infec. Dis, vol. 171, pp. 1995; 464-469.
- ❖ Nataro, JP., Enteroaggregative *Escherichia coli* pathogenesis, Curr Opin Gastroenterol, vol. 21, pp. 2005; 4-8.
- ❖ Nataro JP, Kaper JB, Robins-Browne R, Prado V, Vial P, Levine MM. Patterns of adherence of diarrheagenic *Escherichia coli* to HEp-2 cells. Pediatr Infect Dis J 1987; 6: 829-831.
- ❖ Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 1998; 11: 142-201.
- ❖ Nataro, JP, Steiner, T and Guerrant, RL, Enteroaggregative *Escherichia coli*, Emerg Infect Dis, vol. 4, pp. 1998; 251-261.
- ❖ Nataro JP, Yikang D, Yingkan D, Walker K. AggR , a transcriptional activator of aggregative adherence fimbria I expression in enteroaggregative *Escherichia coli*. J Bacteriol 1994; 176: 4691-4699.
- ❖ Nataro, J.B., Diarrheagenic *Escherichia coli* . Clin. Microbiol. Rev. 11, 1998; 142-201.
- ❖ Nataro, JP, Seriwatana J, Fasano A, Maneval D, Guers LD, Noriega F, Dubovsky F, Levine MM, Morris Jr. JG. Identification and cloning of a novel plasmid-encoded enterotoxin of enteroinvasive *Escherichia coli* and *Shigella* strains. Infect Immun 1995; 63: 4721-4728.
- ❖ Navas J, Ortiz S, Lopez P, Jantzen MM, Lopez V, Martinez-Suarez JV . Evaluation of effects of primary and secondary enrichment for the detection of *Listeria monocytogenes* by real-time PCR in retail ground chicken meat. Foodborne Pathog Dis 3: (2006); 347–354.

References

- ❖ Naves P, del Prado G, Huelves L, Gracia M, Ruiz V, Blanco J, et al. Measurement of biofilm formation by clinical isolates of *Escherichia coli* is method-dependent. *J Appl Microbiol* 2008; 105: 585-590.
- ❖ Neill MA, Tarr PI, Taylor DN, Trofa AF. *Escherichia coli*. In Hui YH, Gorham JR, Murrell KD, Cliver DO (eds.): *Foodborne Disease Handbook: Diseases Caused by Bacteria*, vol. 1. New York: Marcel Dekker, 1994:169-213.
- ❖ Nessa, k, Dilruba, A, Islam, J, Kabir, L and Hossain, MA , Usefulness of a Multiplex PCR for Detection of Diarrheagenic *Escherichia coli* in a Diagnostic Microbiology Laboratory Setting, Bangladesh, *J Med Microbiol*, vol. 01, no. 2, pp. 2007 ; 38-42.
- ❖ Newitts, MacGregor V, Robbins V, et al. Two linked enteroinvasive *Escherichia coli* outbreaks, Nottingham, UK, June 2014. *Emerg Infect Dis*. 2016; 22(7): 1178-1184.
- ❖ Nguyen TV, Le Van P, Le Huy C, Gia, K N, Weintraub, A. Detection and characterization of diarrheagenic *Escherichia coli* from young children in Hanoi, Vietnam. *J Clin Microbiol*. 2005;43(2):755-760.
- 300- Blum, S.; Sela, N.; Heller, E.D.; Sela, S.; Leitner, G. Identification of a bovine mastitis *Escherichia coli* subset. *Vet. Microbiol*. 2008, 132, 135–148.
- ❖ Nguyen, R.N., Taylor, L.S., Tauschek, M., Robins-Brown, R.M., Atypical enteropathogenic *Escherichia coli* infection and prolonged diarrhea in children. *Emerging Infectious Diseases* 12, 2006; 597-603.
- ❖ Nguyen, T.V., Le Van, P., Le Huy, C., Gia, K.N., Weintraub. A. Detection and characterization of diarrheagenic *Escherichia coli* from young children in Hanoi, Vietnam. *J. Clin. Microbiol.*, 43: 2005; 755–60.
- ❖ Nguyen, TV, Le Van, P, Le Huy, C, Gia, KN and Weintraub, A, Etiology and epidemiology of diarrhea in children in Hanoi, Vietnam. *J Infect dis*, vol. 10, pp. 2006; 298-308.
- ❖ Nishi J, Sheikh J, Mizuguchi K, Luisi B, Burland V, Boutin A, et al. The Export of Coat Protein from Enterohaggregative *Escherichia coli* by a Specific ATP-binding Cassette Transporter System. *J Biol Chem* 2003; 278: 45680-45689.

References

- ❖ Nowicki B, Barrish JP, Korhonen T, Hull RA, Hull SI. Molecular cloning of the *Escherichia coli* O75X adhesin. *Infect Immun* 1987; 55: 3168-3173.
- ❖ Nowicki B, Labigne, A, Moseley S, Hull R, Hull S, Moulds J. The Dr hemagglutinin, afimbrial adhesins Afa-I and Afa-III, and F1845 fimbriae of uropathogenic and diarrhea-associated *Escherichia coli* belong to a family of hemagglutinins with Dr receptor recognition. *Infect Immun* 1990; 58: 279-281.
- ❖ Nunes Mdo, R., Magalhaes, P.P., Macedo Ada, S., Franco, R.T., Penna, F.J., Mendes, E.N., Attaching and effacing *Escherichia coli* and Shiga toxin-producing *E. coli* in children with acute diarrhea and controls in Teresina/PI, Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 106, 2012; 43-47.



- ❖ Ochman H, Selander RK. Standard reference strains of *Escherichia coli* from natural populations. *J Bacteriol* 1984;157:690-693.
- ❖ Ochoa TJ, Barletta F, Contreas C, Mercado E. New insight into the epidemiology of enteropathogenic *Escherichia coli* infection. *Trans R Soc Trop Med Hyg* 2008; 102: 852-856.
- ❖ Oliveira MG, Pessoa GVA, Nakahara LK. Enteropathogenic bacteria occurrence in diarrheic children living in Juiz de Fora municipality Minas Gerais Brazil. *Rev Ins Adolfo Lutz*. 1989; 49: 161-168.
- ❖ Oliver, S. P., Jayarao, B. M. and Almeida, R. A. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. *Food borne Pathogenic Dis.* 2(2): (2005) ; 115-129.
- ❖ Olney JW. New insights and new issues in developmental neurotoxicology. *Neurotoxicology*. 23: 2002; 659-668.
- ❖ Orskov F, Orskov I, Evans Jr DJ, Sack RB, Wadstorm T. Special *Escherichia coli* serotypes among enterotoxigenic strains from diarrhea in adults and children. *Med Microbiol Immunol* 1978; 162: 73-80.

References

- ❖ O'Ryan, M, Lucero, Y, O'Ryan-Soriano, MA and Ashkenazi, S, An update on management of severe acute infectious gastroenteritis in children, *Expert Rev Anti Infect* , vol. 8, pp. 2010; 671-682.
- ❖ Osaili, M., Alaboud, R. and Rahahlah, M.: Prevalence and antimicrobial susceptibility of *E. coli* O157:H7 on beef cattle slaughtered in Amman abattoir. *Meat.Sci.*, 93: (2013); 463-468.



- ❖ Panchuk-Voloshina, N., Haugland, R.P., Bishop-Stewart, J., Bhalgat, M.K., Millard, P.J., Mao, F., Leung, W.-Y., Haugland, R.P., Alexa dyes, a series of new fluorescent dyes that yield exceptionally bright, photostable conjugates. *J. Histochem. Cytochem.* 47, 1999; 1179-1188.
- ❖ Paneto, B. R., Schocken-Iturrino, R. P., Macedo, C., Santo, E. and Marin, J. M. Occurrence of toxigenic *Escherichia coli* in raw milk cheese in Brazil. *Arq. Bras. Med. Vet. Zootec.* 59: (2007) ; 508-512.
- ❖ Parsot C. *Shigella* spp. and enteroinvasive *Escherichia coli* pathogenicity factors. *FEMS Microbiol Lett* 2005; 252: 11-18.
- ❖ 146- Sansonetti, PJ. *Escherichia coli*, *Shigella*, antibiotic associated diarrhea, and prevention and treatment of gastroenteritis. *Curr Opin Infect Dis* 1992; 5: 66-73.
- ❖ Paton, A.W., Paton, J.C., Detection and characterisation of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx1*, *stx2*, *eaeA*, enterohaemorrhagic *E. coli* *hlyA*, *rfbO111*, and *rfbO157*. *J. Clin. Micro.* 36, 1998; 598–602.
- ❖ Pejman Abbasi; Mohammad Kargar, Abbas Doosti , Jalal Mardaneh, Mohammad Ali, Dehyadegari , Sadegh Ghorbani-Dalini. Multiplex Real-Time PCR Assay for the Detection of LT, STIa and STIb Genes in Enterotoxigenic *Escherichia coli*. 2013.
- ❖ Peng J, Yang J, Jin Q. The molecular evolutionary history of *Shigella* spp. and enteroinvasive *Escherichia coli*. *Infect Genet Evol* 2009; 9: 147-152.
- ❖ Pereira, AC, Britto-Filho, JD, Jose de Carvalho, J, de Luna, MG and Rosa, AC, Enterotoxigenic *Escherichia coli* (EPEC) strains

References

- enter and survive within cultured intestinal epithelial cells, *Microb Pathog*, vol. 45, pp. 2008; 310-314.
- ❖ Peter, A.C. Method available for the detection of E.coliO157 in clinical food and environmental samples. *World J. Microbiology and Biotechnology*. 16, (2000); 733-740.
 - ❖ PHAC. Public Health Agency of Canada,. Annual Summary of Laboratory Surveillance Data. Last accessed March 5, 2013 http://www.nml-lnm.gc.ca/NESP-PNSME/surveillance-2009-eng.html#_Toc300651044.
 - ❖ PHAC. Public Health Agency of Canada,. Annual Summary of Laboratory Surveillance Data. Last accessed March 5, 2013 http://www.nml-lnm.gc.ca/NESP-PNSME/surveillance-2010-eng.html#_Toc300651044.
 - ❖ Picard B, Garcia JS, Gouriou S, et al. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. *Infect Immun* 1999;67:546-553.
 - ❖ Presterl E, Zwick RH, Reichmann S, Aichelburg A, Winkler S, Kreamsner PG, et al. Frequency and virulence properties of diarrheagenic *Escherichia coli* in children with diarrhea in Gabon. *Am J Trop Med Hyg*. 2003;69(4):406–10.



- ❖ Qadri F, Svennerholm A-M, Faruque ASG, Sack RB. Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment and prevention. *Clin Microbiol Rev*. 2005; 18; 465-483.
 - ❖ Quinn,P.J., Carter,M.E., Markey, B and carter.G.R.: *Enterobacteriaceae*.In:*Clinical Veterinary Microbiology*. .(2002); 209-236.
- 38- Frenzen, P. and Drake, A.: Economic Cost of Illness due to *Escherichia coli* O157 infections in the United States. *J. FoodProt.*,68: (2005); 2623-2630.

References



- ❖ Rahimi, E., Momtaz, H., Mohammad, M., Hosseini, A., Alimoradi, M., Momeni, M., Riahi, M.: Isolation and genomic characterization of *Escherichia coli* O157:H7 and *Escherichia coli* O157:H7 in minced meat and some traditional dairy products in Iran. *African Journal of Biotechnology*. 11(9): (2012); 2328-2332.
- ❖ Rajeev K , Amit P . Detection of *E. coli* and *Staphylococcus* in milk and milk products in and around Pantnagar .*Vet World* 2010;3(11):495-6.
- ❖ Rajendran, P., Ajjampur, S.S., Chidambaram. D., Chandrabose, G., Thangaraj, B., Sarkar, R., et al. Pathotypes of diarrheagenic *Escherichia coli* in children attending a tertiary care hospital in South India. *Diagn. Microbiol. Infect. Dis.*, 68: 2010; 117–22.
- ❖ Rappelli, P., Folgo,sa , E, Solinas , ML, DaCosta , JL and Colombo, MM, Pathogenic enteric *Escherichia coli* in children with and without diarrhea in Maputo, Mozambique , *FEMS Immun and Medical Microbiol*, vol. 43, pp. 2005; 67-72.
- ❖ Rappelli, P., G. Maddau, F. Mannu, M.M. Colombo, P.L. Fiori and P. Cappuccinelli, . Development of a set multiplex PCR for the simultaneous identification of enterotoxigenic, enteropathogenic, enterohemorrhagic and enteroinvasive *Escherichia coli*. *Microbiologica*. 24: 2001; 77-83.
- ❖ Regua-Mangia, A.H., Gomes, T.A., Vieira, M.A., Irino, K., Teixeira, L.M., Molecular typing and virulence of enteroaggregative *Escherichia coli* strains isolated from children with and without diarrhea in Rio de Janeiro city, Brazil. *Journal of Medical Microbiology* 58, 2009; 414-422.
- ❖ Reuben, R. and Owuna, G.: Antimicrobial resistance patterns of *E. coli* O157:H7 from Nigerian fermented milk samples in Nasarawa state, Nigeria. *Int. J. Pharma. Sci. Invention.*, 2: (2013); 2319–6718.
- ❖ Riss TL and Moravec RA. Use of multiple assay endpoint to investigate the effects of incubation time, dose of toxin, and plating

References

- density in cell based cytotoxicity assays. *Assay Drug Dev Technol.* 2: 2004; 51-62.
- ❖ Roca-Fernández, A.I. Sustainable Milk Production Systems in Humid Areas Using Farm Resources. Ph.D. Thesis, Universidad de Santiago de Compostela, Santiago de Compostela. (2011).
 - ❖ Rodriguez-Lazaro D, Hernandez M Isolation of *Listeria monocytogenes* DNA from meat products for quantitative detection by real-time PCR. *J Rapid Method Automat Microbiol* 14: (2006) ; 395–404.
 - ❖ Rosales RL, Arimura K, Takenaga S and Osame M. Extrafusal and intrafusal muscle effects in experimental botulinum toxin A injection. *Muscle Nerve.* 19: 1996; 488-496.
 - ❖ Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. *Microbes Infect* 2003;5:449-456.
 - ❖ Russo TA, Johnson JR. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli* ExPEC. *J Infect Dis* 2000;181:1753-1754.32-Ochman H, Selander RK. Standard reference strains of *Escherichia coli* from natural populations. *J Bacteriol* 1984;157:690-693.
 - ❖ Russo TA, Diseases caused by gram-negative enteric bacteria, *J Clin. Microbiol*, vol. 13, pp. 2006; 1-5 .
 - ❖ Ryser ET , Public health concerns . In : Marth EH, Steele JL , editors . *Applied dairy microbiology* . New York : Marcel Dekker , Inc ;1998 . P. 263 – 403.



- ❖ S.A. Fazlani, S.A. Khan, S. Faraz, M.S. Awan, Antimicrobial susceptibility of bacterial species identified from mastitic milk samples of camel, *Afr. J. Biotechnol.* 10(2011) 2959-2964.

References

- ❖ Sack SB. The discovery of cholera-like enterotoxins produced by *Escherichia coli* causing secretory diarrhea in humans. *Indian J Med Res* 2011; 133: 171-178.
- ❖ Salmanzadeh-Ahrabi S, Habibi E, Jaafari F, Zali MR. Molecular epidemiology of *Escherichia coli* diarrhoea in children in Tehran. *Ann Trop Med* 2005; 25: 35-39.
- ❖ Samira Badri, Ingrid Filliol, Isabelle Carle, Mohammed Hassar, Aziz Fassouane, Nozha Cohen. Prevalence of virulence genes in *Escherichia coli* isolated from food in Casablanca (Morocco). 2008.
- ❖ Samuel R, March SB, Ahmed R, Bezanson GS and Kasatiya S. Characterization of *Escherichia coli* Serotype O157:H7. *J Clin Microbiol.* 26: 1988; 2006-2012.
- ❖ Sansonetti, PJ, Molecular and cellular biology of *Shigella flexneri* invasiveness: from cell assay systems to shigellosis, *Curr Top Microbiol Immunol*, vol. 180, pp. 1992; 1-19.
- ❖ Savarino SJ, McVeigh A, Watson J, Cravioto A, Molina J, Echeverria P, et al. Enteraggregative *Escherichia coli* heat-stable enterotoxin is not restricted to enteraggregative *E. coli*. *J Infect Dis* 1996; 173: 1019-1022.
- ❖ Scaletsky IC, Silva ML, Trabulsi LR. Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells. *Infect Immun* 1984; 45: 534-536.
- ❖ Scaletsky ICA, Aranda KRS, Souza TB, Silva NP. Adherence factors in atypical enteropathogenic *Escherichia coli* strains expressing the localised adherence-like pattern in Hep-2 cells. *J Clin Microbiol* 2010; 48: 302-306.
- ❖ Scaletsky, IC, Fabbriotti, SH, Aranda, KR, Morais, MB and Neto, U, Comparison of DNA hybridization and PCR assays for detection of putative pathogenic enteroadherent *Escherichia coli*, *J Clin Microbiol*, vol. 40, pp. 2002; 1254-1258.
- ❖ Scavia, G., Staffolani, M., Fisichella, S., Striano, G., Colletta, S., Ferri, G., Escher, M., Minelli, F., Caprioli, A., Enteraggregative *Escherichia coli* associated with a foodborne outbreak of gastroenteritis. *Journal of Medical Microbiology* 57, 2008; 1141-1146.

References

- ❖ Schmidt H, Knop C, Franke S, Aleksic S, Heeseman J, Karch H. Development of PCR for screening of enteroaggregative *Escherichia coli*. *J Clin Microbiol* 1995; 33: 701-705.
- ❖ Schmidt MA. LEEways: tales of EPEC, ATEC and EHEC. *Cell Microbiol* 2010; 12: 1544-1552.
- ❖ Schubert S, Rakin A, Karch H, Carniel E, Heesemann J. Prevalence of the “high-pathogenicity island” of *Yersinia* species among *Escherichia coli* strains that are pathogenic for humans. *Infect Immun* 1998; 66: 480-485.
- ❖ Schultsz C, Pool GJ, van Ketel R, de Wever B, Speelman P, Dankert J. Detection of enterotoxigenic *Escherichia coli* in stool samples by using nonradioactively labeled oligonucleotide DNA probes and PCR. *J Clin Microbiol* 1994; 32: 2393-2397.
- ❖ Scotland, S.M., G.A. Willshaw, H.R. Smith and B. Rowe. Properties of strains of *Escherichia coli* O26:H11 in relation to their enteropathogenic or enterohemorrhagic classification. *J. Infect. Dis.* 162: 1990; 1069-1074.
- ❖ Scotter, S.; Aldridge, M. ; Capps, K. Validation of Method for the detection of *E.coli* O157:H7 in food . *Food control* , 11, (2000); 85-95.
- ❖ Servin A. Pathogenesis of Afa/Dr diffusely adhering *Escherichia coli*. *Clin Microbiol Rev.* 2005; 18: 264-292.
- ❖ Servin AL. Pathogenesis of Afa/Dr diffusely adhering *Escherichia coli*. *Clin Microbiol Rev.* 18: 2005; 264-292.
- ❖ Shaheen, H.I., Abdel Messih, I.A., Klena, J.D., Mansour, A., El-Wakkeel, Z., Wierzba, T.F., Sanders, J.W., Khalil, S.B., Rockabrand, D.M., Monteville, M.R., Rozmajzl, P.J., Svennerholm, A.M., Frenck, R.W., Phenotypic and genotypic analysis of enterotoxigenic *Escherichia coli* in samples obtained from Egyptian children presenting to referral hospitals. *J. Clin. Microbiol.* 47, 2009; 189-197.
- ❖ Shahrokhi N, Bouzari S, Jafari A. Comparison of virulence markers and antibiotic resistance in enterotoxigenic *Escherichia coli* isolated ten years apart in Tehran. *J Infect Dev Ctries* 2011; 5: 248-254.
- ❖ Sharada, R., Ruban, S.W. and Waran, M.T. Isolation, characterization and antibiotic resistance pattern of *Escherichia coli*

References

- isolated from poultry. American-Eurasian J Scientific Research.5: (2010); 18-22.
- ❖ Sharifi-Yazdi, M.K., A. Akbari and M.M. Soltan Dallal, Multiplex Polymerase Chain Reaction (PCR) assay for simultaneous detection of Shiga-like toxin (Stx1 and Stx2), intimin (eae) and invasive plasmid antigen H (ipaH) genes in diarrheagenic *Escherichia coli*. Afr. J. Biotechnol., 10: 2011; 1522-1526.
 - ❖ Sheehan, W.J. and Phipatanakul, W. Tolerance to Water Buffalo Milk in a Child with Cow Allergy. Annals of Allergy, Asthma & Immunology, (2009) ; 102-349. [http://dx.doi.org/10.1016/S1081-1206\(10\)60342-0](http://dx.doi.org/10.1016/S1081-1206(10)60342-0).
 - ❖ Sheikh J, Hicks S, Dall'Agnol M, Phillips AD, Nataro JP. Roles for Fis and YafK in biofilm formation by enteroaggregative *Escherichia coli*. Mol Microbiol 2001; 41: 983-997.
 - 166- Sheikh J, Czczulin JR, Harrington S, Hicks S, Henderson IR, Le Bouguénec C, et al. A novel dispersin protein in enteroaggregative *Escherichia coli*. J Clin Invest 2002; 110: 1329-1337.
 - ❖ Silva RM, Toledo MR, Trabulsi LR. Biochemical and cultural characteristics of invasive *Escherichia coli*. J Clin Microbiol 1980; 11: 441-444.
 - ❖ Singh, P. and Prakash, A. Isolation of *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* from milk products sold under market conditions at agra region. Acta. Agriculturae Slovenica. 92(1): (2008); 83–88.
 - ❖ Smith HR, Scotland SM. Isolation and identification methods for *Escherichia coli* O157 and other Vero cytotoxin producing strains. J Clin Pathol 1993; 46: 10-17.
 - ❖ Sommerfelt H, Steinsland H, Grewal HM, Viboud GI, Bhandari N, Gaastra W et al. Colonization factors of enterotoxigenic *Escherichia coli* isolated from children in north India. J Infect Dis 1996; 174: 768-776.
 - ❖ Soomro AH , Arain MA , Khaskheli M , Bhutto B . Isolation of *Escherichia coli* from raw milk and milk products in relation to public health sold under market conditions at Tandojam . pak J Nut 2002;1:151-2.

References

- ❖ Soomro, A. H., Arain, M. A., Khaskheli, M. and Bhutto, B. Isolation of *Escherichia coli* from raw milk and milk products in relation to public health sold under market condition at Tandojam. *Pak. J. Nutr.* 13: (2002) ; 151–152.
- ❖ Spencer PS, Schaumburg HH and Ludolph AC. (eds). *Experimental and Clinical Neurotoxicology*, pp 2000; 13-10. Oxford University Press.
- ❖ Srinivasan, V., Nguyen, L., Headrick, S., Murinda, S. and Oliver, S.: Antimicrobial resistance patterns of Shiga toxin-producing *E. coli* O157:H7 and O157:H7 from different origins. *Microbiol. Drug Resist.*, 13: (2007); 44-51.
- ❖ Srinu, B., Vijaya Kumar, A., Shashi Kumar, M., Narayana, B. V. L. and Madhava Rao T. Assessment of microbiological quality and associated health risks of raw milk sold in and around Hyderabad city. *Int. J. Pharm. Bio. Sci.* 3(4): (2012); 609-614.
- ❖ Staley TE, Jones EW, Corley LD. Attachment and penetration of *Escherichia coli* into intestinal epithelium of the ileum in newborn pigs. *Am J Pathol* 1969; 56: 371-392.
- ❖ Stannard C . Development and use of microbiological criteria for foods. *Food Sci Technol Today* 11: (1997); 137–177.
- 14- Jasson V, Jacxsens L, Luning P, Rajkovic A, Uyttendaele M Review. Alternative microbial methods: An overview and selection criteria. *Food Microbiol* 27: (2010) ; 710–730.
- ❖ Stapleton A, Moseley S, Stamm WE. Urovirulence determinants in *Escherichia coli* isolates causing first episode and recurrent cystitis in women. *J Infect Dis* 1991; 163: 773-779.
- ❖ Stephan, A.; Enabulet , I. ; Nduka, U. Enterohaemorrhagic *E. coli* O157:H7 prevalence in meat vegetables sold in Benin. *Clin. Microbiol. Infect.*, 55, (2009); 264-279.
- ❖ Stipcevic T, Piljac T and Isseroff RR. Di-rahmanolipid from *Pseudomonas aeruginosa* displays differential effects on human keratinocyte and fibroblast cultures. *J Dermatol Sci.* 40: 2005; 141-143.
- ❖ Svenungsson B, Lagergren A, Ekwall E, et al. Enteropathogens in adult patients with diarrhea and healthy control subjects: a 1-year prospective study in a Swedish clinic for infectious diseases. *Clin Infect Dis.* 2000;30(5):770-778.

References



- ❖ Tapping RI, Akashi S, Miyake K, Godowski PJ and Tobias PS.. Toll-like receptor 4, but not toll-like receptor 2, is a signaling receptor for Escherichia and Salmonella lipopolysaccharides. J Immunol. 165: 2000; 5780-5787.
- ❖ Taye, M, Berhanu. T, Berhanu .Y, Tamiru,F. and Terefe. D: Studyon Carcass Contaminating Escherichia coli in Apparently Healthy SlaughteredCattle in Haramaya University Slaughter House with Special Emphasison Escherichia coliO157:H7. Ethiopia. J Veterinar Sci Technol. 4: (2013); 132.
- ❖ Taye, M., Tamiru, B., Yenehiwot, B., Firao, T. and Dechassa, T. Study on Carcass Contaminating Escherichia coli in Apparently Healthy Slaughtered Cattle in Haramaya University Slaughter House with Special Emphasis on Escherichia coli O157:H7, Ethiopia. J Veterinar Sci Technolo., 4: (2013); 1.
- ❖ Taylor DN, Escheverria P, Pal T, et al. The role of Shigella sp, enteroinvasive Escherichia coli, and other enteropathogens as causes of childhood dysentery in Thailand. J Infect Dis. 1986; 153: 1132-1138.
- ❖ Temelli, S., A. Eyigor and S. Anar,. Prevalence of Escherichia coli O157 in red meat and meat products determined by VIDAS ECPT and light cyclor PCR. Turk J. Vet. Anim. Sci., 36: 2012; 305-310.
- ❖ Tieng V, Bougue' nec CL, Du Merle L, Bertheau P, Desreumaux P, Janin A, Charron D and Toubert A. Binding of Escherichia coli adhesin AfaE to CD55 triggers cell-surface expression of the MHC classI-related molecule MICA. Proc Natl Acad Sci. 99: 2002; 2977-2982.
- ❖ Toma, C, Lu, Y, Higa, N, Nakasone, N, Rivas, M and Iwanaga, M. Multiplex PCR Assay for Identification of Human Diarrheagenic Escherichia coli, J Clin Microbiol, vol. 41, no. 6, pp. 2003; 2669-2671.
- ❖ Torkar, K. G. and Teger S. G. The Microbiological quality of raw milk after introducing the two day's milk collecting system. Acta Agri. Slovenica. 92(1): (2008) ; 61–74.

References

- ❖ Trabulsi LR, Keller R, Gomes TAT. Typical and atypical enteropathogenic *Escherichia coli*. *Emerg Infect Dis* 2002; 8: 508-513.
- ❖ Trabulsi, L.R., Keller, R., Gomes, T.A.T.,. Typical and atypical enteropathogenic *Escherichia coli*. *Emerging Infectious Disease* 2002; 5, 508-513.



- ❖ Usein CR, Tatu-Chitoiu D, Ciontea S, Condei M. Damian M. *Escherichia coli* pathotypes associated with diarrhea in Romanian children younger than 5 years of age. *Jpn J Infect Dis*. 2009;62(4):289-293.
- ❖ Utsunomiya A, Elio-Calvo D, Reyes AA, et al. Major enteropathogenic bacteria isolated from diarrheal patients in Bolivia a hospital-based study. *Microbiol Immunol*. 1995; 39: 845-851.
- ❖ Uttley AH, Collins CH, Naidoo Jand George RC. 1998. Vancomycin- resistant enterococci. *Lancet*. I: 1998; 57-58.



- ❖ Valentini SR, Gomes TAT, Falcao DP. Lack of virulence factors in *Escherichia coli* strains of enteropathogenic serogroups isolated from water. *Appl Environ Microbiol*. 1992; 58: 412-414.
- ❖ Vallance, B.A., Finlay, B.B., 2000. Exploitation of host cells by enteropathogenic *Escherichia coli* . *Proc. Natl. Acad. Sci*. 97(16), 2000. 8799-8806.
- ❖ Van den Beld MJC, Reubsaet FAG. Differentiation between *Shigella*, enteroinvasive *Escherichia coli* (EIEC) and noninvasive *Escherichia coli*. *Eur J Clin Microbiol Infect Dis* 2012; 31: 899-904.
- ❖ Varela-Hernandez, J.J., Cabrera-Diaz, E., Cardona-Lopez, M.A., Ibarra-Velazquez, L.M., Rangel-Villalobos, H., Castillo, A., Torres-Vitela, M.R., Ramirez-Alvarez, A., Isolation and characterization of Shiga toxin-producing *Escherichia coli*

References

- O157:H7 and non-O157 from beef carcasses at a slaughter plant in Mexico. *International Journal of Food Microbiology* 113, 2007; 237-241.
- ❖ Veilleux S, Dubreuil D . Presence of *Escherichia coli* carrying the EAST1 toxin gene in farm animals. *Vet. Res.* 37: (2005); 3-13.
 - ❖ Venkatesan MM, Buysse JM, Kopecko DJ. Use of *Shigella flexneri* ipaC and ipaH gene sequences for the general identification of *Shigella* spp and enteroinvasive *Escherichia coli*. *J Clin Microbiol* 1989; 27: 2687-2691.
 - ❖ Viazis S, Diez-Gonzalez F. Enterohemorrhagic *Escherichia coli*: the twentieth century's emerging foodborne pathogen: a review. *Adv Agronom* 2011; 111: 1-50.
 - ❖ Viboud GI, Jouve MJ, Binsztein N, Vergara M, Rivas M, Quiroga M, et al. Prospective cohort study of enterotoxigenic *Escherichia coli* infections in Argentinean children. *J Clin Microbiol.* 1999;37(9):2829-2833.
 - ❖ Voetsch, AC, Angulo, FJ, Rabatsky-Her, T, et al. 2004, Laboratory practices for stool specimen culture for bacterial pathogens, including *Escherichia coli* O157:H7, in the Food Net sites, 1995-2000, *Clin Infect Dis* , vol. 38, pp. 190-7 .



- ❖ Wakimoto N, Nishi J, Sheikh J, Nataro JP, Sarantuya J, Iwashita M, et al. Quantitative biofilm assay using a microtiter plate to screen for enteroaggregative *Escherichia coli*. *Am J Trop Med Hyg* 2004; 71: 687-690.
- ❖ Wei. Z, Weihong.Q, Thomas, J. A, Alifiya.S.M, David.A, Eija. K.H,Efrain. M.R, Patricia. I.F, Thomas .S.W and Bala.S. Probing genomic diversity and evolution of *Escherichia coli* O157 by single nucleotide polymorphisms. *Cold Spring Harbor Laboratory.*16: (2006); 757–767.
- ❖ Wei. Z, Weihong.Q, Thomas, J. A, Alifiya.S.M, David.A, Eija. K.H,Efrain. M.R, Patricia. I.F, Thomas .S.W and Bala.S. Probing genomic diversity and evolution of *Escherichia coli* O157 by single nucleotide polymorphisms. *Cold Spring Harbor Laboratory.*16: (2006); 757–767.

References

- ❖ Weintraub A. Enteroaggregative *Escherichia coli*: epidemiology, virulence and detection. *J Med Microbiol* 2007; 56: 4-8.
- ❖ Wellington EMH and van Elsas JD. Genetic interactions between microorganisms in the natural environment Editors: Pergamon Press.USA. 1992.
- ❖ Werber, D., Mason, B.W., Evans, M.R. and Salmon, R.L. Preventing household transmission of Shiga toxin-producing *Escherichia coli* O157 infection: promptly separating siblings might be the key. *Clin Infect Dis*. 2008; 46(8):1189-96.
- ❖ Wiedermann U and Kollaritsch H. Vaccines against traveler's diarrhea and rotavirus disease – a review. *Wein Klin Wochenscher* 2006; 118: 2-8.
- ❖ Winfield, M.D. and E.A. Groisman. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Appl. Environ. Microbiol.* 69: 2003; 3687-3694.
- ❖ Wolk M, Ohad E, Shafran R, et al. Epidemiological aspects of enterotoxigenic *Escherichia coli* diarrhoea in infants in the Jerusalem area. *Public Health Rev.* 1995;23(1):25-33.
- ❖ world health organization.bridging the gaps 20- Alikhani MY, Mirsalehian A, Aslani MM. Detection of typical and atypical enteropathogenic *Escherichia coli* (EPEC) in Iranian children with and without diarrhoea. *J Med Microbiol.* 2006;55((Pt9)).
- ❖ Wu C-J, Hsueh P-R, Ko W-C. A new health threat in Europe: Shiga toxin producing *Escherichia coli* O104:H4 infections. *J Microbiol Immunol Infect* 2011; 44: 390-393.



- ❖ Xicohtencatl-Cortes J, Monteiro-Neto V, Saldaña Z, Ledesma MA, Puente JL, Girón JA. The type 4 Pili of enterohemorrhagic *Escherichia coli* O157:H7 are multipurpose structures with pathogenic attributes. *J Bacteriol* 2009; 191: 411-421.

References



- ❖ Yamazaki Y, Fukasawa A. Multiplex polymerase chain reaction method discriminating *Escherichia coli* and *Shigella* sp. *Arch Microbiol* 2011; 193: 83-87. 108. Pavlovic M, Luze A, Konrad R, Berger A, Sing A, Busch U, Huber I. Development of a duplex real-time PCR for differentiation between *E. coli* and *Shigella* spp. *J Appl Microbiol* 2011; 110: 1245-1251.
- ❖ Yatsuyanagi, J., Saito, S., Miyajima, Y., Amano, K., Enomoto, K., Characterization of atypical enteropathogenic *Escherichia coli* strains harboring the *astA* gene that were associated with a waterborne outbreak of diarrhea in Japan. *Journal of Clinical Microbiology* 41, 2003; 2033-2039.
- ❖ Yolken RH, Greenberg HB, Merson MH, Sack RB, Kapikian AZ. Enzyme-linked immunosorbant assay for detection of *Escherichia coli* heat-labile enterotoxin. *J Clin Microbiol* 1977; 6: 439-444.



- ❖ Zhang W, Bielaszewska M, Kuczius T, Karch H. Identification, characterization, and distribution of a Shiga toxin 1 gene variant (*stx1c*) in *Escherichia coli* strains isolated from humans. *J Clin Microbiol* 2002; 40: 1441-1446.
- ❖ Zhang Y, Laing C, Steele M, Ziebell K, Johnson R, Benson AK, et al. Genome evolution in major *Escherichia coli* O157:H7 lineages. *BMC Genomics* 2007; 8: 121.

الخلاصة

اجريت الدراسة لعزل بكتريا الايشريشيا القولونية المسببة للإسهال من اللحوم النيئة والحليب والجبن، والكشف عن جينات الضراوة باستخدام تفاعل البلمرة المتسلسلة والبحث عن انماط حساسيتها للمضادات الحيوية. تم جمع 300 عينة تتألف من 100 لحوم نيئة، 100 حليب خام، 100 جبن، جمعت من باعة الحليب، ومحللات الجزارة الواقعة حول ذي قار، تحت الاحتياطات المعقمة. لاغناء الكائن من العينات التي تم جمعها، تم استخدام وسط MacConkey broth وتم اجراء التلقيح على وسط MacConkey agar و EMB. لاحقا، لتأكيد العزلات اجريت مجموعة من الاختبارات الكيموحيوية على سبيل المثال اختبار IMViC، اختبار urease، واختبار نظام Api-20E. نمط حساسية الايشريشيا القولونية للمضادات الحيوية تم تقييمها بواسطة طريقة نشر القرص. اخيرا عزلت الايشريشيا القولونية تم فحصها للبحث عن جينات الضراوة باستخدام تقنية تفاعل البلمرة المتسلسلة. لوحظ انتشار الايشريشيا القولونية بنسبة 16.81% في العينات وبواقع 25% في اللحم، 17% في الحليب، 13% في الجبن. الحساسية للمضادات سجلت بنسبة عالية لمضاد الامبيسيم بنسبة 100%، تبعه مضاد سيفالوثين 100%، كلورامفينيكول 100%، نتيلمايسين 100%، تتراسايكلين 81.8%، سايبيروفلوكساسين 72.27%، جنتاميسين 87.27%، حامض النالديسيك 56.36%. بينما هذه المضادات الاخيرة سجلت فيها نسبة مقاومة وهي الامبيسيلين 67.27%، السيفوتاكزام 45.45%، السيفترايكون 66.6%. في هذه الدراسة، من بين 55 عزلة للايشريشيا القولونية كان هناك 7 عزلة بنسبة 12.7% موجبة لجين *rfbO157*، منها 4 عزلات بنسبة 16% كانت ايجابية للحوم، في حين كانت 2 عزلات بنسبة 11.7% ايجابية للحليب، و 1 عزلات بنسبة 7.6% ايجابية للجبن. 18 عزلة بنسبة 32.72% موجبة لجين *eaeA* : من بينها 6 عزلات بنسبة 24% موجبة للحم، و 5 عزلات بنسبة 29.41% موجبة للحليب، و 7 عزلات بنسبة 53.84% موجبة للجبن. 8 عزلات بنسبة 14.5% موجبة لجين *bfpA* : منها 2 بنسبة 8% موجبة للحم، و 2 بنسبة 11.76% موجبة للحليب، و 4 عزلات بنسبة 30.76% موجبة للجبن. 7 عزلة بنسبة 12.72% موجبة لجين *ial* : منها 2 عزلات بنسبة 8% موجبة للحم، و 2 عزلات بنسبة 11.7% موجبة للحليب، و 3 عزلات بنسبة 23.07% موجبة للجبن. 6 عزلة بنسبة 10.90% موجبة لجين *CVD432* : منها 2 عزلات بنسبة 8% موجبة للحم، و 2 عزلات بنسبة 11.7% موجبة للحليب، و 2 عزلات بنسبة 15.3% موجبة للجبن. 9 عزلة بنسبة 16.36% موجبة لجين

STLa : منها 3 عزلة بنسبة 12% موجبة للحم , و 3 عزلات بنسبة 17.6% موجبة للحليب , و
3 عزلات بنسبة 23.07% موجبة للجبن.



وزارة التعليم العالي والبحث العلمي

جامعة ذي قار

كلية العلوم

قسم علوم الحياة

عزل وتشخيص بكتريا الايشريشيا القولونية المسببة للاسهال
من اللحم الخام, الحليب, الجبن والكشف عن بعض عوامل
الضراوة بتقنية تفاعل البلمرة المتسلسل في ذي قار

رسالة مقدمة إلى

مجلس كلية العلوم - جامعة ذي قار

وهي جزء من متطلبات نيل درجة ماجستير علوم في علوم الحياة

من قبل

أثمار ظاهر مطشر

بكالوريوس علوم حياة

2007

أشرف

الاستاذ المساعد الدكتور

راجي طعمه ناصر

1437 هجري

2017 ميلادي