UNIVERSITI PUTRA MALAYSIA

A COMPARATIVE STIJDY ON TIIE MOLECULAR CHARACTERIZATION OF E. COLI 0157 ISOLATED FROM IMPORTED BEEF IN MALAYSIA AND IN LOCAL AND IMPORTED LAMB IN UNITED ARAB EMIRATES

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By

SARA ABDALLAH A. ABUDAFEERA

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

April 2004

UPM

DEDICATION

This piece of work is dedicated to my great parents, sisters, brothers and my dear husband, not forgetting my lovely nephew and nieces, Mahmoude, Samah and Mozan

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

A COMPARATIVE STUDY ON THE MOLECULAR CHARACTERIZATION OF *E. COLI* O157 ISOLATED FROM IMPORTED BEEF IN MALAYSIA AND IN LOCAL AND IMPORTED LAMB IN UNITED ARAB EMIRATES

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April 2004

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This study was conducted to determine the incidence of *E. coli* O157 in beef in Malaysia and lamb in United Arab Emirates. A total of 113 samples of beef (n = 25) and lamb (n = 88) were examined for the presence of *E. coli* O157 which yielded 130 positive isolates for *E. coli* O157. All the isolates were found to be resistant to one or more of the fifteen antimicrobial agents tested. The strains isolated from lamb samples showed a high occurrence in multiple drug resistance. Plasmid analysis showed that most of the *E. coli* strains isolated from beef samples in Malaysia contained plasmids with size ranging from 1.3 to 60 MDa. The commonest plasmids of 60 MDa were observed in 31.8% of the strains. On the other hand, representative patterns for plasmid profiles among *Escherichia coli* O157 isolates from lamb possessed plasmid with molecular size ranging from 1.2 to 35.8 MDa. However, there did not appear to be a consistent relationship between a particular plasmid and resistance to an antibiotic. Two types of specific primer

encoding the Shiga-like toxins gene, the *SLT I* and *SLT II* gene were utilized in the multiplex PCR assay. Analysis carried out demonstrated that *Escherichia coli* O157 strains were positive for the presence of Shiga–like toxins (*stx*) genes. Thus, analysis of all the *E. coli* genome showed that most of isolates carried either both *SLT I* and *SLT II* genes or only the *SLT II* gene. RAPD-PCR was used to generate polymorphic genomic fingerprints to discriminate the *E. coli* O157 isolates. After a set of ten-mer oligonucleotides of 50% G+C contents were screened, primers Gen1-50-9 (5′AGAAGCGATG 3′) and Gen1-50-10 (5′CCATTTACGC 3′) were chosen whereby the primers generated reproducible and typeable results with bands ranging from 0.25 to 4.0 kilobase pairs. All the dendrogram were constructed utilizing the Gel Compar and RAPDistance software package based on the data retrieved from the presence or absence of banding pattern. Based on the dendrogram generated, there appears to be a genetic similarity at 70% among the strains isolated from beef and lamb. Two main groups were observed at 10% similarity level, group one consists of *E. coli* O157 isolates from imported beef in Malaysia and a few of isolates of *E. coli* O157 from imported lamb in UAE. On the other hand, group two consists mainly of eight isolates of *E. coli* O157 from imported lamb in UAE. In the present study, all the molecular typing techniques have been used generally provided an additional value for assisting the molecular characterization of isolates of *Escherichia coli* O157 utilized.

KAJIAN PERBANDINGAN TERHADAP PENCIRIAN MOLEKUL *E. COLI* O157 DALAM DAGING LEMBU DI MALAYSIA SERTA DAGING BIRI-BIRI TEMPATAN DAN YANG DI IMPORT DI UNITED ARAB EMIRATES

Oleh

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Pengerusi: **Profesor Madya Mariana Nor Shamsudin, Ph.D.**

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Kajian ini dijalankan untuk mengenalpasti kejadian *E. coli* O157 dari daging lembu dan kambing biri-biri di Malaysia dan Emiriyah Arab Bersatu (UAE). Sejumlah 113 sampel daging lembu (n=25) dan kambing biri-biri (n=88) yang diciri untuk kehadiran *E. coli* O157 menghasilkan 130 pencilan. Semua pencilan didapati rintang terhadap satu atau lebih antibiotik yang diuji. Pencilan daripada sampel kambing biri-biri UAE menunjukkan kerintangan yang tinggi kepada pelbagai antibiotik. Analisis plasmid menunjukkan kesemua pencilan *E. coli* daripada sampel daging lembu di Malaysia mengandungi plasmid yang bersaiz 1.3 ke 60 MDa. Plasmid 60 MDa adalah plasmid yang paling kerap ditemui dalam 31.8% pencilan. Sebaliknya, pencilan dari UAE masing masing mempunyai plasmid yang bersaiz 1.2 ke 35.8 Mda. Walau bagaimanapun, tidak wujud hubung kait yang konsisten di antara sesuatu plasmid dan rintang kepada satu antibiotik. Dua jenis spesifik primer untuk gen *SLT I* dan *SLT II* telah digunakan dalam multiplek PCR. Analisis yang dijalankan menunjukkan pencilan *E. coli* O157 adalah positif untuk kehadiran toksin *Shiga-like* (*stx*). Dengan itu, analisis untuk semua genom

E. coli menunjukkan kesemua pencilan membawa kedua-dua *SLT I* dan *SLT II* atau *SLT II* sahaja. RAPD-PCR telah digunakan untuk menghasilkan genomik polimorfik untuk membezakan pencilan *E. coli* O157. Selepas penjaringan satu set sepuluh-mer oligonucleotida yang mempunyai kandungan G+C 50%, primer Gen 1-50-9 (5′AGAAGCGATG 3′) dan Gen 1-50-10 (5′CCATTTACGC 3′) telah dipilih untuk menghasilkan keputusan bagi pencilan yang berjalur 0.25-4.0 kb. Kesemua dendrogram dihasilkan dengan menggunakun Gel-Compar dan RAPDistance software berdasarkan dengan hadir atau ketidakhadiran jalur. Berdasarkan dendrogram yang dihasilkan, terdapat 70% kesamaan genetik diantara pencilan daging lembu dan kambing biri-biri dari Malaysia dan UAE, masing-masing. Dua kumpulan utama telah diperhatikan mempunyai tahap kesamaan 10%, dimana kumpulan pertama terdiri dari *E. coli* O157 yang diperolehi dari daging lembu di Malaysia dan beberapa pencilan *E. coli* O157 yang diperolehi dari daging kambing biri-biri di UAE. Manakala, kumpulan kedua terdiri daripada lapan pencilan *E. coli* O157 yang diperolehi dari daging kambing biri-biri di UAE. Di dalam kajian ini, kesemua teknik pencirian yang digunakan telah membantu di dalam mencirikan semua pencilan *E. coli* O157.

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LIST OF ABBREVIATIONS

%	Percent
°C	degree Celsius
µg	Microgram
µl	Microliter
AOAC	Association Official Analytical Chemistry
CDC	Center for Disease Control
CEN	Comite European de Normalization (France)
CFU	Colony Forming Unit
CT-SMAC	Cefixime and Tellurite-Sorbitol MacConkey agar
DAEC	Diffuse-adhering *E. coli*
DH_2O	Distilled Water
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide Triphosphates
E. coli	*Escherichia coli*
EAEC	Enteroaggregative *E. coli*
EDTA	Ethylenediamine tetra-acetic acid
EHEC	Enterohemorrhagic *E. coli*
EIEC	Entroinvasive *E. coli*
EMB	Eosin Methylene Blue agar
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
FDA	Food and Drug Administration
HACCP	Hazard Analysis Critical Control Point
HCI	Hydrochloric acid
HUS	Haemolytic uraemic syndrome
ISO	International Organization for standardization
Kb	Kilobase pair (number of bases in thousands)
KCI	Potassium chloride
Kda	Kilodalton
Kg	Kilogram
Lab	Laboratory
LB	Luria Bertani
M	Molar or molarity
MAR	Multiple Antibiotic Resistant
MDa	MegaDalton
$MgCl_2$	Magnesium chloride
min	minute(s)
ml	milliliter
mm	millimeter
mM	milliMolar
NFLX	Norfloxacin
PCA	Plate count agar
PCI	Phenol-Chloroform-Isoamyl alcohol

PCR	Polymerase Chain Reaction
PFGE	Pulsed-field gel electrophoresis
R	Resistant
RAN	Ribonucleic acid
RAPD	Random Amplification of Polymorphic DNA
rpm	Revolution per minute
S	Susceptible
S.h	Slaughterhouse
SDS	Sodium dodecyl sulphate
SMAC	Sorbitol MacConkey agar
Spp.	Species
STEC	Shiga-Like Toxins *E. coli*
Stx	Shiga toxins
TBE	Tris borate EDTA electrophoresis buffer
TE	Tris-EDTA
Tris	Tris (hydroxymethyl) methylamine
TSB	Tryptone Soya Broth
TTP	Thrombotic thrombocytopenic purpura
UAE	United Arab Emirates
UV	Ultraviolet
V	Volts
VTEC	Vero cytotoxin producing *E. coli*

CHAPTER 1

INTRODUCTION

Escherichia coli (*E. coli*) forms part of the normal facultative anaerobic microflora in the intestinal tract of humans and warm-blooded animals. Most *E. coli* strains are harmless; however, some are pathogenic and cause diarrheal disease. Commensal strains of *E. coli* may perform essential functions for a host, but they can also cause fatal diseases. Strains of *E. coli* show a high degree of species specificity, and strains, which reside in the gut of one species, may cause fatal diseases in another. In common with many pathogenic bacteria, strains of *E. coli* must colonize a given host as a prelude to pathogenesis, and the receptors in the gut may dictate the degree of host species specificity. Once strains of *E. coli* have competed successfully with other gut microbes and are attached firmly to the intestinal mucosa, the strains produce an array of toxins to infect their host.

E. coli isolates are serologically differentiated based on three major surface antigens, which enable serotyping: the O (somatic (membrane proteins)), H (flagella), and K (capsule polysaccharides) antigens. At present, a total of 167 O antigens, 53 H antigens, and 74 K antigens have been identified (Lior *et al.*, 1994). Currently, it is considered necessary only to determine the O and the H antigens to serotype strains of *E. coli* associated with diarrheal disease. The O

antigen identifies the serogroup of a strain, while the H antigen identifies the serotype of a strain. Diarrheagenic *E. coli* isolates are categorized into specific groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes, and distinct O: H serotypes. These categories include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and enterohemorrhagic *E. coli* (EHEC).

Michael *et al.* (2001) prefer to reserve the term EHEC for those *E. coli* that cause more severe illnesses characterized by bloody diarrhea or the haemolytic uraemic syndrome (HUS). Enterohemorrhagic *E. coli* (EHEC) was first recognized as a cause of infectious diarrheal disease as a result of several outbreaks of severe bloody diarrhea (hemorrhagic colitis) in the early 1980s. Since then EHEC strains, particularly serotype O157, have been implicated worldwide in outbreaks of food and water borne disease in developed countries. EHEC belongs to a larger group of pathogenic strains known as Shiga toxin-producing *E. coli* (STEC), which are defined by their ability to produce Shiga toxins (Stx). (For historical reasons these same toxins are alternatively referred to as verotoxins and the organisms that produce them as VTEC). EHEC strains of serotype O157 have caused both the largest number of outbreaks and the outbreaks that have involved the greatest numbers of patients. Strains with this serotype have also caused the majority of sporadic STEC infections (Griffin *et al.*, 1991; Boyce *et al.*, 1995). EHEC isolates were first recognized as human

pathogens in 1982 when *E. coli* O157 was identified as the cause of two outbreaks of hemorrhagic colitis (Riley *et al.*, 1983). All EHEC strains produce factors cytotoxic to African green monkey kidney cells, and have been named verotoxins or Shiga toxins (*Stx*) because of similarity to the Shiga toxin produced by *Shigella dysenteria* type 1 (Calderwood *et al.*, 1996). EHEC strains associated with human disease have genetic properties in common. One of these properties is that the strains possess the *stx* genes coding for the Shiga toxins *Stx1* and *Stx2*, found on temperate lambdoid bacteriophages, in the bacterial chromosome (Francois *et al.*, 2001). Subsequently, associated with Shiga toxin-producing *E. coli* infections is a severe and sometimes fatal condition known as hemolytic-uremic syndrome (HUS).

Risk factors, which have been strongly associated with human infection, include the likelihood of contact with farm animals or their feces (Locking *et al.*, 2001) and the consumption of ground beef (Slutsker *et al.*, 1998). The abattoir is a major link in the transmission of *E. coli* O157 to the food chain and cross-contamination of the carcass with feces (Richards *et al.*, 1998), and the return of waste to the fields (Hepburn *et al.*, 2002; Jones 1999) is a major concern. Although EHEC infections are mostly foodborne, there have also been several waterborne outbreaks of *E. coli* O157:H7 (Swerdlow *et al.*, 1992; Ackman *et al.*, 1997). EHEC infections have been linked to a variety of products, including foods of animal origin, apple juice, apple cider, sprouts, and lettuce (Ackman *et al.*, 1997; Ackers *et al.*, 1998; Cody *et al.*, 1999; Michino *et al.*, 1999). *Stx*

production has been shown in *E. coli* strains from a large number of serotypes, isolated from a wide variety of animals, foods, and environmental samples (Seriwatana *et al.*, 1988; Sekla *et al.*, 1990; Suthienkul *et al.*, 1990; Wells *et al.*, 1991; Bell *et al.*, 1994; Samadpour *et al.*, 1994; Pradel *et al.*, 2000).

Currently accepted methods for the isolation of O157 serotype consist of assays for the detection of Shiga-like toxins (*SLTs*), either directly or at the genomic level. There are varieties of rapid methods that have been developed for the detection of *E. coli* O157. Molecular techniques of polymerase chain reaction (PCR) and DNA probes as a tool for detection of *E. coli* O157 have been developed. Fast laboratory tests are needed to facilitate early diagnosis, expedite outbreak investigations and establish the influence of SLT-EC and different serotypes carried in humans and animals. PCR methods have been developed to detect Shiga-like toxin gene sequences in SLT-EC (Ramotar *et al.*, 1995; Deng *et al.*, 1996; Tsen *et al.*, 1996; Alexa *et al.*, 1997). Typically, target PCR products are identified by standard gel electrophoresis using ethidium bromide to stain target DNA. Moreover, determination of plasmid profiles can aid in the differentiation of isolates and has been shown to be useful tool in investigating the epidemiology and histories of individual endemic strains.

For the past three decades, the role of antibiotics in promoting the emergence and spread of drug-resistant pathogenic bacteria, which infect humans through the food chain, has been a contentious issue in clinical microbiology and food

hygiene (Hayashi *et al.*, 1985). Antibiotic resistance can be transferred under appropriate conditions, from one organism to another, especially through the extra chromosomal plasmid. Cells indicating the existence of plasmids in *E. coli*, especially *E. coli* O157:H7 have been determined largely in strains from clinical materials (Xu *et al.*, 1990; O'Brien *et al.*, 1993). In view of the importance of *E. coli* O157 in human disease, molecular characterization techniques was carried out to biotype the isolates of *Escherichia coli* in this study.

The incidences of *E. coli* O157 in UAE is not well documented and not well understood. Since lamb is one of the major food in UAE, and beef is one of the major food in Malaysia, this research was undertaken to determine the presence of *E. coli* O157 in beef from Malaysia and lamb from United Arab Emirates. Identification of the source whether, human, animal or water of contamination of foods with *E. coli* O157 is important in understanding of the epidemiology of human infection and devising strategies for its control.