Investigation and Characterization of *Escherichia coli* O45, O103, O111 and O145 from Raw Meats, Hat-Yai City, Thailand

Aphisara Sae-lim

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Microbiology (International Program) Prince of Songkla University 2019

Investigation and Characterization of *Escherichia coli* O45, O103, O111 and O145 from Raw Meats, Hat-Yai City, Thailand

Aphisara Sae-lim

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Microbiology (International Program) Prince of Songkla University 2019

Thesis Title	Investigation and Characterization of *Escherichia coli* O45, O103, O111 and O145 from Raw Meats, Hat-Yai City, Thailand
Author	Miss Aphisara Sae-lim
Major Program	Microbiology (International Program)

Major Advisor

…………………………………………..

(Assoc. Prof. Dr. Pharanai Sukhumungoon)

Examining Committee：

.……………………….………Chairperson

(Dr. Saowapar Khianngam)

……………………...……Committee

(Dr. Rattanaruji Pomwised)

……………………...……Committee

(Assoc. Prof. Dr. Pharanai Sukhumungoon)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Science Degree in Microbiology (International Program)

………………………………………

(Prof. Dr. Damrongsak Faroongsarng)

Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

…………………….………Signature

(Assoc. Prof. Dr. Pharanai Sukhumungoon)

Major Advisor

……………………………….Signature

(Miss Aphisara Sae-lim)

Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

………………………….. Signature

(Miss Aphisara Sae-lim)

Candidate

Thesis Title	Investigation and Characterization of *Escherichia coli* O45, O103, O111 and O145 from Raw Meats, Hat-Yai City, Thailand
Author	Miss Aphisara Sae-lim
Major Program	Microbiology (International Program)
Academic Year	2018

ABSTRACT

Non-O157 Shiga toxin-producing *Escherichia coli* (STEC) are gradually become a major public health concern worldwide. One of the STEC potential vehicles carrying them to humans are raw meats. Thus, this current study investigates the STEC in an important serogroup, O45, O103, O111 and O145, from raw meats in southern Thailand by immunomagnetic separation technique (IMS). The prevalence of *E. coli* O45, O103, O111 and O145 were found to be 12.4%, 42%, 0%, and 3%, respectively. Most of them were not members of STEC including other five diarrheagenic *E. coli* (DEC) pathotypes except serogroup O145 that belonged to atypical enteropathogenic *E. coli* (aEPEC) and one O103 strain (strain 103.10) from chicken that identified to be extraintestinal pathogenic *E. coli* (ExPEC). Investigation of other virulence genes exhibited that *fimH*, responsible for bacterial adherence, was the gene that found in all strains in all serogroups while *astA* encoding for EAST-1 toxin was found in O45 and O103 as 63.2% and 20.8%, respectively. In addition, *lpf* encoding long polar fimbriae was found in O45 and O145 as 30.6% and 100%, respectively. Phylogenetic group analysis demonstrated that the majority of *E. coli* O45 belonged to group D (88%) followed by group A (8%) and B1 (4%) but none belonged to group B2. Contrary, *E. coli* O103 showed that 3%, 5%, 30% and 62% of strains belonged to group B2, D, B1 and A, respectively. While all *E. coli* O145 isolates belonged to phylogenetic group D. Intactness of stx_2 phage integration sites revealed that in O45 and O103, *sbcB* was occupied by some prophages in highest rate, followed by Z2577. No prophage integration was detected in all *E. coli* O145. Antimicrobial susceptibility assay of O45 and O103 showed the high proportion of resistance to cephalothin (78% and 78%), streptomycin (51% and 93%),

cotrimoxazole (39% and 28%), tetracycline (31% and 48%) and chloramphenical (23% and 33%), respectively. Furthermore, multi-drug resistant ability was found in 49% and 59.2% of O45 and O103, respectively. DNA typing of *E. coli* O45 and O103 surrogates by BOX-PCR analyzed at 80% genetic similarity exhibited 5 and 22 distinct clusters. Interestingly, in each serogroup, some strains from different samples and different time intervals demonstrated the identical fingerprint, suggesting that they are genetically closely related or they may be originated from the same bacterial clones.

Keywords: *Escherichia coli* O45, *Escherichia coli* O103, *Escherichia coli* O111, *Escherichia coli* O145, atypical enteropathogenic *Escherichia coli*, raw meat, southern Thailand

ชื่อวิทยานิพนธ์	การตรวจสอบและการอธิบายลักษณะของเชื้อ *Escherichia coli* O45,
	O103, O111 และ O145 จากเนื้อสัตว์ดิบ อำเภอหาดใหญ่ ประเทศไทย
ผู้เขียน	นางสาวอภิสรา แซ่ลิ่ม
สาขาวิชา	จุลชีววิทยา (นานาชาติ)
ปีการศึกษา	2561

บทคัดย่อ

Shiga toxin-producing *Escherichia coli* (STEC) ในกลุ่ม non-O157 เริ่มกลายเป็น
กลุ่มที่สร้างความวิตกกังวลทางด้านสาธารณสุขทั่วโลก โดยมีเนื้อสัตว์เป็นหนึ่งในพาหะสำคัญที่
สามารถถ่ายโอนเชื้อเหล่านี้สู่คนได้ทางการบริโภค การศึกษานี้ได้ทำการตรวจหาเชื้อ STEC ทั้ง 4 ซี
โรไทป์ที่สำคัญได้แก่ O45, O103, O111 และ O145 จากเนื้อสัตว์ดิบ ทางภาคใต้ของประเทศไทย
โดยวิธี Immunomagnetic separation (IMS) จากการตรวจหาพบว่าความชุกของ *E. coli* O45, O103,
O111 และ O145 เป็น 12.4, 42, 0 และ 3 เปอร์เซ็นต์ ตามลำดับ แม้ว่าเชื้อ *E. coli* เหล่านี้ไม่ได้จัดอยู่
ในทั้ง 5 กลุ่มของ diarrheagenic *E. coli* (DEC) ยกเว้น *E. coli* O145 ที่จัดอยู่ในกลุ่ม atypical
enteropathogenic *E. coli* (aEPEC) และ *E. coli* O103 จำนวน 1 ไอโซเลทซึ่งแยกได้จากเนื้อไก่ที่จัด
อยู่ในกลุ่ม extraintestinal pathogenic *E. coli* (ExPEC) การตรวจหายีนก่อโรคอื่นๆ พบว่าทุกไอโซ
เลทในทุกซีโรไทป์ ตรวจพบยีน *fimH* ซึ่งเกี่ยวข้องกับการยึดเกาะของแบคทีเรีย นอกจากนี้ *E. coli*
O45 และ O103 ยังตรวจพบยีน *astA* ซึ่งเกี่ยวข้องกับการสร้าง enteroaggregative heat stable
enterotoxin 1 (EAST-1) ในอัตรา 63.2% และ 20.8% ตามลำดับ อีก 30.6% ของ *E. coli* O45 และ
100% ของ *E. coli* O145 ตรวจพบยีน *lpf* (ถอดรหัสสร้าง long polar fimbria) ผลการวิเคราะห์
phylogenetic group ของ *E. coli* O45 พบว่าจัดอยู่ใน phylogenetic group D (88%), A (8%) และ B1
(4%) แตกต่างจาก *E. coli* O103 ที่พบ 3%, 5%, 30% และ 62% จัดอยู่ใน phylogenetic group B2, D,
B1 และ A ตามลำดับ ในขณะที่ aEPEC O145 อยู่ใน phylogenetic group D ทั้งหมด การตรวจหา
การแทรกแซงของ *stx₂* phage พบว่า *E. coli* O45 และ O103 ถูกแทรกแซงด้วย phage ในตำแหน่ง
sbcB ในอัตราที่สูงที่สุด ตามมาด้วยตำแหน่ง Z2577 อย่างไรก็ตามพบว่าทั้งห้าตำแหน่งใน *E. coli*
O145 ยังไม่เคยถูกแทรกแซงด้วย prophage การตรวจสอบการดื้อต่อยาต้านจุลชีพของ *E. coli* O45
และ O103 แสดงให้เห็นว่ามีการดื้อต่อยาเหล่านี้ในอัตราที่ค่อนข้างสูงได้แก่ cephalothin (78% และ

78%), streptomycin (51% และ 93%), cotrimoxazole (39% และ 28%), tetracycline (31% และ 48%) และยา chloramphenical (23% และ 33%) ตามลำดับ นอกจากนี้พบว่าเชื้อบางสายพันธุ์ของ *E. coli* O45 และ O103 มีความสามารถในการดื้อต่อยาต้านจุลชีพหลายชนิด (multi-drug resistance) คิดเป็นร้อยละ 49 และ 59.2 ตามลำดับ การตรวจสอบรูปแบบลายพิมพ์ดีเอ็นเอของสายพันธุ์ที่ใช้ เป็นตัวแทนใน *E. coli* O45 และ O103 โดยวิธี BOX-PCR ที่ 80% similarity พบว่าสามารถแยกได้ 5 และ 22 cluster ตามลำดับ นอกจากนี้ยังมีบางสายพันธุ์ซึ่งแยกได้จากตัวอย่างและช่วงเวลาที่แตกต่าง กัน ที่ให้รูปแบบลายพิมพ์ดีเอ็นเอเหมือนกันทุกประการบ่งบอกได้ว่าสายพันธุ์เหล่านี้มีความใกล้ชิด กันทางพันธุกรรม หรือมีต้นกำเนิดมาจากโคลนเดียวกัน

คำสำคัญ: *Escherichia coli* O45, *Escherichia coli* O103, *Escherichia coli* O111, *Escherichia coli* O145, atypical enteropathogenic *Escherichia coli*, เนื้อสัตว์ดิบ, ภาคใต้ของประเทศไทย

ACKNOWLEDGEMENTS

CONTENTS

CONTENTS (continued)

LIST OF TABLES

XIII

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

%	percentage
°C	degree Celsius
μl	microliter
AK	amikacin
C	chloramphenicol
CLSI	Clinical Laboratory Standards Institute
CN	gentamicin
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotides
E. coli	*Escherichia coli*
EHEC	enterohemorrhagic *Escherichia coli*
EMB	eosin methylene blue agar
ExPEC	extraintestinal pathogenic *Escherichia coli*
aEPEC	atypical enteropathogenic *Escherichia coli*
FOS	fosfomycin
K	kanamycin
KF	cephalothin
IMS	immunomagnetic separation technique
IPM	imipenem
ml	milliliter

LIST OF ABBREVIATIONS (continued)

mM   millimolar

PCR   polymerase chain reaction

rpm   rotation per minute

S   streptomycin

STEC   Shiga toxin-producing *Escherichia coli*

SXT   trimethoprim/ sulfamethoxazole

T   tetracycline

UPGMA   unweighted pair group method with arithmetic mean

CHAPTER 1

INTRODUCTION

BACKGROUND AND RATIONALE

Escherichia coli are normal microbiota in gastrointestinal tract of humans and warm-blood animals which provide the benefits to its host (Nataro and Kaper, 1998). Nonetheless, some *E. coli* strains contain virulence factors and post the health risk to the host. Shiga toxin-producing *E. coli* (STEC) contain the cardinal virulence factors, *stx* genes, coding for Shiga toxins. These toxins are able to cause the food poisoning outbreaks in several countries all around the world (Michino *et al.*, 1999; Dundas *et al.*, 2001; Rangel *et al.*, 2005). The patients infected by STEC are not only display the intestinal symptoms but also the disorders in other organs especially the kidney due to the excess of Stx receptor (Nataro and Kaper, 1998). Renal failure occurs after Stx internalized into kidney cell through the cellular receptor called globotriaocylcreamide (Gb3) and then it removes 1 adenine residue from 28S ribosomal RNA of 60S ribosomal subunit, resulting in inhibition of protein synthesis (Karmali *et al.*, 1983). Even though the STEC serotype O157:H7 is the most important, recently the serotypes other than O157:H7 gradually play the role. Currently, more than 40 serotypes of STEC have been reported to be involved with the severe human diseases worldwide (Paton and Paton, 1996). In addition to the STEC, another group of *E. coli* called extraintestinal pathogenic *E. coli* (ExPEC) also plays a pivotal role in human diseases (Smith *et al.*, 2007). This *E. coli* group resides in the human gut without causing symptoms but maintains an ability to disseminate, colonize, and infect other host organs (Wiles *et al*, 2008). ExPEC carries a great numbers of virulence genes, responsible for several aspects of its course of pathogenesis. Type 1 fimbriae (encoded from *fimH*), P fimbriae (encoded by *pap*), and S fimbriae (*sfaDE*) are the important gene responsible for ExPEC adherence. Afterwards, the pathogenesis is committed by the crucial effector proteins such as protectins or toxins (Köhler and Dobrindt, 2011).

Natural reservoirs of STEC are ruminants, especially cattles that carry STEC in their gut without pathological symptoms (Arthur *et al.*, 2002). There are many routes of STEC transmission from animals to humans and STEC contamination on raw meats during slaughtering processes is one of the potential routes, capable of transferring STEC to human. Likewise in ExPEC, raw meats are also shown to be the vehicle of ExPEC, especially chicken meat which is likely to be a primary reservoir (Johnson *et al*, 2006; Cortés *et al*, 2010). Therefore, raw meats are the important STEC/ExPEC vehicles (Barkocy-Gallagher *et al.*, 2003, Bosilevac and Koohmaraie, 2011).

Collectively, due to a great impact of STEC/ExPEC on public health and the lack of information regarding STEC serotype O45, O103, O111 and O145 prevalence including their characteristics in Thailand, this study aimed to investigate their prevalence, virulence profile, antimicrobial susceptibility and the genetic similarity of STEC/ExPEC strains from meats marketed throughout Hat-Yai city, southern Thailand. The data obtained may provide the information on possible infections and outbreaks including the way to cope with STEC/ExPEC in this area in the future.

LITERATURE REVIEWS

Enterobacteriaceae

Enterobacteriaceae is a large family of Gram negative, rod-shape, non-sporulating, and facultative anaerobes. Some are present in intestinal tract as the gut microbiota but some can be pathogens. Bacteria in this family can be divided into 2 groups by the ability to ferment lactose, lactose fermenters (LF) and non-lactose fermenter (NLF) and can be speculated on MacConkey agar. This family includes more than 15 different genera such as *Salmonella* spp*., Citrobacter* spp*., Yersinia pestis, Klebsiella* spp., *Shigella* spp*., Proteus* spp*., Enterobacter* spp*., Serratia* spp*.,* and *Escherichia coli.* Furthermore, some genera act as pathogen in humans. Common antigens of *Enterobacteriaceae* are O (somatic O antigen), H (flagella antigen), and K (capsular antigen) (Figure 1).

Coliform bacteria are groups of bacteria in *Enterobacteriaceae* which are defined as rod-shaped, Gram-negative, non-spore forming and motile or non-motile bacteria. They can be divided into 2 groups, fecal coliform and non-fecal coliform. *E. coli* is belonged to fecal coliform which is used as indicators for water and food sanitary quality.
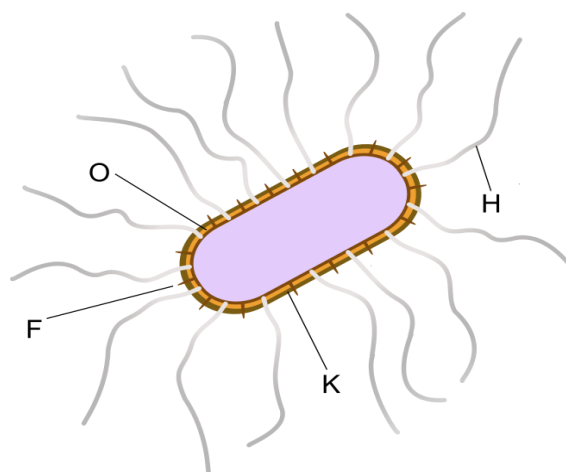
Figure 1 Common antigens of *Enterobacteriaceae* (Enterobacteriaceae Antigen/© Matthias M. / CC BY-SA 3.0)

General characteristics of *Escherichia coli*

E. coli are normal microbiota in gastrointestinal tract of humans and warm-blood animals. *E. coli* is Gram negative, rod shape (Figure 2), oxidase negative, non-spore forming, and facultative anaerobic bacteria. *E. coli* in general has size about 1.1-1.5 x 2.0-6.0 μm, and use peritrichous flagella and fimbriae for movement. These microorganisms can grow within temperature ranged from 10ºC to 47ºC and pH ranged from 5 to 9, but can grow well at 35 ºC to 37 ºC and can still survive after a short exposure to a pH level as low as 2 (Small *et al.*, 1994) like in a stomach that is the organ for these bacteria to pass through to the intestine where they can cause the diseases.

E. coli strains of significance to humans can be genetically and clinically classified into three groups. The first is commensal *E. coli* follows by intestinal pathogenic *E. coli* (IPEC) which obtain virulence factors and make them cause diseases in gastrointestinal tract of human and animals such as diarrhea, called diarrheagenic *E. coli* (DEC) (Nataro and Kaper, 1998). Moreover, the last group is the extraintestinal pathogenic *E. coli* (ExPEC) (Smith *et al.*, 2007). Most of them are harmless, but some can cause diseases in humans as well as mammals and birds (Bélanger *et al.*, 2011). Three general sets of symptoms resulted from infections with pathogenic *E. coli* strains are urinary tract infection, sepsis/meningitis, and enteric/diarrheal disease (Nataro and Kaper, 1998). Besides, these microorganisms are nosocomial and an opportunistic pathogens which have the potential to cause diseases in the immunocompromised host (Sousa, 2006).

Scientific classification

Domain:	Bacteria
Kingdom:	Eubacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Enterobacteriales
Family:	Enterobacteriaceae
Genus:	*Escherichia*
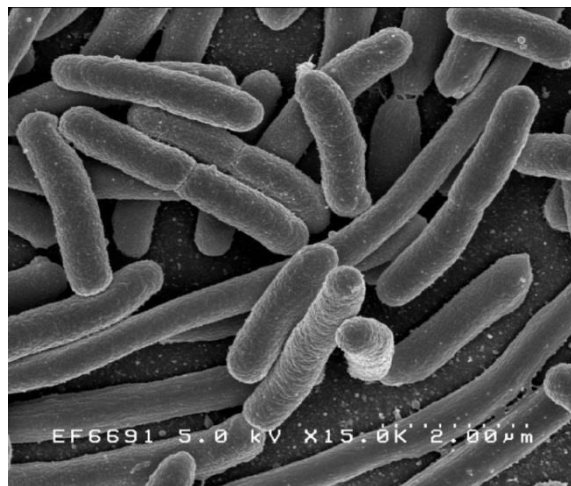
Figure 2 An image of *E. coli* using Scanning electron microscope (SEM) (Escherichia Coli NIAID/Rocky Mountain Laboratories, NIAID, NIH/Public domain)

Classification of pathogenic *E. coli*

It can be classified by pathogenesis into large 2 groups;

1. Diarrheagenic *E. coli* (DEC)

DEC is *E. coli* group that is the main cause of human gastrointestinal illnesses. It has been divided into six major categories (Nataro and Kaper, 1998):

1.1) Enteropathogenic *E. coli* (EPEC) is important bacteria that can cause infantile diarrhea especially in developing countries (Hicks *et al.*, 1998). EPEC induces a watery, sometimes bloody, diarrhea (Bielaszewska *et al.*, 2007). Consumption of contaminated drinking water and some meat products is the cause in

EPEC outbreaks. Mechanism of their pathogenesis is the production of attaching and effacing (A/E) lesion, which is characterized by pedestal formation, intimate adherence of bacteria to the intestinal epithelium, microvilli destruction, and some promoting aggregation of polarized actin and other elements of the cytoskeleton between cell membrane of host and bacteria at bacterial attachment sites (Trabulsi *et al.*, 2002). EPEC has been divided into typical EPEC (tEPEC) and atypical EPEC (aEPEC) which differ from genetic characteristics, serotypes, and virulence properties. As mentioned, tEPEC has *bfp* (coding for bundle-forming pili) and *eae* (encoding intimin, an adherent protein) genes. The aEPEC has the *eae* gene alone (Campos *et al.*, 2004). The aEPEC is more closely related to enterohaemorrhagic *E. coli* (EHEC) (Trabulsi *et al.*, 2002) and can be changed to be EHEC by the gain of *stx* genes through *stx* phages integration.

1.2) Enterotoxigenic *E. coli* (ETEC) can cause diarrhea in traveler, so it is called traveler's diarrhea. It also causes the symptoms in infants in the developing countries (Stratton *et al.*, 2000). Main virulence factors of ETEC are the production of toxins and colonization factors which allow these microorganisms to colonize in the small intestine, resulting in diarrhea (Sack, 1980). The toxins that are produced by ETEC are heat stable enterotoxin (ST) and heat-labile enterotoxin (LT). These toxins are able to disturb the absorption of some elements of intestinal epithelial cells. Thus, the clinical symptoms of ETEC infection are mild diarrhea to a severe cholera-like syndrome (Qadri *et al.*, 2005). ETEC is transmitted by food or drinking water that contaminated with animal or human feces.

1.3) Enteroinvasive *E. coli* (EIEC) can cause an invasive, dysenteric form of diarrheal illness in volunteers (DuPont *et al.*, 1971). The pathogenesis of these microorganisms is closely related to *Shigella* spp. Factually, *Shigella* spp. and *E. coli* have originated from the same ancestor (van den Beld and Reubsaet, 2012). They give infection by invading using adhesin proteins to bind and enter into intestinal cells, multiplying within epithelial cells of the colonic mucosa, evading from phagosome (Bando *et al.*, 2010), and severely damage intestinal wall. As a result, a strong inflammatory response appears and then develops to abscesses and ulcerations.

1.4) Enteroaggregative *E. coli* (EAEC or EAggEC) can cause acute and persistent diarrhea in children, travelers, and patients with AIDS in developed and developing countries (Elias and Navarro-Garcia, 2016). EAEC is defined by a phenotypic assay and their aggregative or stacked-brick pattern of adhesion to the human laryngeal epithelial cell line HEp-2 (Steiner *et al.*, 2000). EAEC pathogenesis is the aggregation and adherence of these bacteria to intestinal mucosa, then, enterotoxins and cytotoxins attack to the host cells then induce inflammation leading to diarrhea.

1.5) Diffusely adherent *E. coli* (DAEC), for diagnosis DAEC, the most useful phenotypic assay is the HEp-2 adherence assay (Donnenberg and Nataro, 1995). DAEC is defined by diffuse adherence (DA) (Nataro and Kaper, 1998) which adheres over the entire surface of the cells. The symptoms are associated with the watery diarrhea that can become persistent in young children. Although some studies have shown a relationship between DAEC infection and presence of symptoms, some have shown these organisms present in children without symptoms (Gióon *et al.*, 1991).

1.6) Enterohemorrhagic *E. coli* (EHEC) is the most important pathotype that emerges worldwide such as North America, Europe, and Japan. EHEC can cause non-bloody diarrhea, bloody diarrhea and hemolytic uremic syndrome (HUS) in humans in all age ranges (Kaper, 1998) until life threatening. This organism can produce Shiga toxins (Stxs) as a main virulence factors and also carry *eae* gene encoding intimin protein, responsible for bacterial adherence (Shiga toxin-producing *E. coli*, STEC, is a bacterium that contains *stx* but no *eae*). Stxs composed of 2 major types which are Stx1 and Stx2. These toxins have 2 subunits consisting of A and B (figure 3). Toxins are able to bind to endothelial cells through the interaction of the B subunit (which forms as a pentamer) with the membrane specific receptor called globotriaosylceramide (Gb3) present on the cell especially on renal cells. After internalization into the cells, the A1 subunit cleaves a specific adenine nucleotide from the 28S rRNA of the 60S ribosomal subunit (O'Brien and Holmes, 1987), thereby inhibiting the protein synthesis (Figure 4), causing kidney cell destruction and failure designated as hemolytic uremic syndrome (HUS) (Karmali *et al.*, 1983). Patients with HUS have high fatality rates. Although EHEC O157: H7 is the most

dominant EHEC serotype associated with the most severe disease (Nataro and Kaper, 1998), in recent years there are reports of 6 important serotypes (also called the big six) that have been demonstrated to carry *stx* genes. These strains in such serotypes show a striking ability to cause sporadic infections and outbreak in many countries all around the world. The big six strains include O26, O45, O103, O111, O121 and O145. Ruminants , espectially cattle are considered to be the most important reservoir for EHEC/STEC O157:H7 and non-O157 serogroups (Chapman *et al.*, 1993). Healthy ruminants carry EHEC/STEC O157:H7 and non-O157 without pathological symptoms. Moreover, these animals can transmit *E. coli* O157:H7 to other hosts and can cause pathological symptoms by contamination of their feces to meat through the slaughtering processes.

EHEC is a subset of STEC that can cause human illness and are cautioned to public health worldwide. Both of them can be distinguished by the presence of *stx* and *eae* gene. The one that carries both genes is defined as EHEC. Any bacterium that carry only *stx* gene is classified as STEC. More than 40 serotypes of STEC have been reported to be causing the severe disease in humans (Paton and Paton, 1996).
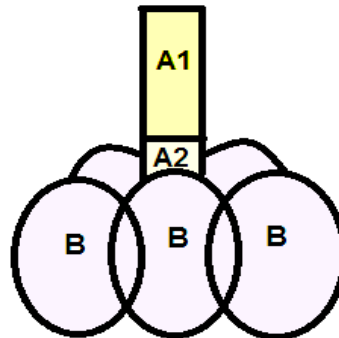
Figure 3 The structure of Shiga toxin protein (modified from Engedal *et al*., 2011).
A1 subunit is linked to the pentameric B subunit by A2 subunit.
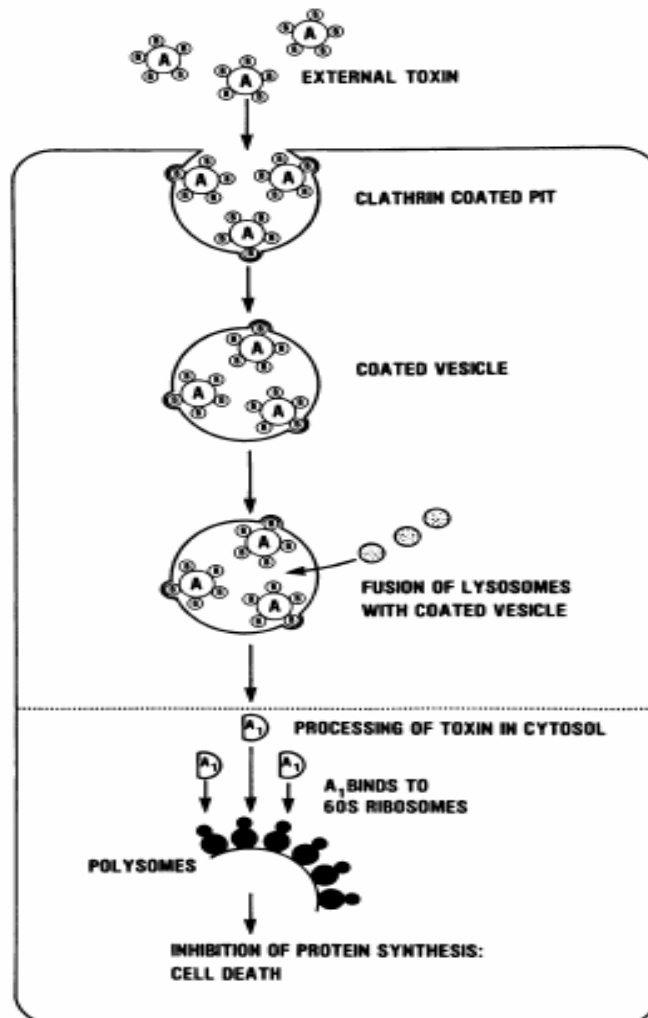
Figure 4 Mechanism of Shiga toxin to destroy mammalian cell (modified from
O'Brien and Holmes, 1987)

Table1 Virulence genes used to classify the diarrheagenic *E. coli* (DEC) pathotypes

DEC categories	Virulence characteristics	Gene (s)
EHEC	Intimin	*eae*
	Shiga toxin1	*stx*$_1$
	Shiga toxin 2	*stx*$_2$
EPEC		
Typical EPEC	Bundle forming pili	*bfpA*
	Intimin	*eae*
Atypical EPEC	Intimin	*eae*
ETEC	Heat-labile enterotoxin	*elt*
	Heat-stable enterotoxin	*est*
EAEC	Transcriptional activator of AAF/I	*aggR*
EIEC	Enteroinvasive mechanism	*ipaH*
DAEC	F1845 fimbriae	*daaE*

2. Extraintestinal pathogenic *E. coli* (ExPEC) possesses virulence factors that allow them to invade, colonize, and cause diseases outside the gastrointestinal tract. This bacterial group can lead to urinary tract infections, neonatal meningitis, bacteremia, sepsis, pneumonia, surgical site infections, and also infections in other extraintestinal sites (Smith *et al.*, 2007). ExPEC is defined as facultative pathogens which are part of normal gut microflora in healthy populations where they live together as a commensal (Köhler and Dobrindt, 2011). These microorganisms include many subgroups which are uropathogenic *E. coli* (UPEC), neonatal meningitis-associated *E. coli* (NMEC), sepsis-associated *E. coli* (SEPEC), and avian pathogenic *E. coli* (APEC, which cause infection in poultry). Besides NMEC, there are two new animal pathogenic subgroups which are mammary pathogenic *E. coli* (MPEC) and endometrial pathogenic *E. coli* (EnPEC) (Shpigel *et al.*, 2008; Sheldon *et al.*, 2010).

ExPEC can be classified by using 6 indicator genes (in five groups) as followed:

Group 1   *papA* (P fimbriae subunit)/*papC* (outer membrane usher protein)

Group 2   *afa* (Afa adhesion)

Group 3   *sfaDE* (S fimbriae)

Group 4   *kpsMTII* (capsular antigen)

Group 5   *iutA* (aerobactin receptor)

For the following 6 groups above, any *E. coli* that carry two or more groups of indicator genes, it is defined as ExPEC (Johnson and Russo, 2005). In addition, ExPEC also equip with some more virulence genes, e.g. *fimH, hlyA, cnf1, traT, agn43, lpf*, they are virulence factors which make *E. coli* to be powerful in human cell destruction. These virulence factors can be grouped by their functions; for example, adhesions, siderophore systems, toxins, surface polysaccharides, invasins and serum resistance as shown in Table 2.

Table 2 Virulence factors of ExPEC categorized by functions (Johnson and Russo, 2005)

Functional category	Virulence characteristic	Gene
Adhesins	type 1 fimbriae	*fim*
	P fimbriae	*pap*
	S fimbriae	*sfa*
	Afa adhesion	*afa*
Toxins	alpha-hemolysin	*hly*
	cytotoxic necrotizing factor 1	*cnf1*
Siderophore	aerobactin receptor	*iutA*
Protectins	surface exclusion	*traT*
	capsular antigen	*kpsMT* II

Other virulence factors of STEC/EHEC O157 and non-O157

Stxs are considered the major virulence factors of STEC and EHEC. This toxic protein consists of two major toxin types which are Stx1and Stx2. For Stx2, there are several Stx2 variants (O'brien *et al.*, 1992). EHEC not only produce Stx but also acquired the ability to adhere to the intestinal mucosa, the presence of other specific virulence factors; hemolysin (most producing an EHEC-specific plasmid-encoded hemolysin, which is not capable to lysing erythrocyte but also granulocytes and epithelial cells, encoded by *hlyA*) (Schmidt *et al.*, 1994). Attaching and effacing (A/E) lesions are also produced by EHEC to attach to the intestinal epithelial cells, causing in the intestinal mucosa. Intimin (encoded by *eae*) is part of the locus for enterocyte effacement (LEE), a pathogenicity Islands found in EHEC and EPEC.

Antibiotic resistance of STEC/EHEC O157 and non-O157

E. coli are commonly found in gastrointestinal of human and warm blood animal as result of fecal contamination or contamination during slaughtering processes. EHEC O157:H7 was first recognized as a pathogen in 1982 by consumption of undercooked ground beef burger (Riley *et al*., 1982). Subsequently, *E. coli* O157 became broadly recognized as an important and threatening pathogen and its was a world public health concern (Rangel *et al.*, 2005).

Improper and overuse of antibiotics are considered the most important factors that can promote the emergence and dissemination of antibiotic-resistant bacteria in both animals and humans. Antimicrobial agents are often used in animal and human therapy, including the food additive in farms. Antimicrobial resistance mechanisms between animals and humans are similar because in animal farms, they use antibiotics for prevention and control of animal infections (e.g. tetracycline, fluoroquinolone). Therefore, antimicrobial-resistant bacteria from livestock may internalize and colonize the intestine of human population especially meats that is essential for human being. Besides the consumption of meats, human can gain antimicrobial-resistant bacteria through other ways, occupational exposure, waste excess from animal production facilities (Schroeder *et al.*, 2002).

There are 4 main antibiotic resistant mechanisms (Cloete, 2003) (Figure 5).

1. Efflux pumps that can propel the antibiotic out from the bacterial cell. Bacteria can produce the protein pumps on their membrane or cell wall. Sometimes, mutated bacterial DNA leads the bacteria to generate more pumps, which increase resistant ability.

2. Decrease in permeability of the cell membrane that surrounds the bacterial cell, resulting in the lesser antibiotics passing through to the cell.

3. Inactivation of antibiotics by bacterial enzyme. Bacteria can produce enzyme that can inactivate antibiotics. For example, β-lactamases destroy β-lactam ring of penicillin.

4. Modification of the target site for antibiotic binding, inhibiting binding between antibiotic and target site of bacterial cell.
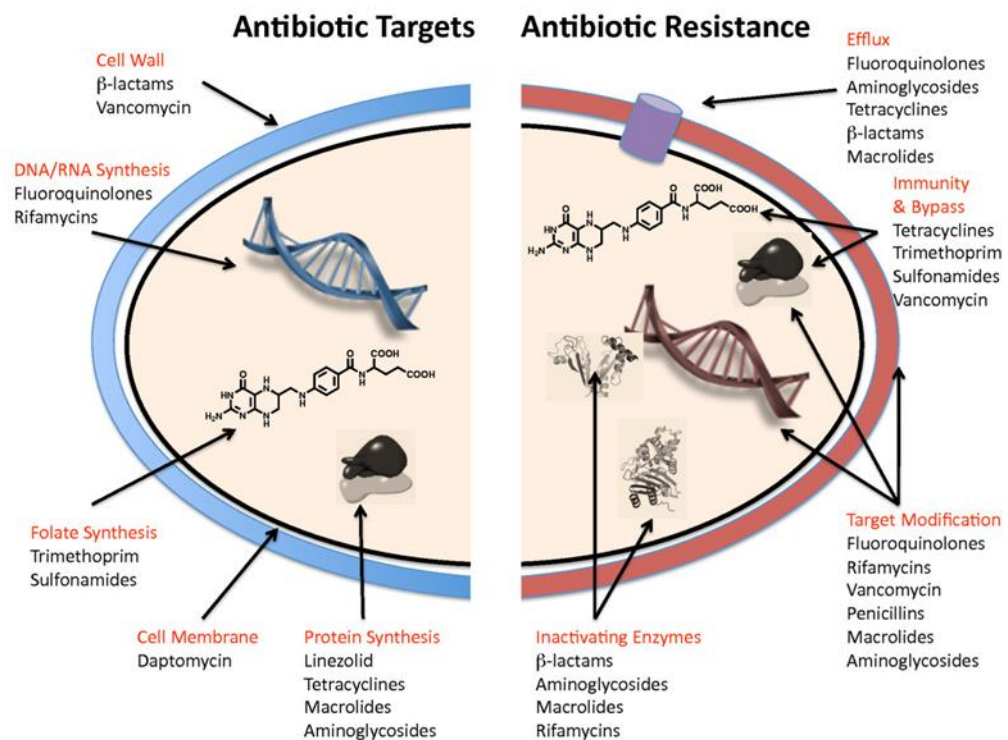
Figure 5 Antibiotic resistance mechanisms in bacteria (Antibiotic targets and mechanisms of resistance/ © Gerard D Wright/ CC BY-SA 2.0)

Antibiotic resistance in *E. coli*, especially multidrug resistance should be aware because *E. coli* is the most common Gram-negative bacteria in humans that are the most common cause of urinary tract infections (UTI), community and hospital-acquired bacteremia (Rasheed *et al.*, 2014). Recently, there are reports about Carbapenamase-producing *Enterobacteriaceae* worldwide. Carbapenem-resistant *Enterobacteriaceae* (CRE) are usually resistant to all β-lactam antibiotics which limit the available treatment options. CRE can produce a carbapenemase or an extended-spectrum cephalosporinase, such as an AmpC-type β-lactamase, in combination with porin loss, leading to the drug resistance.

Even though the therapy by antibiotics is essential in the case of bacterial infection, however, the use of antibiotics in the case of STEC/EHEC infection must be aware because misuse of antibiotics may lead to the exacerbation of the disease. Some antibiotics that affect the DNA of bacteria such as mitomycin C and fluoroquinolones, can induce the DNA repair and result in Shiga toxin-encoding bacteriophage propagation, resulting to increase the expression of Shiga toxin genes (Zhang *et al.*, 2000). Furthermore, some antibiotics can lyse bacterial cell walls, then release free Shiga toxins (Stxs) into the host (Karch *et al.*, 1986).

Immunomagnetic separation (IMS) technique

IMS is a laboratory tool that is efficient for the concentration and purification of target pathogen from heterogeneous sample matrices. IMS works by using magnetic beads coated with antibody specific for targeted bacteria (Figure 6) (Jones, 2015). Generally, IMS is coupled to Polymerase Chain Reaction (PCR) (IMS-PCR). IMS-PCR has been used successfully to increase the detection of *E. coli* O26 (Hara-Kudo *et al.*, 2000), *E. coli* O157:H7 (Wright *et al.*, 1994; Fu *et al.*, 2005), *Salmonella* spp. (Skjerve and Olsvik, 1991), *Staphylococcus aureus* (Johne *et al.*, 1989), *Listeria monocytogenes* (Hsih and Tsen, 2001), *Helicobacter pylori* (Enroth and Engstrand, 1995), *Vibrio cholerae* (Cheng *et al.*, 2014) and other pathogens. Importantly, IMS has high efficiency to obtain the target pathogenic strain around 100-fold more than direct culture (conventional method) (Chapman *et al.*, 1994). Therefore, IMS is indispensable for the isolation of target microorganisms.
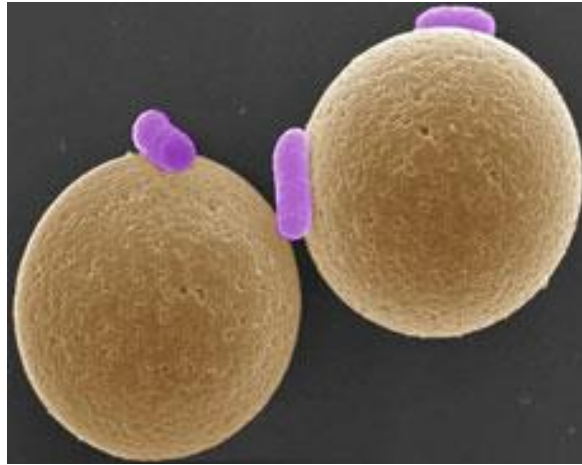
Figure 6 Capture *E. coli* by immunomagnetic beads. *E. coli* are in violet, IMS are in brown (Immunomagnetic bead selection of *E. coli* bacteria/ © NIAID/ CC BY-SA 2.0)

Phylogenetic group analysis

E. coli strains can be assigned to one of the main phylogenetic groups: A, B1, B2 or D (Lecointre *et al*., 1998), according to the combination of the three genetic markers *chuA*, *yjaA*, and TspE4.C2 fragment (Clermont *et al*., 2000). Necessarily, based on ExPEC strains, pathogenic strains are more likely to be members of phylogenetic groups B2 or D (Picard *et al.*, 1999, Johnson and Stell, 2000). Besides, commensal strains tend to be belonged mainly to phylogenetic groups A and B1 (Picard *et al.*, 1999). Moreover, resistance to multiple antibiotics is most prevalent in group B2 and D (Chakraborty *et al.*, 2015). Groups B2 and D consist of different evolutionary lineages because their regular association with various extraintestinal infection syndromes have come to be considered as "virulent clones" as commonly defined by O: K: H serotypes (Selander *et al.*, 1986) and showed least antimicrobial resistance among the groups (Chakraborty *et al.*, 2015).

Bacterial typing methods

1. Pulsed-field gel electrophoresis (PFGE)

PFGE was first described by Schwartz and Cantor (Schwartz and Cantor, 1984). It is the current "gold standard" for DNA typing which uses restriction enzyme to digest the genomic DNA. Then, the digested products are run under the electric field in more than one direction through a solid matrix to reach the separation of very large DNA fragments (Kaufmann, 1998). DNA molecules larger than 25 kilobase pair (kb) are difficult to be resolved by standard agarose gel electrophoresis. However, PFGE can separate DNA molecules up to 10 megabase pair (Mb) (Herschleb *et al.*, 2007). The time required to separate is depended on DNA fragments of different sizes and their molecular weight (Kaufmann, 1998). PFGE is the genomic DNA analysis of both microorganisms and in mammalian cells. It takes more time than repetitive element sequence-based PCR (rep-PCR). Although PFGE is a gold standard for bacterial typing, it is time comsuming taking around 2-3 days for all processes (Herschleb *et al.*, 2007) and it needs expensive and sophisticated machine.

2. Repetitive element sequence-based PCR (rep-PCR)

The rep-PCR technique uses primers that bind to repetitive sequence elements (non-coding sequences) in the bacterial genome. DNA between adjacent repetitive elements is amplified using PCR technique. Subsequently, PCR-products are resolved by agarose gel electrophoresis, generating fingerprinting profile. There are many types of rep-PCR for example the repetitive extragenic palindromic (REP) sequence, enterobacterial repetitive intergenic consensus (ERIC), $(GTG)_5$-PCR and BOX-PCR. The advantage of rep-PCR over PFGE are the ease to perform, lower cost and it needs short period of time to get the result (Sabat *et al.*, 2013). Additionally, rep-PCR uses high annealing temperature to proceed (around 50ºC), suggesting that this rep-PCR has high reproducibility compared to other DNA fingerprinting methods such as random amplification of polymorphic DNA (RAPD).

1.) ERIC-PCR; ERIC sequences are present in many copies in the genomes of enterobacteria such as *E. coli*, *S. typhimurium*, and other members of *Enterobacteriaceae* (Hulton *et al.*, 1991). ERIC contains conserved regions for primer targeting and variable region for polymorphism detection. In *E. coli* genome, there is the number of copies in ERIC sequence around 30 copies. Furthermore, in *Salmonella* Typhimurium, there is the number of copy in ERIC sequence around 150 copies. In the study of Nath *et al.* (2010), they concludes that this technique shows excellent discriminatory power and can be reproduced when analyzed in *S. enterica* serotype Typhi isolated from patients with typhoid in 1987 to 2006.

2.) BOX-PCR: Originally, it is found in *Streptococcus pneumoniae* (Van Kessel *et al.*, 2005). Box has three subunits, *boxA* (59 bp), *boxB* (45 bp), and *boxC* (50 bp) but only *boxA* is conserved. Therefore, *boxA* can be used for bacterial typing. Recently, there are development of a fluorescent-BOX-PCR for subtyping *E. coli* and *Bacillus cereus* (Brusetti *et al.*, 2008). The result shows the resolution power and discriminatory power higher than traditional BOX-PCR. This technique uses single primer, BOXA1R, which targets the repeated sequence.

3.) $(GTG)_5$-PCR: $(GTG)_5$-PCR amplifies the polytrinucleotide GTG, the repetitive element that spreads throughout the bacterial genome. Besides, it shows the high discriminatory power (Kathleen *et al.*, 2014). $(GTG)_5$-PCR fingerprint analysis has been used for molecular typing of *Enterococcus* spp. (Švec *et al.*, 2005), *Lactobacillus* spp. (Gevers *et al.*, 2001), *Staphylococcus* spp. (Švec *et al.*, 2010), *Salmonella enterica* serotype Enteritidis (Fardsanei *et al.*, 2016), *Cryptococcus neoformans* (Meyer *et al.*, 1993), *Mycobacterium tuberculosis* (Wiid *et al.*, 1994), *E. coli* (Sirikaew *et al.*, 2015, Sukhumungoon *et al.*, 2016, Sae-lim *et al.*, 2017).

OBJECTIVES

1) To investigate the existence and prevalence of *E. coli* O45, O103, O111 and O145 from raw meats in Hat-Yai city, Songkhla, Thailand.

2) To classify the pathotype and investigate the virulence genes of isolated *E. coli*.

3) To determine antimicrobial susceptibility of the isolated *E. coli* strains by disk diffusion approach.

4) To determine genetic relatedness of *E. coli* O45, O103, O111 and O145 by BOX-PCR

CHAPTER 2

MATERIALS AND METHODS

MATERIALS AND EQUIPMENTS

Microbiological Media

Agar	HiMedia, India
Tryptic Soy Broth	Difco, France; HiMedia, India
Eosin Methylene Blue Agar	Difco, France
Luria Bertani Broth	Difco, France; HiMedia, India
Mueller-Hinton Broth	Difco, France; HiMedia, India

Immunomagnetic beads

Immunomagnetic beads	Dynabeads, Thermo Sciencetific, USA

Antimicrobial disks

Antimicrobial disks	Oxiod, United Kingdom

Chemicals

Tris base	Amresco, USA
Ethidium Bromide	Sigma, USA
Ethylenediaminetetraacetic Acid (EDTA)	Merck, Germany
Sodium Chloride	Thermo Fisher, USA
Sodium Hydroxide	Merck, Germany
Boric Acid	Merck, Germany

Hydrochloric Acid   Merck, Germany

PCR Reagents

dNTPs   Thermo Scientific, USA

Go*Taq* DNA polymerase buffer   Promega, Madison, USA

Go*Taq* DNA polymerase   Promega, Madison, USA

Magnesium Chloride   Promega, Madison, USA

Miscellaneous

Agarose   OmniPur, USA

Genomic DNA extraction kit   Geneaid, Taiwan

PCR tubes, strips   ExtraGene, Taiwan

Pipette Tips   Axygen, China

1.5 ml microcentrifuge tubes   Axygen, China

2-log DNA Ladder (0.1-10.0 kb)   Biolabs, United Kingdom

Equipments and instruments

Autoclave   Tomy, Japan

AutoPipette   Sartorious, Germany

Bioprofile software   France

Digital Heat Block BSH1002   Benchmark, USA

Densitometer   Biosan, Latvia

Freezer (-20 °C)   Panasonic, Japan

Freezer (-80 °C)   New Brunswick Sciencetific,
                                            USA

Hot Air Oven	BINDER, Germany
Incubator	Sanyo, Japan
Larminar airflow cabinet	Astec microflow, USA
Microcentrifuge	Sigma, USA
Microwave	Toshiba, Japan
pH meter	Sartorious, Germany
Shaker incubator	New Brunswick Sciencetific, USA
T100^{TM} Thermal cycler	Bio-Rad, USA
Vortex Mixer	Scientific Industries, USA
Water bath	Sheldon manufacturing, USA
WSE-5200 Printgraph gel imaging system	ATTO, Japan

METHODS

1) Sample collection and immunomagnetic separation (IMS) of *E. coli* O45, O103, O111 and O145 from raw meats

In order to isolate *E. coli* O45, O103, O111 and O145 from raw meats, IMS technique was employed as previously described (Sukhumungoon *et al.*, 2011). Raw meat samples, including beef, chicken, and pork were collected from 8 fresh markets throughout Hat-Yai city, Songkhla province, Thailand (Table 3) and processed within 2 hours after purchased. Briefly, a 50 g of raw meat was homogenized with 450 ml of tryptic soy broth (TSB) for 1 minute. Liquid phase was rinsed back aseptically to a sterile bottle and incubated at 37ºC for 6 hours without shaking. Subsequently, a 1 ml of the enriched culture was transferred to a new sterile tube (1.5 ml-sized plastic tube) and mixed with 15-µl immunomagnetic beads (Dynabeads, Thermo Scientific, USA) specific for each somatic O antigen for 30 minutes with gently tube inverted every 5 minutes. Immunomagnetic beads-bacteria complex was harvested by magnetic concentrator and washed with 500 µl of phosphate buffer saline, pH 7.4 (PBS). The recovered magnetic beads-bacteria complex was re-suspended in 100 µl PBS and streaked on eosin methylene blue (EMB) agar and incubated at 37ºC for 18 hours. Ten to twenty green metallic sheen colonies were selected and kept at -80ºC [glycerol final concentration of 10% (v/v)] for further analyses.

Table 3 Samples collected in this study

O-serotype	Types of meats (Samples)			Total	Duration of sample collection
	Beef	Chicken	Pork		
O45	50	27	28	105	July, 2018 to February, 2019
O103	54	43	43	140	July, 2016 - February, 2017
O111	86	-	-	86	July, 2016 – November, 2016 and August, 2017 to December, 2017
O145	100	-	-	100	July, 2016 - February, 2017

2) Genomic DNA preparation

Bacterial genomic DNA (gDNA) was extracted using a boiling method (Pannuch *et al.*, 2014). In short, a single colony was inoculated into 3 ml of TSB and incubated at 37ºC for 3 hours with 150 rpm shaking. One ml of bacterial culture was boiled for 10 minutes and immediately immersed on ice for 5 minutes, then centrifuged at 11,000 *g* for 5 minutes. Ten-fold dilution of boiled supernatant was performed with sterile deionized water (DI) (10-µl boiled supernatant: 90-µl sterile deionized water). Afterwards, the mixed solution was used as a PCR template.

3) Identification of *E. coli* O45, O103, O111 and O145

For identification of *E. coli* O45, O103, O111 and O145, PCR targeting to *wzy*O45, *wzx*O103, *rfb*O111 and *wzx*O145 genes were carried out (Table 4). PCR reaction was performed in a 25-µl reaction mixture consisting of 3.0 mM $MgCl_2$, 0.1 mM of dNTPs, 0.4 µM of forward and reverse primers (Table 4), 1X Go*Taq* Flexi green buffer, 0.5 unit of Go*Taq* DNA polymerase (Promega, USA) and 2 µl of DNA template. Subsequently, PCR product was analyzed in 1.0% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV light. To confirm the being of *E. coli*, the presence of *uidA* gene specific for *E. coli* was performed (Table 4). A bacterial strain that is positive to *wzy*O45, *wzx*O103, *rfb*O111, or *wzx*O145, with the presence of *uidA* was categorized as *E. coli* O45, O103, O111 and O145, respectively.

4) *E. coli* pathotype classification and detection of other virulence genes

Since *E. coli* in the group of O45, O103, O111 and O145 tend to be members of enterohaemorrhagic and Shiga toxin-producing *E. coli* (STEC) group, thus, this study aimed to investigate the pathotype of the isolated strains from all 4 serotypes. Indicator genes for 6 diarrheagenic *E. coli* (DEC) categories were investigated by PCR as following criteria, *bfp+eae* for typical enteropathogenic *E. coli* (tEPEC) or *eae* alone for atypical enteropathogenic *E. coli* (aEPEC); *stx+eae* for EHEC; *aggR* for EAEC; *ipaH* for enteroinvasive *E. coli* (EIEC); *est/elt* for enterotoxigenic *E. coli* (ETEC); *daaE* for diffusely adherent *E. coli* (DAEC) (Table 4). Moreover, extra PCR

reactions were included to identify the presence of extraintestinal pathogenic *E. coli* (ExPEC) by targeting 6 genes in 5 groups (group 1, *papA* and/or *papC*; group 2, *sfaDE*; group 3, *afa*; group 4, *kpsMTII*; and group 5, *iutA*). Any strains that were positive for two or more groups were judged as ExPEC (Johnson *et al*., 2003). Thermal cycling conditions were as followed: pre-heated at 95ºC for 3 minutes followed by 35 cycles of denaturation at 94ºC for 1 minute, annealing at 40ºC (for *est*), 50ºC (*elt*, *aggR*, *stx₂*, *kpsMTII*), 55ºC (*stx₁*, *eae*, *bfp*, *daaE*), 60ºC (for *ipaH*, *papA*, *papC*, *afa*), 58ºC (for *iutA*, *sfaDE*), and extension at 72ºC for 1 minute except *eae* for 1.15 minutes. The reactions were finalized at 72ºC for 5 minutes. PCR products were analyzed as described above. Other *E. coli* virulence genes composed of *astA*, *agn43*, *cnf1*, *hlyA*, *fimH*, and *lpf* was investigated using PCR with appropriate primer pairs (Table 4). PCR components and conditions were the same as mentioned above except the annealing temperature as followed: 50ºC (for *astA*), 55ºC (*fimH*, *lpf*), 58ºC (*cnf1*, *hlyA*), and 67ºC (for *agn43*) for 1 minute.

5) Phylogenetic group classification

Phylogenetic group might, in part, indicate the virulence capability of *E. coli*. Therefore, *E. coli* in all 4 serotypes in this study were investigated for their phylogenetic group. Determination of phylogenetic group was carried out by PCR targeting *chuA*, *yjaA*, and TspE4.C2 fragment. Thermal cycling condition was as followed: pre-heated at 95ºC for 3 minutes followed by 35 cycles of denaturation at 94ºC for 50 seconds, annealing at 54ºC for 50 seconds, and extension at 72ºC for 30 seconds. The reaction was finalized at 72ºC for 5 minutes. PCR products were analyzed as described above. Bacterial phylogenetic group classification was interpreted through the key from Clermont *et al*. (2000). Starting at the *chuA* result, if *chuA* is positive, it could be group B2 or D. However, if it is negative, it could be group B1 or A. The presence of *yjaA* and TspE4.C2 are applied for the next step of identification. If *yjaA* is positive, it is identified as group B2 but if negative, identified as group D. Similarly, if TspE4.C2 is positive, it is the group B1 but if negative, it is group A (Figure 7).
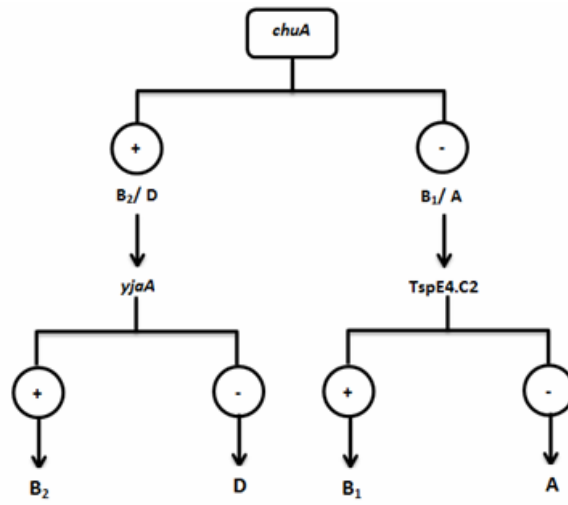
Figure 7 Dichotomous decision tree to determine the phylogenetic group of an *E. coli* strain by using the results of PCR amplification of the *chuA*, *yjaA*, and TSPE4.C2 fragment (Clermont *et al.*, 2000).

Table 4 Oligonucleotide primers used in this study

Gene	Virulence factor	Primer name	Sequence (5' to 3')	Amplicon size (bp)	References
*wzy*O45	O45 antigen	5'O45	GGCTCATCATTTGGTGCTTTGTG	404	Ju *et al.*, 2012
		3'O45	ATAAGGATTTTCAGCGCCCCTG		
*wzx*O103	O103 antigen	5'O103	TATCCTTCATAGCCTGTTGTT	320	Monday *et al.*, 2007
		3'O103	AATAGTAATAAGCCAGACACCTG		
*rfb*O111	O111 antigen	O111-F	AGAGAAATTATCAAGTTAGTTCC	406	Durso *et al.*, 2007
		O111-R	ATAGTTATGAACATCTTGTTTAGC		
*wzx*O145	O145 antigen	O145.6	TTGAGCACTTATCACAAGAGATT	418	Monday *et al.*, 2007
		O145.B	GATTGAATAGCTGAAGTCATACTAAC		
bfpA	Bundle forming pili	EP-1	AATGGTGCTTGCGCTTGCTGC	326	Gunzburg *et al.*, 1995
		EP-2	GCCGCTTTATCCAACCTGGTA		
eae	Intimin	AE-19	CAGGTCGTCGTGTCTGCTAAA	1,087	Gannon *et al.*, 1993
		AE-20	TCAGCGTGGTTGGATCAACCT		

Table 4 Oligonucleotide primers used in this study (continued)

Gene	Virulence factor	Primer name	Sequence (5' to 3')	Amplicon size (bp)	References
stx_1	Shiga toxin 1	EVT-1 EVT-2	CAACACTGGATGATCTCAG CCCCCTCAACTGCTAATA	350	Sukhumungoon *et al.*, 2011
stx_2	Shiga toxin 2	EVS-1 EVS-2	ATCAGTCGTCACTCACTGGT CCAGTTATCTGACATTCTG	404	Sukhumungoon *et al.*, 2011
ipaH	Enteroinvasive mechanism	ipaIII ipaIV	GTTCCTTGACCGCCTTTCCGATACCGTC GCCGGTCAGCCACCCTCTGAGAGTAC	603, 619	Sethabutr *et al.*, 1993
daaE	F1845 fimbriae	daaF-F daaF-R	GAACGTTGGTTAATGTGGGGTAA TATTCACCGGTCGGTTATCAGT	542	Vidal *et al.*, 2005
elt	Heat-labile enterotoxin	TW20 JW11	GGCGACAGATTATACCGTGC CGGTCTCTATATTCCCTGTT	450	Stacy-Phipps *et al.*, 1995
est	Heat-stable enterotoxin	JW14 JW7	ATTTTTACTTTCTGTATTAGTCTT CACCCGGTACAAGGCAGGATT	190	Stacy-Phipps *et al.*, 1995
astA	EAST1	EAST11a EAST11b	CCATCAACACAGTATATCCGA GGTCGCGAGTGACGGCTTTGT	111	Yamamoto *et al.*, 1995
aggR	Transcriptional activator of AAF/I	AggR-1 AggR-2	CAGAATACATCAGTACACTG GAAGCTTACAGCCGATATAT	433	Tsukamoto, 1996

Table 4 Oligonucleotide primers used in this study (continued)

Gene	Virulence factor	Primer name	Sequence (5' to 3')	Amplicon size (bp)	References
agn43	Antigen 43	1-Kpn 2-Bam	GAACCTGTCGGTACCGATGCCCTCCC CGGGATCCGTTGCCACTGTACCGGGCTTGACGACC	≈900	Danese *et al.*, 2000
chuA	Heme transport	chuA1 chuA2	GACGAACCAACGGTCAGGAT TGCCGCCAGTACCAAAGACA	279	Clermont *et al.*, 2000
yjaA	Unknown	yjaA1 yjaA2	TGAAGTGTCAGGAGACGCTG ATGGAGAATGCGTTCCTCAAC	211	Clermont *et al.*, 2000
TspE4.C2	Unknown	TspE4.C2-1 TspE4.C2-2	GAGTAATGTCGGGGCATTC A CGCGCCAACAAAGTATTACG	152	Clermont *et al.*, 2000
uidA	β-glucoronidase	*uidA* –F *uidA* -R	ATCACCGTGGTGACGCATGTCGC CACCACGATGCCATGTTCATCTGC	486	Heininger *et al.*, 1999
pet	Plasmid encoded toxin	pet-F pet-R	ACTGGCGGACTCATTGCTGT GCGTTTTTCCGTTCCCTATT	832	Vila *et al.*, 2000
fimH	Type 1 fimbriae	*fimH* –F *fimH* -R	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	508	Johnson and Stell, 2000
cnf1	Cytotoxic necrotizing factor-1	*cnf1*-F *cnf1*-R	GGCGACAAATGCAGTATTGCTTGG GACGTTGGTTGCGGTAATTTTGGG	552	Yamamoto *et al.*, 1995

Table 4 Oligonucleotide primers used in this study (continued)

Gene	Virulence factor	Primer name	Sequence (5' to 3')	Amplicon size (bp)	References
lpf	Long polar fimbriae	*lpf* A1-F *lpf* A1-R	GGTCGTTTTTGCCTTAACCGC AGGTTGAAATCGACCTGCGC	≈500	Torres *et al.*, 2004
wrbA	Quinone oxidoreductase	wrbA1 wrbA2	ATGGCTAAAGTTCTGGTG CTCCTGTTGAAGATTAGC	600	Toth *et al.*, 2003
yecE	Unknown	EC10 EC11	GCCAGCGCCGAGCAGCACAATA GGCAGGCAGTTGCAGCCAGTAT	400	DeGreve *et al.*, 2002
sbcB	Exonuclease I	sbcB1 sbcB2	CATGATCTGTTGCCACTCG AGGTCTGTCCGTTTCCACTC	1,800	Ohnishi *et al.*, 2002
yehV	Transcriptional regulator	Primer A Primer B	AAGTGGCGTTGCTTTGTGAT AACAGATGTGTGGTGAGTGTCTG	340	Shaikh and Tarr., 2003
Z2577	Oxidoreductase	Z2577F Z2577R	AACCCCATTGATGCTCAGGCTC TTCCCATTTTACACTTCCTCCG	909	Koch *et al.*, 2003

Table 4 Oligonucleotide primers used in this study (continued)

Gene	Virulence factor	Primer name	Sequence (5' to 3')	Amplicon size (bp)	References
papA	P fimbriae subunit	papA f papA r	ATGGCAGTGGTGTCTTTTGGTG CGTCCCACCATACGTGCTCTTC	720	Johnson and Stell, 2000
papC	Outer membrane usher protein	papC f papC r	GTGGCAGTATGAGTAATGACCGTTA ATATCCTTTCTGCAGGGATGCAATA	200	Johnson and Stell, 2000
afa	Afa adhesin	afa1 afa2	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	750	Le Bouguenec *et al.*, 1992
sfaDE	S fimbriae	sfaDE-F sfaDE-R	CTCCGGAGAACTGGGTGCATCTTAC CGGAGGAGTAATTACAAACCTGGCA	408	Le Bouguenec *et al.*, 1992
kpsMTII	Capsular amtigen	kpsII f kpsII r	GCGCATTGCTGATACTGTTG CATCCAGACGATAAGCATGAGCA	153	Johnson and Stell, 2000
iutA	Aerobactin	AerJ f AerJ r	GGCTGGACATCATGGGAACTGG CGTCGGGAACGGGTAGAATCG	300	Johnson *et al.*, 1998
hlyA	α-hemolysin	hly1 hly2	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	1,177	Yamamoto *et al.*, 1995
	Repetitive sequence	BOXA1R	CTACGGCAAGGCGACGCTGACG	variable	Versalovic *et al.*, 1994

6) Investigation of *stx₂* phages occupancy in *E. coli* O45, O103 and O145

The five *E. coli* specific integration sites for *stx₂* phages, namely, *sbcB*, *wrbA*, *yecE*, *yehV*, and Z2577 are essential for investigating *stx₂* phages occupancy. (Sirikaew *et al*., 2015). The intactness of all such 5 genes was performed by PCR using the components and condition described above except the different primers (Table 4) with the following annealing temperatures, 47ºC for *wrbA*, 50ºC for *sbcB* and *yehV*, 53ºC for Z2577, and 60ºC for *yecE*. The amplicons were analyzed as described above. If the *stx₂* phage occupied in a particular locus, PCR amplification was not allowed to be amplified because of large *stx₂* phage genome. *E. coli* O157:H7 strain PSU55 (Sukhumungoon and Nakaguchi, 2013) was used as the positive controls for intact *wrbA* and *yehV*. *E. coli* O157:H7 strain EDL933 (O'Brien *et al*., 1983) was used as the positive controls for inatact *sbcB*, *yecE* and Z2577.

7) Antimicrobial susceptibility assay (CLSI, 2015)

Antimicrobial susceptibility assay was performed by disk diffusion method (CLSI, 2015). Shortly, an individual colony was inoculated into 1 ml of Mueller-Hinton broth (MHB) and incubated at 37ºC for 3 hours with 150 rpm shaking, then centrifuged at 8,000 *g* for 30 seconds. Supernatant was poured out and 1 ml of 0.85% (w/v) sodium chloride solution (NSS) was added and mixed with vortex. Solution was adjusted to 0.5 McFarland turbidity standards (approximately 1.5×10^8 cfu/ml) by densitometer (Biosan, Latvia). The adjusted bacteria were swab on the surface of Mueller-Hinton agar (MHA) using sterile cotton swab. Ten common antimicrobial agents, amikacin (30 μg), cephalothin (30 μg), chloramphenical (30 μg), fosfomycin (200 μg), gentamicin (10 μg), imipenem (10 μg), kanamycin (30 μg), streptomycin (10 μg), tetracycline (30 μg) and trimethoprim/sulfamethoxazole (25 μg) were then applied onto MHA and incubated at 37ºC for 18 hours. Diameter of clear zone was measured by vernier caliper.

8) Genetic relationship of *E. coli* O45, O103, O111 and O145 by BOX-PCR

DNA profiling in each serogroup of *E. coli* was performed using BOX-PCR (Sukhumungoon *et al.*, 2016). Genomic DNA (gDNA) was extracted by using a mini-

prep spin column method (Geneaid, Taipei, Taiwan). PCR was performed in a 25-µl reaction mixture consisting of 0.2 µM of BOXA1R primer (Table 4), 0.2 mM dNTPs, 1X Go*Taq* Flexi green buffer, 3.0 mM of $MgCl_2$, 1.25 units of Go*Taq* DNA polymerase, and 50 ng of DNA template. Thermal cycler condition was performed with an initial denaturation step (95ºC for 3 minutes) followed by 30 cycles of denaturation at 94ºC for 3 seconds and 92ºC for 30 seconds, annealing at 50ºC for 1 minute, and extension at 65ºC for 8 minutes. The amplification products were analyzed using 1.5% agarose gel electrophoresis for 1.5 hours at 90V and imaging as described above. Dendrograms for O45 and O103 were constructed using unweighted pair-group method of arithmetic average (UPGMA) (Bioprofile software, France).

9.) Statistical analysis

Data were analyzed using SPSS for Windows version 11.0 (SPSS, USA). One-way ANOVA was employed to analyze significant difference in *E. coli* O45 and O103 prevalence among meat types. Significance was set at *p*-value < 0.05.

CHAPTER 3

RESULTS

1.) Prevalence of *E. coli* O45, O103, O111 and O145 in raw meat samples

Using IMS, *E. coli* O45, O103 and O145 could be isolated from meat samples while *E. coli* O111 was unable to be obtained. A total number of 1,890, 1,498, 1,541 and 1,470 suspected isolates were obtained from 105, 140, 86 and 100 meat samples for the investigation of *E. coli* O45, O103, O111 and O145, respectively. It exhibited that 49/1,890 from 13 samples (prevalence of 12.4%), 125/1,498 from 59 samples (prevalence of 42.1%), 0/1,541 from 0 sample (prevalence of 0%) and 10/1,470 from 3 samples (prevalence of 3%), were positive for O45, O103, O111 and O145, respectively (Table 5). These results suggest that *E. coli* O45, O103 and O145 did exist in southern Thai environment, especially O103 which exists in very high prevalence.

2.) DEC pathotype examination and ExPEC identification of *E. coli* O45, O103, and O145 from raw meats.

DEC pathotype examination and ExPEC identification by PCR technique using specific primer pair as listed in Table 4 revealed all *E. coli* O45 and 124/125 of *E. coli* O103 did not belong to any DEC categories and ExPEC groups. However, one *E. coli* O103 isolate (strain 103.10 from chicken sample) was identified as ExPEC carrying *iutA* (encoding aerobactin) and *kpsMTII* (encoding capsular antigen). On the other hand, all 10 isolates of *E. coli* O145 from 3 beef samples carried *eae* gene (encoding intimin) alone, classifying as atypical enteropathogenic *E. coli* (aEPEC) (Table 6). This suggests that *E. coli* O45, O103 and O145 in Thai environment are not STEC, which is different from the studies from other countries.

Table 5 Prevalence of *E. coli* O45, O103, O111 and O145 in raw meats, Hat-Yai, Thailand during July, 2016-February, 2019.

O-serotype	Source	Number of positive samples/ Total number of samples (%)	Number of positive isolates/ Total number of isolates (%)	Duration of sample collection
O45	Beef	1/50^A (2)	2/933 (0.2)	July, 2018 to February, 2019
	Chicken	8/27^B (29.6)	41/495 (8.3)	
	Pork	4/28^{AB} (14.3)	6/462 (0.9)	
	Total	**13/105 (12.4)**	**49/1,890 (2.6)**	
O103	Beef	22/54^A (40.7)	45/638 (7.1)	July, 2016 to February, 2017
	Chicken	21/43^A (48.8)	40/430 (9.3)	
	Pork	16/43^A (37.2)	40/430 (9.3)	
	Total	**59/140 (42.1)**	**125/1,498 (8.3)**	
O111	Beef	0/86 (0)	0/1,541 (0)	July, 2016 to November, 2016 and
	Chicken	-	-	August, 2017 to December, 2017
	Pork	-	-	
	Total	**0/86 (0)**	**0/1,541 (0)**	
O145	Beef	3/100 (3)	10/1,470 (0.7)	July, 2016 to February, 2017
	Chicken	-	-	
	Pork	-	-	
	Total	**3/100 (3)**	**10/1,470 (0.7)**	

Uppercase letters (A, B, AB) indicate significant difference among 3 types of raw meat in *E. coli* O45 and O103.

Table 6 DEC pathotype classification and ExPEC identification of *E. coli* O45, O103 and O145 from meats.

O-serotype	Diarrheagenic *E. coli* (DEC)	Extraintestinal pathogenic *E. coli* (ExPEC)	Classified as *E. coli*
O45	-	-	49
O103	-	1	124
O145	10[*]	-	-

[*]All *E. coli* O145 strains were classified as atypical EPEC.

3.) Detection of other *E. coli* virulence genes of *E. coli* O45, O103 and O145

Despite the fact that *E. coli* O45, O103 and O145 from meats in this study were not STEC and most of them were not members of DEC and ExPEC, they may equipped with some other virulence genes. The results revealed that *fimH* (responsible for bacterial adherence) was the gene that found in all strains in all serogroups. The *astA* gene encoding for EAST-1 toxin was found in O45 and O103 as 31/49 (63.2%) and 26/125 (20.8%), respectively. Additionally, *lpf* encoding long polar fimbriae, was found in both O45 and O145 as 15/49 (30.6%) and 10/10 (100%), respectively (Figure 8). This suggests that in some *E. coli* strains that are not members of DEC and ExPEC, they frequently carry certain virulence factors that can cause illnesses in human.

4.) Phylogenetic group classification of *E. coli* O45, O103 and O145 in raw meat samples

Phylogenetic group based on the data from ExPEC could be, in part, used to indicate their virulence capability. ExPEC in group B2 and D are considered highly virulence compared to group B1 and groupA. In this study, phylogenetic group was performed by PCR-based analysis using *chuA*, *yjaA*, and TspE4.C2 fragment as the targets and determined by dichotomous decision (Figure 7). The results demonstrated that the majority (43/49 strains) of *E. coli* O45 belonged to group D (88%) followed by group A (4/49 strains, 8%) and B1 (2/49 strains, 4%) but no strains belonged to

group B2 (Figure 9A). Whereas, *E. coli* O103 showed that 3% (4/125 strains), 5% (6/125 strains), 30% (37/125 strains) and 62% (78/125 strains) belonged to group B2, D, B1 and A, respectively (Figure 9B). All *E. coli* O145 belonged to group D (Figure 9C). Based on only the sole data of phylogenetic group examination, these results suggest that most of *E. coli* O45 and O145 are virulent strains (group D).

5.) Investigation of *stx*$_2$ phages occupancy in *E. coli* O45, O103 and O145

Due to the Stx2, one of the major virulence factors of STEC, is encoded by *stx*$_2$ phages and this type of phage tend to be integrated into these following genes; *wrbA, sbcB*, *yehV*, *yecE*, and Z2577. When *stx*$_2$ phage integrates into the specific gene, such a gene is intact no more. The absence of *stx*$_2$ gene in *E. coli* O45, O103 and O145 in this study may be explained by investigation of these genes by PCR. The results revealed that in O45, the highest non-intact gene was *sbcB* (47 strains), followed by Z2577 (13 strains) (Figure 10). In O103 serogroup, *sbcB* gene also showed the highest rate of prophage integration (86 strains), followed by Z2577 (32 strains). However, no prophages integration was detected in all *E. coli* O145.
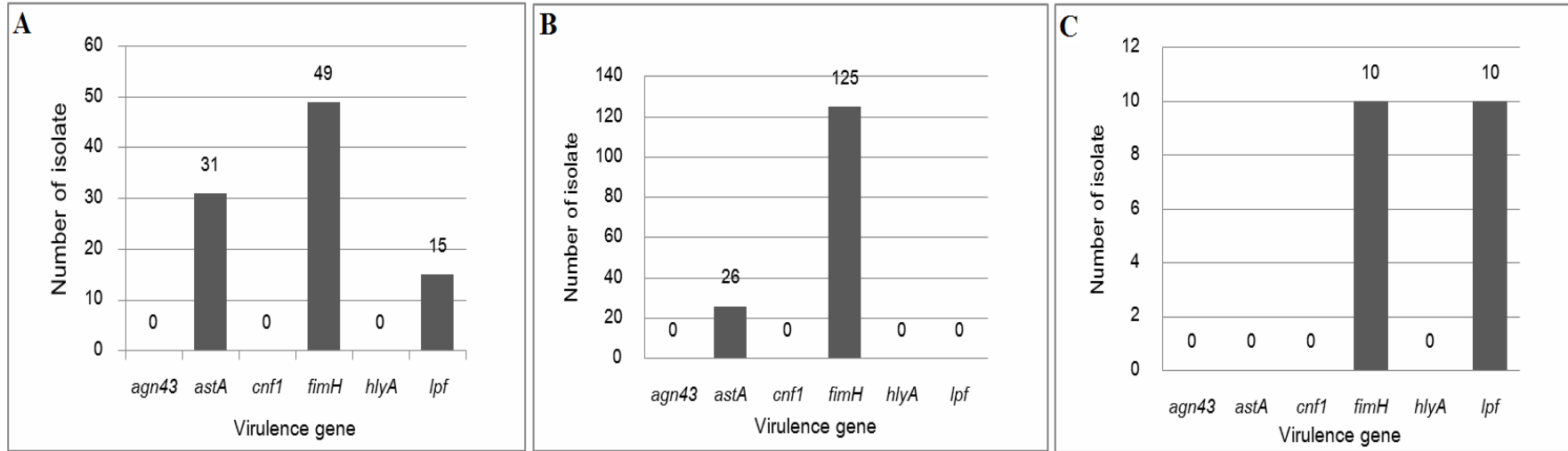
Figure 8 Virulence genes detection of *E. coli* O45, O103 and O145 from meats. A, forty-nine *E. coli* O45 strains; B, 125 *E. coli* O103 strains; C, 10 *E. coli* O145 strains.
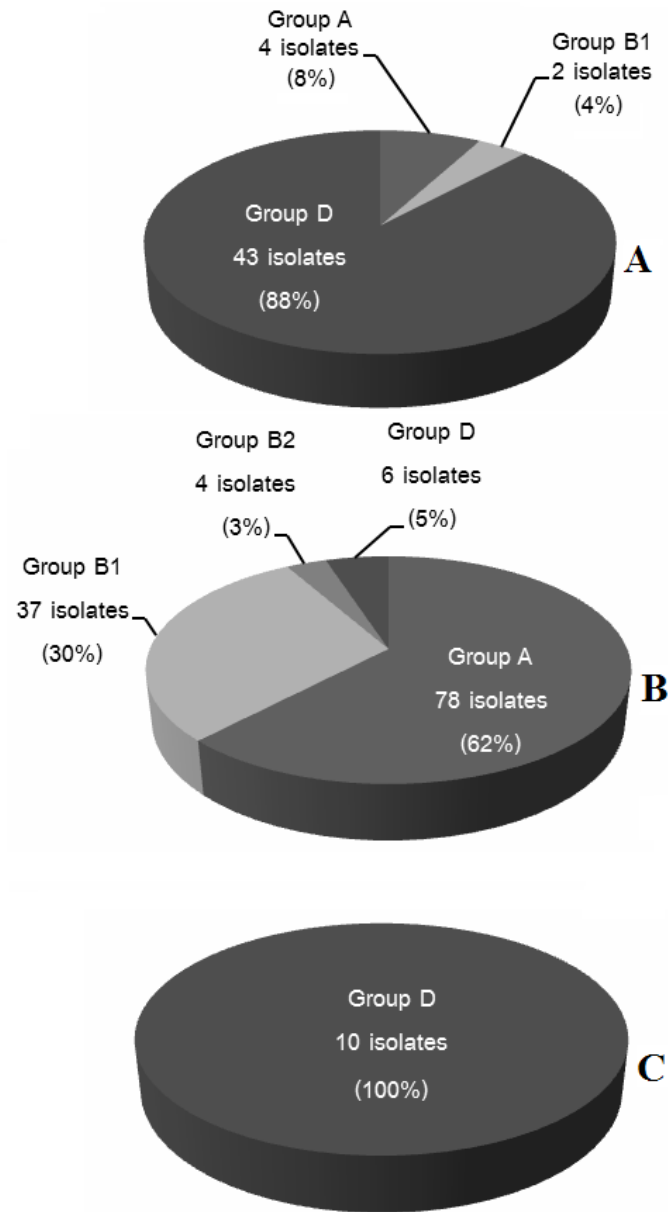
Figure 9 Phylogenetic group of *E. coli* O45, O103 and O145 from meat samples collected during July, 2016 to February, 2019. PCR was done based on *chuA*, *yjaA* and TspE4.C2 fragment. A, forty-nine *E. coli* O45 strains; B, 125 *E. coli* O103 strains; C, 10 *E. coli* O145 strains.
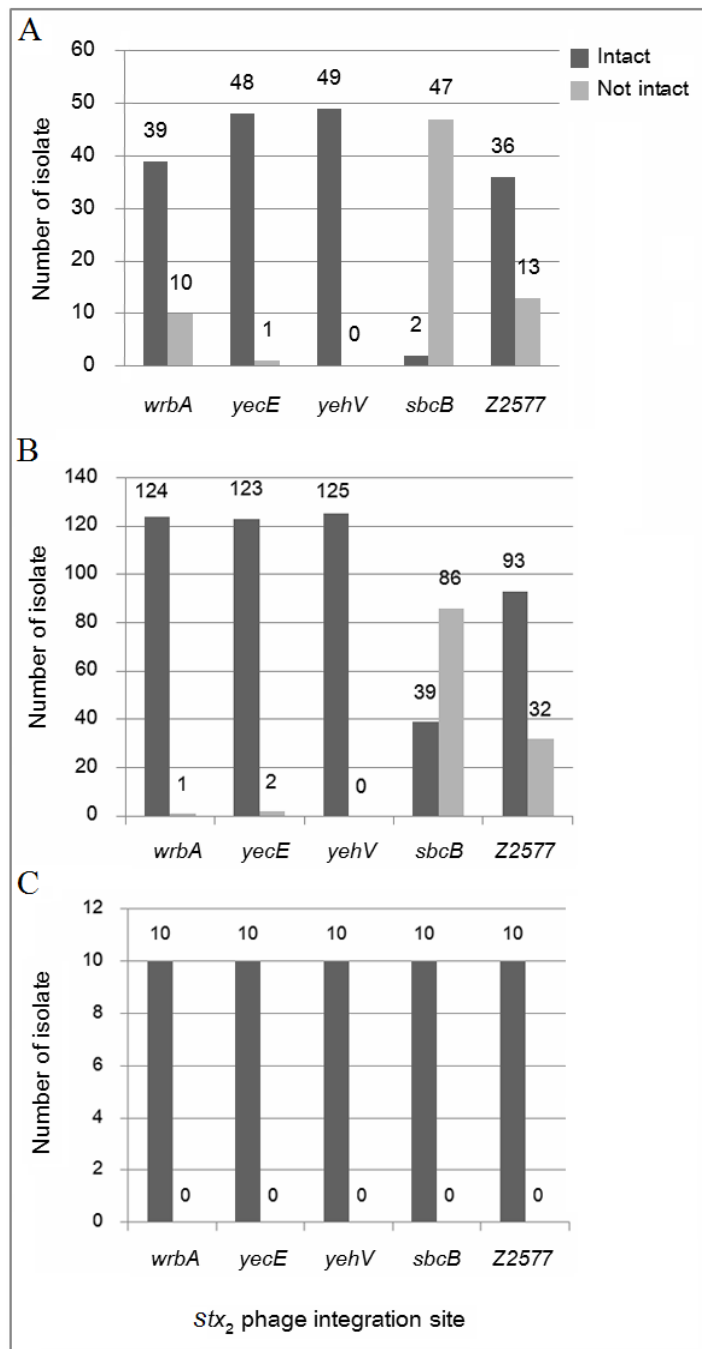
Figure 10 Intactness of stx_2 phage insertion genes in *E. coli* O45, O103 and O145 from raw meat samples collected during July, 2016 to February, 2019. A, forty-nine *E. coli* O45 strains; B, 125 *E. coli* O103 strains; C, 10 *E. coli* O145 strains.

6.) Antimicrobial susceptibility assay of *E. coli* O45, O103 and O145

Antimicrobial susceptibility of bacteria can inform the promising agents that are able to cope with the pathogen in the case of infection. This study tested the susceptibility of *E. coli* O45, O103 and O145 using 10 common antimicrobial agents. The results exhibited the high proportion of resistant strains in O45 and O103 serogroups, respectively; cephalothin (78% and 78%), streptomycin (51% and 93%), cotrimoxazole (39% and 28%), tetracycline (31% and 48%) and chloramphenical (23% and 33%). On the other hand, for gentamicin, 33% of resistance was found in O45 but only 2% in O103 (Figure 11). In addition, all 10 aEPEC O145 isolates were still susceptible to 10 antimicrobial agents tested. Interestingly, one O103 strain showed the ability to resist to imipenem. This strain might be classified as an imipenem-resistant *E. coli*. When focused on the being of multi-drug resistant strain which defines the strain that has the ability to resist to at least 3 antimicrobial classes, this study found multidrug-resistant (MDR) strains of 49% and 59.2% in O45 and O103, respectively.
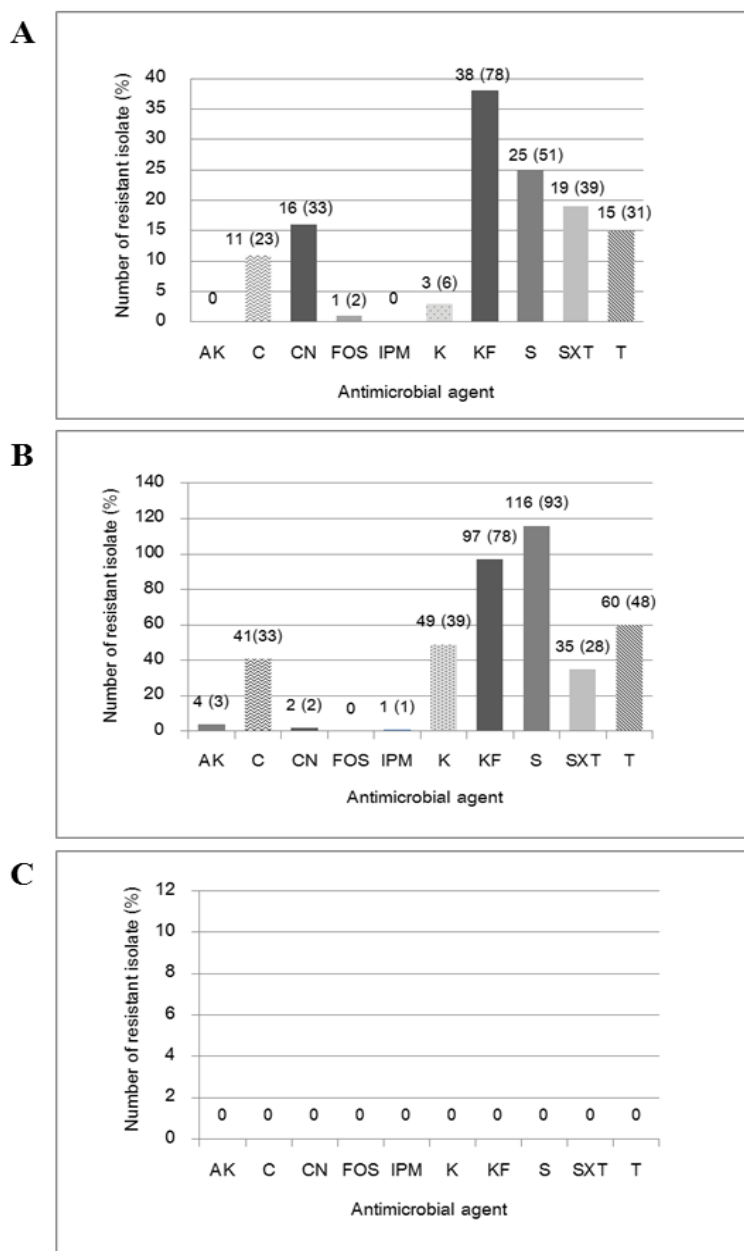
Figure 11 Antimicrobial susceptibility assay by disc diffusion method of *E. coli* O45, O103 and O145 from raw meat samples collected during July, 2016 to February, 2019. A, forty-nine *E. coli* O45 strains; B, 125 *E. coli* O103 strains; C, 10 *E. coli* O145 strains. AK, amikacin (30 μg); C, chloramphenical (30 μg); CN, gentamicin (10 μg); FOS, fosfomycin (200 μg); K, kanamycin (30 μg); KF, cephalothin (30 μg); IPM, imipenem (10 μg); S, streptomycin (10 μg); SXT, trimethoprim/sulfamethoxazole (25 μg); T, tetracycline (30 μg).

7.) Genetic relationship of *E. coli* O45, O103 and O145

To investigate the genetic relatedness of *E. coli* strains in the same serotype that circulate in Thai environment, DNA typing was carried out for the strains collected from meats in different samples and different time intervals. Using BOX-PCR as a source tracking tool, it was found that in *E. coli* O45 could be distinguished into 5 clusters based on 80% genetic similarity (Figure 13). Dendrogram analysis exhibited the identical DNA fingerprint among strains from beef, pork and chicken collected from different samples and different times, suggested that they were closely related or may be the same bacterial clone. Likewise for *E. coli* O103, it showed that all 68 surrogated *E. coli* O103 were categorized in 22 distinguishable fingerprint clusters (cluster I-XXII) (Figure 15). Focusing on all 11 identical clusters , 10 of 11 comprised the strains within the same meat types, for example, O103 strains 93.6 and strain 104.9 were from pork; O103 strains 22.8, 27.7, and 49.1, were from beef. This was except for cluster I that composed of strains from 2 meat sources, beef and pork. Interestingly, all EPEC O145 demonstrated identical fingerprint profile (Figure 16). These results suggest that *E. coli* in the same O serotype are closely related or they are the same clone which disperse in Thai environment.
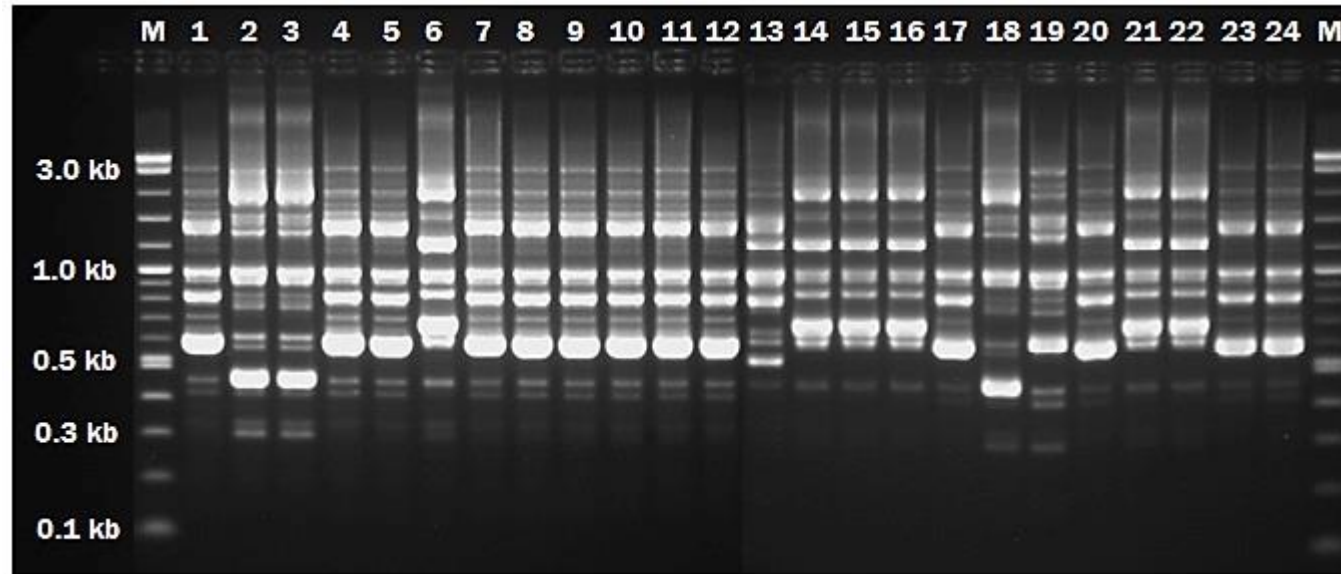
Figure 12 DNA fingerprint generated by BOX-PCR of 24 surrogates *E. coli* O45 isolated from raw meat samples collected during July, 2018 to February, 2019, Hat-Yai, southern Thailand. PCR was performed using primers listed in Table 4 and analyzed by 1.5% agarose gel electrophoresis. Lane M, 2-log DNA markers; lane 1 to 24 are M48.5, C57.1, C57.2, C57.9, C58.18, P60.5, P63.5, C64.5, C64.6, C64.8, C71.6, C72.7, C72.19, P76.4, P76.20, C78.6, C78.10, C78.16, C80.1, C80.4, C80.15, C80.18, C87.1, P95.1, respectively.
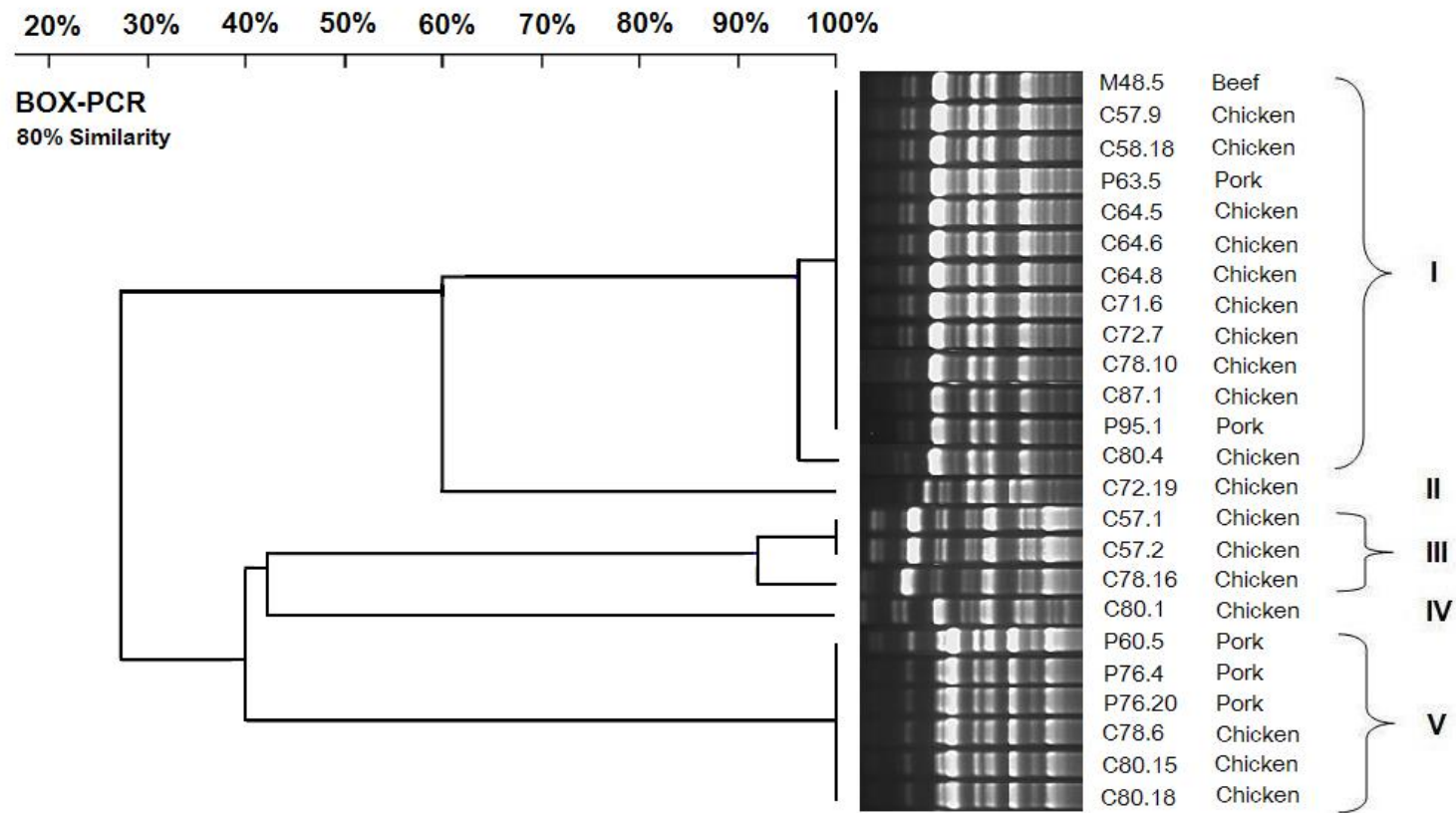
Figure 13 BOX-PCR-based dendrogram of 24 surrogates *E. coli* O45 strains from raw meat samples collected during July 2018 to February 2019, Hat-Yai, Songkhla, southern Thailand. DNA profiles were generated by BOX-PCR using boxA. The dendrogram was constructed using unweighted pair-group method of arithmetic average (UPGMA) (BioProfile software; Vilber Lourmat, Torey, France) and cut off at 80% similarity.
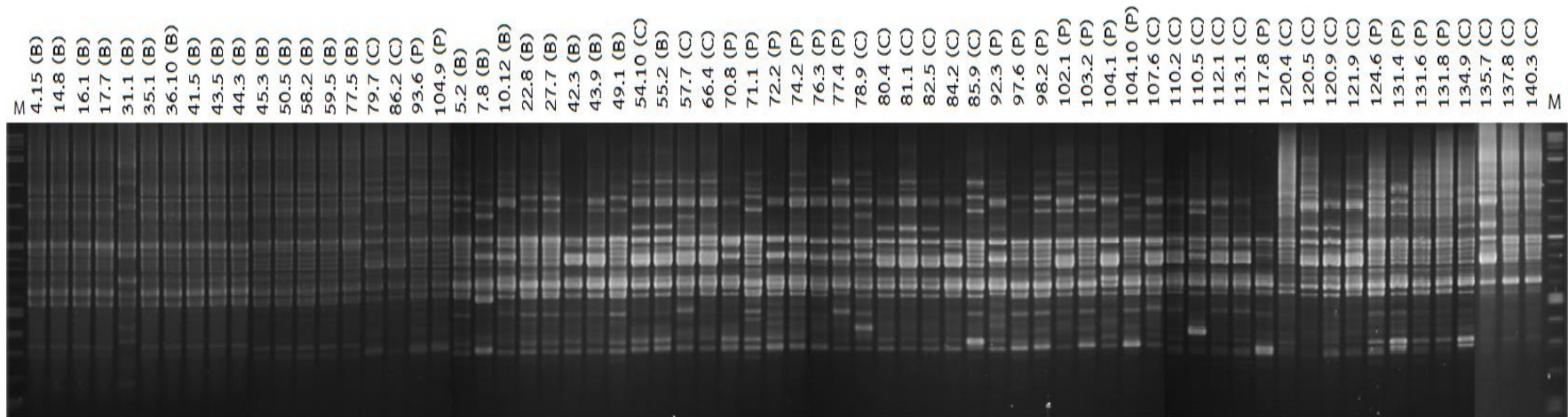
Figure 14 DNA profiles generated by BOX-PCR of 68 surrogates *E. coli* O103 isolated from raw meat samples collected during July, 2016 to February, 2017, Hat-Yai, Songkhla, southern Thailand. PCR was performed using primers listed in Table 4 and analyzed by 1.5% agarose gel electrophoresis. Lane M, 2-log DNA markers; lane 1 to 68 are B4.15, B14.8, B16.1, B17.7, B31.1, B35.1, B36.10, B41.5, B43.5, B44.3, B45.3, B50.5, B58.2, B59.5, B77.5, C79.7, C86.2, P93.6, P104.9, B5.2, B7.8, B10.12, B22.8, B27.7, B42.3, B43.9, B49.1, C54.10, B55.2, C57.7, C66.4, P70.8, P71.1, P72.2, P74.2, P76.3, P77.4, C78.9, C80.4, C81.1, C82.5, C84.2, C85.9, P92.3, P97.6, P98.2, P102.1, C110.5, C112.1, C113.1, P117.8, C120.4, C120.5, C120.9, C121.9, P124.6, P131.4, P131.6, P131.8, C134.9, C135.7, C137.8, C140.3, respectively.

40% 50% 60% 70% 80% 90% 100%

BOX-PCR
80% Similarity

Strain	Sample type	Group
4.15	Beef	
14.8	Beef	
16.1	Beef	
17.7	Beef	
35.1	Beef	
36.10	Beef	I
41.5	Beef	
43.5	Beef	
44.3	Beef	
45.3	Beef	
50.5	Beef	
58.2	Beef	
59.5	Beef	
77.5	Pork	
79.7	Chicken	
86.2	Chicken	II
93.6	Pork	
104.9	Pork	
5.2	Beef	III
10.12	Beef	
22.8	Beef	
27.7	Beef	IV
49.1	Beef	
54.10	Chicken	
55.2	Beef	
42.3	Beef	
43.9	Beef	V
70.8	Pork	
72.2	Pork	
57.7	Chicken	
66.4	Chicken	
74.2	Pork	VI
76.3	Pork	
78.9	Chicken	
77.4	Pork	
7.8	Beef	VII
71.1	Pork	VIII
80.4	Chicken	
81.1	Chicken	
82.5	Chicken	
84.2	Chicken	IX
110.2	Chicken	
112.1	Chicken	
113.1	Chicken	
92.3	Pork	X
97.6	Pork	
98.2	Pork	XI
103.2	Pork	
110.5	Chicken	XII
120.4	Chicken	XIII
120.5	Chicken	
124.6	Pork	
131.6	Pork	
131.8	Pork	XIV
131.4	Pork	
134.9	Chicken	
120.9	Chicken	XV
121.9	Chicken	
135.7	Chicken	
137.8	Chicken	XVI
140.3	Chicken	
85.9	Chicken	XVII
102.1	Pork	XVIII
104.1	Pork	
107.6	Chicken	XIX
104.10	Pork	XX
31.1	Beef	XXI
117.8	Pork	XXII

Figure 15 BOX-PCR-based dendrogram of 68 surrogates of *E. coli* O103 strains from raw meat samples collected during July 2016 to February 2017, Hat-Yai, Songkhla, southern Thailand. *E. coli* O103 surrogates are classified into 22 distinct fingerprint profiles based on 80% similarity.
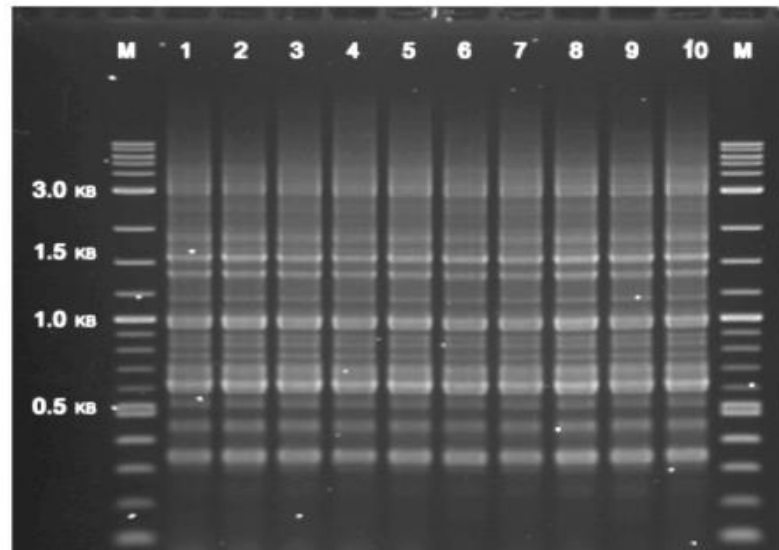
Figure 16 DNA fingerprint generated by BOX-PCR of 10 aEPEC O145 isolated from beef during July, 2016 to February, 2017, Hat-Yai, southern Thailand. PCR was performed using primers listed in Table 4 and analyzed by 1.5% agarose gel-electrophoresis. Lane M, 2-log DNA markers; lane 1 to 10 are PJP-1-10, respectively.

CHAPTER 4

DISCUSSION

Diarrheal disease plays an important role as the public health problems accounting about 11% of child death worldwide, making diarrhea the second leading cause of mortality among children under 5 years of age (CDC, 2013). Among DEC group, STEC (including EHEC) is the most important pathotype in human's infections. Only approximately 100 cells of STEC may be enough for causing illness. Although raw meats especially beef are the important vehicles of STEC, conventional method comprising enrichment and isolation on selective media are not enough to isolate the bacteria from meats because of low amount of STEC contamination in each meat sample. Thus, IMS method using antibody-coated magnetic bead is applied for STEC isolation. This method is shown to be approximately 100 folds more effective than the conventional method (Chapman *et al*, 1994). *E. coli* O26, O104 and O157 were found to be successfully isolated from raw meats by this IMS approach in this geographical area (Sirikaew *et al*., 2015; Wamaedesa *et al*., 2017; Sukhumungoon *et al*., 2011). Therefore, this study also employed IMS to isolate *E. coli* O45, O103, O111 and O145. Prevalence of *E. coli* O45, O103 and O145 in this study was 12.4%, 42.1%, 3%, respectively, while *E. coli* O111 was not obtained. Prevalence of serogroup O45 and O103 is considered relatively high, supporting the other reports in the same area. Sirikaew *et al*. (2015) investigated *E. coli* O26 from raw meats and found that the prevalence of O26 was 12%. Moreover, the work from Wamaedesa *et al*. (2017) revealed 17% prevalence of O104 from beef. In addition, Sukhumungoon *et al*. (2011) showed 18% prevalence of O157 from beef. These results suggest that *E. coli* in the serogroup O45 and O103 exist in southern Thailand in relatively high prevalence. The absence of O111 in meats was a surprise. We did confirm the absence of *E. coli* O111 by MPN-PCR and it exhibited the negative result as well (data not shown). Supporting data from Arthur *et al.* (2002) and Pearce *et al.* (2006) also described no *E. coli* O111 from beef carcasses and cattle shedding in USA and Scotland, respectively. However, in contrast with the study of Kayali *et al*. (2015) who investigated 12 STEC serogroups from beef marketed in Hat-yai city, southern

Thailand in 2015 by the pickpen-assisted IMS in combination with loop-mediated isothermal amplification (LAMP). They were capable of isolating STEC O111 carrying stx_1 from beef. This may suggest that IMS in combination with techniques that have the high sensitivity and specificity such as LAMP are able to help isolating a very low bacterial contamination from the sample. Howsoever, it was also clearly shown that there were different *E. coli* O111 strains in southern Thailand.

The lack of *stx* genes in all serogroups in this current study is surprising. The result was also in contrast with the report of Kayali *et al*. (2015) that found STEC O103, and O111 carrying stx_1 from beef. The presence of different O103 and O111 strains in southern Thai area might be the explanation. Another reason to explain the lack of *stx* might be because the stx_1 and stx_2 specific primer pairs, EVT-1 and EVT-2 and EVS-1 and EVS-2, respectively, are use to detect only limited gene variants. EVT-1 and EVT-2 primers are able to detect one form of stx_1 and EVS-1 and EVS-2 are able to detect stx_2, stx_{2vha}, stx_{2vhb} and stx_{2vp1}. Surprisingly, we firstly showed that *E. coli* O103 which tend to be STEC but in this study, it was shown to be a member of ExPEC. Such a strain of O103 was designated as strain 103.10 carrying *iutA* and *kpsMTII*. This suggests that *E. coli* O103 in this study is also dangerous for human organs outside the intestine.

Although almost all strains of *E. coli* O45 and O103 in this study were absent in term of indicative genes for DEC, these strains carried other virulence genes that responsible for pathogenesis. The *fimH* (encoding type 1 fimbrail tip), *lpf* (encoding long polar fimbria) and *astA* (encoding enteroaggregative heat stable enterotoxin 1, EAST-1) are found in our study. Especially *fimH* that is positive in all strains from all O-serogroups. Our results are in concordance with the study of Tiba *et al*. (2008) and Van *et al*. (2008) that revealed the high prevalence of this gene as 97.5% of 162 UPEC strains isolated from patients with cystitis and 92.1% of 38 multidrug-resistant strains isolated from raw meats and shellfishes sold in Vietnam. Hence, the presence of these genes in *E. coli* O45, O103 and O145 in the current study suggests that they are virulent strains and may cause the problem after consumption.

Based on the information from ExPEC group, bacteria in phylogenetic group B2 and D are pathogenic strains while group A and B1 are commensal strains (Picard *et al.*, 1999). Thus, this criterion is also applied to *E. coli* strains in this current study to indicate the pathogenic potentials. The data exhibited that most of *E. coli* O45 and all strains in O145 are in group D, accompanied by the results of virulence genes examined, suggest that they are pathogenic to some extent.

Integration of prophages is reported to play a key role in *E. coli* O157:H7 evolution and can increase their pathogenesis (Ooka *et al.*, 2009; Sukhumungoon and Nakaguchi, 2013). Prophage integration happens through site-specific recombination or transposition. Prophage integration sites are located in housekeeping genes or the regions in the close proximity to tRNA genes (Schmidt, 2001). Although the preference site of *stx$_2$* phage integration in *E. coli* O157:H7 from Spain and sorbitol-fermenting *E. coli* O157:NM (non-motile) are *yehV* and *yecE*, respectively (Serra-Moreno *et al.*, 2007; Mellmann *et al.*, 2008), *E. coli* O157:H7 strains from beef in Thailand seem to integrate into *sbcB* (Sukhumungoon and Nakaguchi, 2013) or to the sites other than reported. In the present study, since *stx$_2$* gene was not detected in all O45, O103 and O145, thus, we tried to elucidate that the integration sites may not be available for *stx$_2$* phage because of pre-integrated prophages. However, it was found that most of *E. coli* O45 and O103 strains had the intact *wrbA*, *yecE*, *yehV*, and Z2577, especially they are all intact in *E. coli* O145 (Figure 10). This suggests that the integration sites are unoccupied and available for *stx$_2$* phage integration in the future. This result leads to the worriment since these bacteria are able to gain *stx$_2$* phage and makes *E. coli* become more powerful. Particularly for aEPEC O145 that has a high dynamic interchange to be EHEC by loss and gain of *stx$_2$* phages (Bielaszewska *et al.*, 2007).

Antimicrobial resistant capability can be emerged and transferred among bacterial species and the spread of this resistant ability is now become the problems worldwide owing to the often use for treatment of infected humans and animals, including for animal growth promotion and prophylaxis (Rasheed *et al*, 2014). The rates of antimicrobial resistance of DEC strains isolated from meats in this southern Thai area has been varied study-to-study in recent years (Sukhumungoon *et*

al., 2011; Sirikaew *et al*, 2014; Sirikaew *et al*, 2015; Sirikaew *et al*, 2016; Wameadesa *et al*, 2017). Thus, the presence of resistant strains in this study was not surprising. We found that *E. coli* O45 and O103 in this study was resistant to chloramphenical, cotrimoxazole, cephalothin, streptomycin and tetracycline, especially the latter three that are corresponded to other works (O26 and O157) in the same area (Sirikaew *et al.*, 2014; Sirikaew *et al.*, 2015). The result of tetracycline resistant was also corresponded to the work from Sirikaew *et al.* (2016) that showed that 3 of 4 (75%) of *stx*-negative *E. coli* O157 were resistant to tetracycline. Furthermore, the study from Vietnam demonstrating that tetracycline is the most frequent antimicrobial resistance in raw meats (Van *et al.*, 2008). These results suggest the ineffectiveness of these antimicrobial agents for treatment in the case of possible infection by *E. coli* from meats

DNA profiling of DEC strains from meats in this geographical area has indicated that the repetitive sequence-PCR is the efficient source-tracking tool (Sirikaew *et al.*, 2015; Sirikaew *et al.*, 2016; Sukkua *et al.*, 2017). DNA fingerprint generated by PCR targeting repetitive sequence regions have proven to be reliable and reproducible for individual bacteria strains (Versalovic *et al.*, 1994). Moreover, the protocol to generate the DNA fingerprint is also simple, cheap, not laborious and no time consuming. In this current study, PCR-based DNA profiling approach such as BOX-PCR is employed and targeted the repetitive sequence called *boxA*, a 59 bp conserved DNA cassette that spread throughout bacterial genome. The finding of the identical DNA fingerprint among bacteria isolated from different sample and different time interval, exhibites that *E. coli* in the same O serotype are closely related or they are the same clone which disperses in Thai environment.

CHAPTER 5

CONCLUSION

Since the presence of STEC in non-O157 group in raw meats is important for public health. Thus, we investigated their presence in raw meats in this study. It is very obvious that *E. coli* O45, O103 and O145 indeed exist in meats sold in Hat-Yai city, southern Thailand, especially for serogroup O103 that shows very high contamination rate. Even though they lack *stx* genes, they carry some other virulence genes that also play a role in human diseases. In addition, in the case of infection, some antimicrobial agents may not be the good choices for therapy. More importantly, the intactness of stx_2 phage integration sites brings the worriment to the population in this area because these integration sites are available for stx_2 phages insertion in the future, making *E. coli* more powerful. The discovery of the identical genetic fingerprint among *E. coli* strains in each serogroup demonstrates that these *E. coli* strains disperse in Thai environment for a period of time and are capable of transferring to the consumers. This study highlights the public health importance of raw meats as potential vehicles and should be beneficial to the people in southern Thai area.

REFERENCES

Arthur, T. M., Barkocy-Gallagher, G. A., Rivera-Betancourt, M., and Koohmaraie, M. 2002b. Prevalence and characterization of non-O157 Shiga toxin-producing *Escherichia coli* on carcasses in commercial beef cattle processing plants. *Appl. Environ. Microbiol*. 68(10): 4847-4852.

Bando, S. Y., Moreno, A. C., Albuquerque, J. A., Amhaz, J. M., Moreira-Filho, C. A., and Martinez, M. B. 2010. Expression of bacterial virulence factors and cytokines during in vitro macrophage infection by enteroinvasive *Escherichia coli* and *Shigella flexneri*: a comparative study. *Memórias do Instituto Oswaldo Cruz*. 105(6): 786-791.

Barkocy-Gallagher, G. A., Arthur, T. M., Rivera-Betancourt, M., Nou, X., Shackelford, S. D., Wheeler, T. L., and Koohmaraie, M. 2003. Seasonal prevalence of Shiga toxin–producing *Escherichia coli*, including O157: H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *Journal of food protection*. 66(11): 1978-1986.

Barrett, T. J., Kaper, J. B., Jerse, A. E., and Wachsmuth, I. K. 1992. Virulence factors in Shiga-like toxin-producing *Escherichia coli* isolated from humans and cattle. *Journal of Infectious Diseases*. 165(5): 979-980.

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

Bielaszewska, M., Prager, R., Köck, R., Mellmann, A., Zhang, W., Tschäpe, H., Tarr, P. I., and Karch, H. 2007. Shiga toxin gene loss and transfer in vitro and in vivo during enterohemorrhagic *Escherichia coli* O26 infection in humans. *Applied and environmental microbiology*. 73(10): 3144-3150.

Bosilevac, J. M., and Koohmaraie, M. 2011. Prevalence and characterization of non-O157 Shiga toxin producing *Escherichia coli* isolated from commercial ground beef in the United States. *Applied and environmental microbiology*.

Brusetti, L., Malkhazova, I., Gtari, M., Tamagnini, I., Borin, S., Merabishvili, M., Chanishvili, N., Mora, D., Cappitelli, F., and Daffonchio, D. 2008.

Fluorescent-BOX-PCR for resolving bacterial genetic diversity, endemism and biogeography. *BMC microbiology*. 8(1): 220.

Campos, L. C., Franzolin, M. R., and Trabulsi, L. R. 2004. Diarrheagenic *Escherichia coli* categories among the traditional enteropathogenic *E. coli* O serogroups: a review. *Memorias do Instituto Oswaldo Cruz*. 99(6): 545-552.

Chakraborty, A., Saralaya, V., Adhikari, P., Shenoy, S., Baliga, S., and Hegde, A. 2015. Characterization of *Escherichia coli* phylogenetic groups associated with extraintestinal infections in South Indian population. *Annals of medical and health sciences research*. 5(4): 241-246.

Chapman, P., Siddons, C., Wright, D., Norman, P., Fox, J., and Crick, E. 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. *Epidemiology & Infection*. 111(3): 439-448.

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

Cheng, J., Zeng, J., Liu, L., Wei, H., Zhao, X., Zhang, X., Zhang, L., and Zhang, H. 2014. The establishment of a novel method of nano-immunomagnetic separation and Real-time PCR for detecting *Vibrio cholerae* from seafood. *Chinese journal of preventive medicine*. 48(2): 133-137.

Clermont, O., Bonacorsi, S., and Bingen, E. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Applied and environmental microbiology*. 66(10): 4555-4558.

Clermont, O., Christenson, J. K., Denamur, E., and Gordon, D. M. 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environmental microbiology reports*. 5(1): 58-65.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; Twenty-third informational supplement M100-S23. Wayne, PA, USA: CLSI, 2013.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; Twenty-third informational supplement M100-S25. Wayne, PA, USA: CLSI, 2015.

Cloete, T. E. 2003. Resistance mechanisms of bacteria to antimicrobial compounds. *International Biodeterioration & Biodegradation*. 51(4): 277-282.

Cortés, P., Blanc, V., Mora, A., Dahbi, G., Blanco, J. E., Blanco, M., López, C., Andreu, A., Navarro, F., and Alonso, M. P. 2010. Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. *Appl. Environ. Microbiol.* 76(9): 2799-2805.

Danese, P. N., Pratt, L. A., Dove, S. L., and Kolter, R. 2000. The outer membrane protein, Antigen 43, mediates cell-to-cell interactions within *Escherichia coli* biofilms. *Molecular microbiology*. 37(2): 424-432.

De Greve, H., Qizhi, C., Deboeck, F., and Hernalsteens, J.-P. 2002. The Shiga-toxin VT2-encoding bacteriophage φ297 integrates at a distinct position in the *Escherichia coli* genome. *Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression*. 1579(2-3): 196-202.

Donnenberg, M. S., and Nataro, J. P. 1995. [26] Methods for studying adhesion of diarrheagenic *Escherichia coli*. In Methods in enzymology. editors. *Elsevier*, City, pp. 324-336.

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

DuPont, H. L., Formal, S. B., Hornick, R. B., Snyder, M. J., Libonati, J. P., Sheahan, D. G., LaBrec, E. H., and Kalas, J. P. 1971. Pathogenesis of *Escherichia coli* diarrhea. *New England Journal of Medicine*. 285(1): 1-9.

Durso, L. M., Bono, J. L., and Keen, J. E. 2007. Molecular serotyping of *Escherichia coli* O111: H8. *Journal of microbiological methods*. 69(2): 381-383.

Elias, W. P., and Navarro-Garcia, F. 2016. Enteroaggregative *Escherichia coli* (EAEC). In *Escherichia coli* in the Americas. editors. *Springer*, City, pp. 27-57.

Enroth, H., and Engstrand, L. 1995. Immunomagnetic separation and PCR for detection of *Helicobacter pylori* in water and stool specimens. *Journal of clinical microbiology*. 33(8): 2162-2165.

Fardsanei, F., Nikkhahi, F., Bakhshi, B., Salehi, T. Z., Tamai, I. A., and Dallal, M. S. 2016. Molecular characterization of *Salmonella enterica* serotype Enteritidis isolates from food and human samples by serotyping, antimicrobial resistance, plasmid profiling,(GTG) 5-PCR and ERIC-PCR. *New microbes and new infections*. 14: 24-30.

Fu, Z., Rogelj, S., and Kieft, T. L. 2005. Rapid detection of *Escherichia coli* O157: H7 by immunomagnetic separation and real-time PCR. *International journal of food microbiology*. 99(1): 47-57.

Galland, J. C., Hyatt, D. R., Crupper, S. S., and Acheson, D. W. 2001. Prevalence, antibiotic susceptibility, and diversity of *Escherichia coli* O157: H7 isolates from a longitudinal study of beef cattle feedlots. *Applied and Environmental Microbiology*. 67(4): 1619-1627.

Gannon, V., Rashed, M., King, R. K., and Thomas, E. 1993. Detection and characterization of the eae gene of Shiga-like toxin-producing *Escherichia coli* using polymerase chain reaction. *Journal of clinical microbiology*. 31(5): 1268-1274.

Gevers, D., Huys, G., and Swings, J. 2001. Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. *FEMS Microbiology Letters*. 205(1): 31-36.

Gillings, M., and Holley, M. 1997. Repetitive element PCR fingerprinting (rep-PCR) using enterobacterial repetitive intergenic consensus (ERIC) primers is not necessarily directed at ERIC elements. *Letters in Applied Microbiology*. 25(1): 17-21.

Gióon, J., Jones, T., Millan-Velasco, F., Castro-Munoz, E., Zarate, L., Fry, J., Frankel, G., Moseley, S., Baudry, B., and Kaper, J. 1991. Diffuse-adhering

Escherichia coli (DAEC) as a putative cause of diarrhea in Mayan children in Mexico. *Journal of Infectious Diseases*. 163(3): 507-513.

Gunzburg, S. T., Tornieporth, N. G., and Riley, L. W. 1995. Identification of enteropathogenic *Escherichia coli* by PCR-based detection of the bundle-forming pilus gene. *Journal of Clinical Microbiology*. 33(5): 1375-1377.

Hara-Kudo, Y., Konuma, H., Nakagawa, H., and Kumagai, S. 2000. *Escherichia coli* O26 detection from foods using an enrichment procedure and an immunomagnetic separation method. *Letters in applied microbiology*. 30(2): 151-154.

Heininger, A., Binder, M., Schmidt, S., Unertl, K., Botzenhart, K., and Döring, G. 1999. PCR and blood culture for detection of *Escherichia coli* bacteremia in rats. *Journal of clinical microbiology*. 37(8): 2479-2482.

Herschleb, J., Ananiev, G., and Schwartz, D. C. 2007. Pulsed-field gel electrophoresis. *Nature protocols*. 2(3): 677.

Hicks, S., Frankel, G., Kaper, J. B., Dougan, G., and Phillips, A. D. 1998. Role of intimin and bundle-forming pili in enteropathogenic *Escherichia coli* adhesion to pediatric intestinal tissue in vitro. *Infection and immunity*. 66(4): 1570-1578.

Hsih, H.-Y., and Tsen, H.-Y. 2001. Combination of immunomagnetic separation and polymerase chain reaction for the simultaneous detection of *Listeria monocytogenes* and *Salmonella* spp. in food samples. *Journal of food protection*. 64(11): 1744-1750.

Hulton, C., Higgins, C., and Sharp, P. 1991. ERIC sequences: a novel family of repetitive elements in the genomes of *Escherichia coli*, *Salmonella typhimurium* and other enterobacteria. *Molecular microbiology*. 5(4): 825-834.

Johne, B., Jarp, J., and Haaheim, L. 1989. *Staphylococcus aureus* exopolysaccharide in vivo demonstrated by immunomagnetic separation and electron microscopy. *Journal of clinical microbiology*. 27(7): 1631-1635.

Johnson, J. R., Brown, J. J., Carlino, U. B., and Russo, T. A. 1998. Colonization with and acquisition of uropathogenic *Escherichia coli* as revealed by polymerase

chain reaction-based detection. *Journal of Infectious Diseases*. 177(4): 1120-1124.

Johnson, J. R., Kuskowski, M. A., Menard, M., Gajewski, A., Xercavins, M., and Garau, J. 2006. Similarity between human and chicken *Escherichia coli* isolates in relation to ciprofloxacin resistance status. *The Journal of infectious diseases*. 194(1): 71-78.

Johnson, J. R., Murray, A. C., Gajewski, A., Sullivan, M., Snippes, P., Kuskowski, M. A., and Smith, K. E. 2003. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. *Antimicrobial agents and chemotherapy*. 47(7): 2161-2168.

Johnson, J. R., and Russo, T. A. 2005. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. *International journal of medical microbiology*. 295(6-7): 383-404.

Johnson, J. R., and Stell, A. L. 2000. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. *The Journal of infectious diseases*. 181(1): 261-272.

Jones, J. 2015. High throughput screening of seafood for foodborne pathogens. In High Throughput Screening for Food Safety Assessment. editors. *Elsevier*, City, pp. 491-505.

Ju, W., Shen, J., Li, Y., Toro, M. A., Zhao, S., Ayers, S., Najjar, M. B., and Meng, J. 2012. Non-O157 Shiga toxin-producing *Escherichia coli* in retail ground beef and pork in the Washington DC area. *Food microbiology*. 32(2): 371-377.

Kaper, J. B. 1998. Enterohemorrhagic *Escherichia coli*. *Current opinion in microbiology*. 1(1): 103-108.

Karch, H., Strockbine, N. A., and O'Brien, A. D. 1986. Growth of *Escherichia coli* in the presence of trimethoprim-sulfamethoxazole facilitates detection of Shiga-like toxin producing strains by colony blot assay. *FEMS microbiology letters*. 35(2-3): 141-145.

Karmali, M., Petric, M., Steele, B., and Lim, C. 1983. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *The Lancet*. 321(8325): 619-620.

Kathleen, M., Samuel, L., Felecia, C., Ng, K., Lesley, M., and Kasing, A. 2014. (GTG) 5-PCR analysis and 16S rRNA sequencing of bacteria from Sarawak aquaculture environment. *International Food Research Journal*. 21(3).

Kaufmann, M. E. 1998. Pulsed-field gel electrophoresis. In Molecular Bacteriology. editors. *Springer*, City, pp. 33-50.

Kayali, A., Escalante-Maldonado, O., Vuddhakul, V., Seto, K., and Nakaguchi, Y. 2015. Development of a Method for Detection of Shigatoxin-Producing *Escherichia coli* Belonging to Clinically Important Twelve O Serotypes Based on the Combination of PickPen-Assisted Immunomagnetic Separation and Loop-Mediated Isothermal Amplification. *Int J Immunol Immunother*. 2(004).

Koch, C., Hertwig, S., and Appel, B. 2003. Nucleotide sequence of the integration site of the temperate bacteriophage 6220, which carries the Shiga toxin gene stx1ox3. *Journal of bacteriology*. 185(21): 6463-6466.

Köhler, C.-D., and Dobrindt, U. 2011. What defines extraintestinal pathogenic *Escherichia coli*? *International Journal of Medical Microbiology*. 301(8): 642-647.

Le Bouguenec, C., Archambaud, M., and Labigne, A. 1992. Rapid and specific detection of the pap, afa, and sfa adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. *Journal of clinical microbiology*. 30(5): 1189-1193.

Lecointre, G., Rachdi, L., Darlu, P., and Denamur, E. 1998. *Escherichia coli* molecular phylogeny using the incongruence length difference test. *Molecular biology and evolution*. 15(12): 1685-1695.

Mellmann, A., Lu, S., Karch, H., Xu, J.-g., Harmsen, D., Schmidt, M. A., and Bielaszewska, M. 2008. Recycling of Shiga toxin 2 genes in sorbitol-fermenting enterohemorrhagic *Escherichia coli* O157: NM. *Appl. Environ. Microbiol*. 74(1): 67-72.

Meng, J., Zhao, S., Doyle, M. P., and Joseph, S. W. 1998. Antibiotic resistance of *Escherichia coli* O157: H7 and O157: NM isolated from animals, food, and humans. *Journal of Food Protection*. 61(11): 1511-1514.

Meyer, W., Mitchell, T. G., Freedman, E., and Vilgalys, R. 1993. Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans*. *Journal of Clinical Microbiology*. 31(9): 2274-2280.

Michino, H., Araki, K., Minami, S., Takaya, S., Sakai, N., Miyazaki, M., Ono, A., and Yanagawa, H. 1999. Massive outbreak of *Escherichia coli* O157: H7 infection in schoolchildren in Sakai City, Japan, associated with consumption of white radish sprouts. *American journal of epidemiology*. 150(8): 787-796.

Monday, S., Beisaw, A., and Feng, P. 2007. Identification of Shiga toxigenic *Escherichia coli* seropathotypes A and B by multiplex PCR. *Molecular and cellular probes*. 21(4): 308-311.

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

Nath, G., Maurya, P., and Gulati, A. K. 2010. ERIC PCR and RAPD based fingerprinting of *Salmonella* Typhi strains isolated over a period of two decades. *Infection, genetics and evolution*. 10(4): 530-536.

O'Brien, A. D., and Holmes, R. K. 1987. Shiga and Shiga-like toxins. *Microbiological reviews*. 51(2): 206.

O'Brien, A. D., Tesh, V., Donohue-Rolfe, A., Jackson, M., Olsnes, S., Sandvig, K., Lindberg, A., and Keusch, G. 1992. Shiga toxin: biochemistry, genetics, mode of action, and role in pathogenesis. In Pathogenesis of Shigellosis. editors. *Springer*, City, pp. 65-94.

Ohnishi, M., Terajima, J., Kurokawa, K., Nakayama, K., Murata, T., Tamura, K., Ogura, Y., Watanabe, H., and Hayashi, T. 2002. Genomic diversity of enterohemorrhagic *Escherichia coli* O157 revealed by whole genome PCR scanning. *Proceedings of the National Academy of Sciences*. 99(26): 17043-17048.

Olorunmola, F. O., Kolawole, D. O., and Lamikanra, A. 2013. Antibiotic Resistance and Virulence Properties in *Escherichia coli* Strains from Cases of Urinary Tract Infections. *African journal of infectious diseases*. 7(1): 1-7.

Ooka, T., Terajima, J., Kusumoto, M., Iguchi, A., Kurokawa, K., Ogura, Y., Asadulghani, M., Nakayama, K., Murase, K., and Ohnishi, M. 2009. Development of a multiplex PCR-based rapid typing method for enterohemorrhagic *Escherichia coli* O157 strains. *Journal of clinical microbiology*. 47(9): 2888-2894.

Pannuch, M., Sirikaew, S., Nakaguchi, Y., Nishibuchi, M., and Sukhumungoon, P. 2014. Quantification of enteropathogenic *Escherichia coli* from retailed meats. *International Food Research Journal*. 21(2).

Paton, A. W., and Paton, J. C. 1996. Enterobacter cloacae producing a Shiga-like toxin II-related cytotoxin associated with a case of hemolytic-uremic syndrome. *Journal of clinical microbiology*. 34(2): 463-465.

Pearce, M., Evans, J., McKendrick, I., Smith, A., Knight, H., Mellor, D., Woolhouse, M., Gunn, G., and Low, J. 2006. Prevalence and virulence factors of *Escherichia coli* serogroups O26, O103, O111, and O145 shed by cattle in Scotland. *Appl. Environ. Microbiol*. 72(1): 653-659.

Picard, B., Garcia, J. S., Gouriou, S., Duriez, P., Brahimi, N., Bingen, E., Elion, J., and Denamur, E. 1999. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. *Infection and immunity*. 67(2): 546-553.

Qadri, F., Svennerholm, A.-M., Faruque, A., and Sack, R. B. 2005. Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clinical microbiology reviews*. 18(3): 465-483.

Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M., and Swerdlow, D. L. 2005. Epidemiology of *Escherichia coli* O157: H7 outbreaks, united states, 1982–2002. *Emerging infectious diseases*. 11(4): 603.

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

Sabat, A., Budimir, A., Nashev, D., Sá-Leão, R., Van Dijl, J., Laurent, F., Grundmann, H., Friedrich, A., and Markers, E. S. G. o. E. 2013. Overview of molecular typing methods for outbreak detection and epidemiological surveillance. *Eurosurveillance*. 18(4): 20380.

Sack, R. B. 1980. Enterotoxigenic *Escherichia coli*: identification and characterization. *The Journal of infectious diseases*. 142(2): 279-286.

Sae-lim, A., Jearanai, P., Rattanachuay, P., and Sukhumungoon, P. 2017. Prevalence, virulence profiles, and genetic relationship of atypical enteropathogenic *Escherichia coli* o145 from beef, southern Thailand. *Southeast asian journal of tropical medicine and public health*. 48(6): 1248-1259.

Schmidt, H., Plaschke, B., Franke, S., Rüssmann, H., Schwarzkopf, A., Heesemann, J., and Karch, H. 1994. Differentiation in virulence patterns of *Escherichia coli* possessing *eae* genes. *Medical microbiology and immunology*. 183(1): 23-31.

Schmidt, H., Zhang, W.-L., Hemmrich, U., Jelacic, S., Brunder, W., Tarr, P., Dobrindt, U., Hacker, J., and Karch, H. 2001. Identification and characterization of a novel genomic island integrated at selC in locus of enterocyte effacement-negative, Shiga toxin-producing *Escherichia coli*. *Infection and immunity*. 69(11): 6863-6873.

Schroeder, C. M., Zhao, C., DebRoy, C., Torcolini, J., Zhao, S., White, D. G., Wagner, D. D., McDermott, P. F., Walker, R. D., and Meng, J. 2002. Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine, and food. *Applied and Environmental Microbiology*. 68(2): 576-581.

Schwartz, D. C., and Cantor, C. R. 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *cell*. 37(1): 67-75.

Selander, R. K., Korhonen, T. K., Väisänen-Rhen, V., Williams, P., Pattison, P., and Caugant, D. 1986. Genetic relationships and clonal structure of strains of *Escherichia coli* causing neonatal septicemia and meningitis. *Infection and immunity*. 52(1): 213-222.

Serra-Moreno, R., Jofre, J., and Muniesa, M. 2007. Insertion site occupancy by *stx₂* bacteriophages depends on the locus availability of the host strain chromosome. *Journal of bacteriology*. 189(18): 6645-6654.

Sethabutr, O., Venkatesan, M., Murphy, G. S., Eampokalap, B., Hoge, C. W., and Echeverria, P. 1993. Detection of Shigellae and enteroinvasive *Escherichia coli* by amplification of the invasion plasmid antigen H DNA sequence in patients with dysentery. *Journal of Infectious Diseases*. 167(2): 458-461.

Shaikh, N., and Tarr, P. I. 2003. *Escherichia coli* O157: H7 Shiga toxin-encoding bacteriophages: integrations, excisions, truncations, and evolutionary implications. *Journal of bacteriology*. 185(12): 3596-3605.

Sharples, G. J., and Lloyd, R. G. 1990. A novel repeated DNA sequence located in the intergenic regions of bacterial chromosomes. *Nucleic acids research*. 18(22): 6503-6508.

Sheldon, I. M., Rycroft, A. N., Dogan, B., Craven, M., Bromfield, J. J., Chandler, A., Roberts, M. H., Price, S. B., Gilbert, R. O., and Simpson, K. W. 2010. Specific strains of *Escherichia coli* are pathogenic for the endometrium of cattle and cause pelvic inflammatory disease in cattle and mice. *Plos one*. 5(2): e9192.

Shpigel, N. Y., Elazar, S., and Rosenshine, I. 2008. Mammary pathogenic *Escherichia coli*. *Current opinion in microbiology*. 11(1): 60-65.

Sirikaew, S., Patungkaro, W., Rattanachuay, P., Sukkua, K., and Sukhumungoon, P. 2014. Enterotoxigenic *Escherichia coli* O169: HUT from a diarrheal patient: phylogenetic group and antimicrobial susceptibility. *Southeast Asian Journal of Tropical Medicine and Public Health*. 45(6): 1376.

Sirikaew, S., Rattanachuay, P., Nakaguchi, Y., and Sukhumungoon, P. 2015. Immuno-magnetic isolation, characterization and genetic relationship of *Escherichia coli* O26 from raw meats, Hat Yai City, Songkhla, Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*. 46(2): 241.

Sirikaew, S., Sukkua, K., Rattanachuay, P., Khianngam, S., and Sukhumungoon, P. 2016. High level of Shiga toxin-producing *Escherichia coli* and occurrence of stx-negative *E. coli* O157 from raw meats: characterization of virulence

profile and genetic relatedness. *Southeast Asian J Trop Med Public Health*. 47: 1008-1019.

Skjerve, E., and Olsvik, Ø. 1991. Immunomagnetic separation of *Salmonella* from foods. *International journal of food microbiology*. 14(1): 11-17.

Small, P., Blankenhorn, D., Welty, D., Zinser, E., and Slonczewski, J. L. 1994. Acid and base resistance in *Escherichia coli* and *Shigella flexneri*: role of rpoS and growth pH. *Journal of bacteriology*. 176(6): 1729-1737.

Smith, J. L., Fratamico, P. M., and Gunther, N. W. 2007. Extraintestinal pathogenic *Escherichia coli*. *Foodborne pathogens and disease*. 4(2): 134-163.

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

Stacy-Phipps, S., Mecca, J. J., and Weiss, J. B. 1995. Multiplex PCR assay and simple preparation method for stool specimens detect enterotoxigenic *Escherichia coli* DNA during course of infection. *Journal of clinical microbiology*. 33(5): 1054-1059.

Steiner, T. S., Nataro, J. P., Poteet-Smith, C. E., Smith, J. A., and Guerrant, R. L. 2000. Enteroaggregative *Escherichia coli* expresses a novel flagellin that causes IL-8 release from intestinal epithelial cells. *The Journal of clinical investigation*. 105(12): 1769-1777.

Stratton, K. R., Durch, J. S., and Lawrence, R. S. 2000. Enterotoxigenic *E. coli*. In Vaccines for the 21st Century: A Tool for Decisionmaking. editors. *National Academies Press* (US), City, pp.

Sukhumungoon, P., and Nakaguchi, Y. 2013. Shiga toxin 2-converting bacteriophages occupy *sbcB* gene as a primary integration site in bovine-origined *Escherichia coli* O157: H7 and non-O157 from Thailand. *Life Sci J*. 10: 2334-2340.

Sukhumungoon, P., Nakaguchi, Y., Ingviya, N., Pradutkanchana, J., Iwade, Y., Seto, K., Son, R., Nishibuchi, M., and Vuddhakul, V. 2011. Investigation of stx_2+ $eae+$ *Escherichia coli* O157: H7 in beef imported from Malaysia to Thailand. *International Food Research Journal*. 18(1).

Sukhumungoon, P., Tantadapan, R., and Rattanachuay, P. 2016. Repetitive sequence based-pcr profiling of *Escherichia coli* O157 strains from beef in southern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*. 47(1): 55.

Sukkua, K., Pomwised, R., Rattanachuay, P., Khianngam, S., and Sukhumungoon, P. 2017. Characterization of extraintestinal pathogenic *Escherichia coli* from meat in southern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*. 48(1): 98-108.

Švec, P., Pantůček, R., Petráš, P., Sedláček, I., and Nováková, D. 2010. Identification of *Staphylococcus* spp. using (GTG) 5-PCR fingerprinting. *Systematic and applied microbiology*. 33(8): 451-456.

Švec, P., Vancanneyt, M., Seman, M., Snauwaert, C., Lefebvre, K., Sedláček, I., and Swings, J. 2005. Evaluation of (GTG) 5-PCR for identification of *Enterococcus* spp. *FEMS Microbiology Letters*. 247(1): 59-63.

Tiba, M. R., Yano, T., and Leite, D. d. S. 2008. Genotypic characterization of virulence factors in *Escherichia coli* strains from patients with cystitis. *Revista do Instituto de Medicina Tropical de Sao Paulo*. 50(5): 255-260.

Torres, A. G., Giron, J. A., Perna, N. T., Burland, V., Blattner, F. R., Avelino-Flores, F., and Kaper, J. B. 2002. Identification and characterization of lpfABCC′ DE, a fimbrial operon of enterohemorrhagic *Escherichia coli* O157: H7. *Infection and immunity*. 70(10): 5416-5427.

Torres, A. G., Kanack, K. J., Tutt, C. B., Popov, V., and Kaper, J. B. 2004. Characterization of the second long polar (LP) fimbriae of *Escherichia coli* O157: H7 and distribution of LP fimbriae in other pathogenic *E. coli* strains. *FEMS microbiology letters*. 238(2): 333-344.

Tóth, I., Schmidt, H., Dow, M., Malik, A., Oswald, E., and Nagy, B. 2003. Transduction of porcine enteropathogenic *Escherichia coli* with a derivative of a Shiga toxin 2-encoding bacteriophage in a porcine ligated ileal loop system. *Appl. Environ. Microbiol*. 69(12): 7242-7247.

Trabulsi, L. R., Keller, R., and Gomes, T. A. T. 2002. 10.321/eid0805. Typical and Atypical Enteropathogenic *Escherichia coli*. *Emerging infectious diseases*. 8(5): 508.

Tsukamoto, T. 1996. PCR methods for detection of enteropathogenic *Escherichia coli* (localized adherence) and enteroaggregative *Escherichia coli*. Kansenshogaku zasshi. *The Journal of the Japanese Association for Infectious Diseases*. 70(6): 569-573.

van den Beld, M., and Reubsaet, F. 2012. Differentiation between *Shigella*, enteroinvasive *Escherichia coli* (EIEC) and noninvasive *Escherichia coli*. *European journal of clinical microbiology & infectious diseases*. 31(6): 899-904.

Van Kessel, J., Karns, J., Gorski, L., and Perdue, M. 2005. Subtyping *Listeria monocytogenes* from bulk tank milk using automated repetitive element–based PCR. *Journal of food protection*. 68(12): 2707-2712.

Van, T. T. H., Chin, J., Chapman, T., Tran, L. T., and Coloe, P. J. 2008. Safety of raw meat and shellfish in Vietnam: an analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. *International journal of food microbiology*. 124(3): 217-223.

Versalovic, J., Schneider, M., De Bruijn, F., and Lupski, J. R. 1994. Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. *Methods in molecular and cellular biology*. 5(1): 25-40.

Vidal, M., Kruger, E., Durán, C., Lagos, R., Levine, M., Prado, V., Toro, C., and Vidal, R. 2005. Single multiplex PCR assay to identify simultaneously the six categories of diarrheagenic *Escherichia coli* associated with enteric infections. *Journal of clinical microbiology*. 43(10): 5362-5365.

Vila, J., Vargas, M., Henderson, I. R., Gascón, J., and Nataro, J. P. 2000. Enteroaggregative *Escherichia coli* virulence factors in traveler's diarrhea strains. *The Journal of infectious diseases*. 182(6): 1780-1783.

Wameadesa, N., Sae-lim, A., Hayeebilan, F., Rattanachuay, P., and Sukhumungoon, P. 2017. Enteroaggregative *Escherichia coli* O104 from Thai and imported

Malaysian raw beef. *The Southeast Asian journal of tropical medicine and public health*. 48(2): 338-350.

Wiid, I., Werely, C., Beyers, N., Donald, P., and van Helden, P. D. 1994. Oligonucleotide (GTG) 5 as a marker for *Mycobacterium tuberculosis* strain identification. *Journal of clinical microbiology*. 32(5): 1318-1321.

Wiles, T. J., Kulesus, R. R., and Mulvey, M. A. 2008. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. *Experimental and molecular pathology*. 85(1): 11-19.

Wright, D., Chapman, P., and Siddons, C. 1994. Immunomagnetic separation as a sensitive method for isolating *Escherichia coli* O157 from food samples. *Epidemiology & Infection*. 113(1): 31-39.

Yamamoto, S., Terai, A., Yuri, K., Kurazono, H., Takeda, Y., and Yoshida, O. 1995. Detection of urovirulence factors in *Escherichia coli* by multiplex polymerase chain reaction. *FEMS Immunology & Medical Microbiology*. 12(2): 85-90.

Zhang, X., McDaniel, A. D., Wolf, L. E., Keusch, G. T., Waldor, M. K., and Acheson, D. W. 2000. Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice. *The Journal of infectious diseases*. 181(2): 664-670.

APPENDIX

APPENDIX A. Culture Media

1. Mueller-Hinton Agar (MHA)

Beef extract	300.0	g
Casamino acids technical	17.5	g
Starch	1.5	g
Agar	15.0	g
Distilled water (dH$_2$O)	1000.0	ml

Dissolve the mixture in dH$_2$O and autoclave at 121ºC, 15 psi for 15 min.

2. Mueller-Hinton Broth (MHB)

Beef extract	300.0	g
Bacto Casamino acids technical	17.5	g
Bacto solutble starch	1.5	g
Distilled Water (dH$_2$O)	1000.0	ml

Dissolve the mixture in dH$_2$O and autoclave at 121ºC, 15 psi for 15 min.

3. Tryptic Soy Agar (TSA)

Pancreatic digest of casein	17.0	g
Papaic digest of soybean	3.0	g
Dextrose	2.5	g
Sodium chloride (NaCl)	5.0	g
Agar	15.0	g
Distilled water (dH$_2$O)	1000.0	ml

Dissolve the mixture in dH$_2$O and autoclave at 121ºC, 15 psi for 15 min.

4. Tryptic Soy Broth (TSB)

Pancreatic digest of casein	17.0	g
Papaic digest of soybean	3.0	g

Dextrose	2.5	g
NaCl	5.0	g
Distilled water (dH$_2$O)	1000.0	ml

Dissolve the mixture in dH$_2$O and autoclave at 121ºC, 15 psi for 15 min.

5. Eosin Methylene Blue Agar

Peptone	10.0	g
Lactose	10.0	g
Dipotassium hydrogen phosphate	2.0	g
Eosin Y	0.4	g
Methylene blue	0.065	g
Agar	15.0	g
Distilled water (dH$_2$O)	1000.0	ml

Dissolve the mixture in dH$_2$O and autoclave at 121ºC, 15 psi for 15 min.

APPENDIX B. Reagents and buffers

1. 0.85% Sodium chloride

NaCl	8.5	g
Distilled water (dH$_2$O)	1000.0	ml

Dissolve NaCl in dH$_2$O and autoclave at 121ºC, 15 psi for 15 min.

2. 0.5 M EDTA (pH 8.0)

EDTA.2H$_2$O	186.1	g
Distilled water (dH$_2$O)	1000.0	ml

Dissolve EDTA.2H$_2$O in dH$_2$O, adjust pH to 8.0 with NaOH and autoclave at 121ºC, 15 psi for 15 min.

3. 6X Loading dye

Bromophenol blue	0.25	g
Sucrose	40.0	g
0.05M EDTA (pH8)	10.0	ml
DI	up to 100.0	ml

Dissolve bromophenol blue and sucrose in DI (around 50 ml), vortex until it was dissolved well, add 10.0 ml of 0.05M EDTA (pH8), and then adjust the volume to 100 ml.

4. 10X Phosphate buffered saline (PBS) (pH7.4)

Na_2HPO_4	14.4	g
KH_2PO_4	2.4	g
NaCl	80.0	g
KCl	2.0	g
Distilled water (dH_2O)	1000.0	ml

Dissolve ingredients in dH_2O, adjust pH to 7.4 with NaCl and autoclave at 121ºC, 15 psi for 15 min.

5. Tris-borate-EDTA (TBE), 10X

Tris base	108.0	g
Boric acid	55.0	g
0.5 M EDTA, pH 8.0	40.0	ml
Distilled water (dH_2O)	1000.0	ml

Dissolve Tris-base and boric acid in dH_2O, and then add 40 ml 0.5M EDTA, pH 8.0 and autoclaving at 121°C, 15 psi for 15 min.

APPENDIX C. Characterization of 49 *E. coli* O45 strains

Sample source	Sample ID	*wzx*O45	*uidA*	DEC indicator gene									ExPEC indicator gene					
													Gr. 1		Gr. 2	Gr. 3	Gr. 4	Gr. 5
				stx$_1$	*stx*$_2$	*eae*	*bfp*A	*elt*	*est*	*agg*R	*ipa*H	*daa*E	*pap*A	*pap*C	*sfa*DE	*afa*	*Kps*MTII	*iut*A
Beef	**48.5**	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	–
	48.20	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	–
Chicken	**57.1**	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	57.2	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	57.7	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	57.9	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	57.13	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
Chicken	**58.18**	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
Pork	**60.5**	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	–
Pork	**63..5**	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	–
Chicken	**64.5**	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	64.6	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	64.7	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	64.8	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	64.9	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	64.10	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	64.12	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	64.15	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	64.18	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	64.19	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	64.20	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+

APPENDIX C. Characterization of 49 *E. coli* O45 strains (continued)

Sample source	Sample ID	*wzx*O45	*uidA*	DEC indicator gene									ExPEC indicator gene					
													Gr. 1		Gr. 2	Gr. 3	Gr. 4	Gr. 5
				stx_1	stx_2	*eae*	*bfp*A	*elt*	*est*	*agg*R	*ipa*H	*daa*E	*pap*A	*pap*C	*sfa*DE	*afa*	*Kps*MTII	*iut*A
Chicken	**71.6**	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	71.19	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
Chicken	**72.7**	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	72.8	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	72.14	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	72.16	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	72.19	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
Pork	**76.4**	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	-
	76.20	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	-
Chicken	**78.6**	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	78.10	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	78.16	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
Chicken	**80.1**	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	80.2	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	80.3	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	80.4	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	80.6	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	80.8	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	80.10	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	80.11	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	80.12	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+

APPENDIX C. Characterization of 49 *E. coli* O45 strains (continued)

Sample source	Sample ID	*wzx*O45	*uidA*	DEC indicator gene									ExPEC indicator gene					
													Gr. 1		Gr. 2	Gr. 3	Gr. 4	Gr. 5
				stx_1	stx_2	*eae*	*bfp*A	*elt*	*est*	*agg*R	*ipa*H	*daa*E	*pap*A	*pap*C	*sfa*DE	*afa*	*Kps*MTII	*iut*A
	80.14	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	80.15	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	80.16	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	80.18	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
Chicken	**87.1**	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
Pork	**95.1**	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+
	95.9	+	+	-	-	-	-	-	-	-	-	-	–	–	–	–	–	+

Number of isolates (bold) = surrogated strains for BOX-PCR analysis

APPENDIX C. Further characterization of 49 *E. coli* O45 strains

Sample source	Sample ID	Other *E. coli* virulence genes						Phylogenetic group	*stx*2 phage integration site					Antimicrobial resistance
		agn43	*ast*A	cnf*1*	*fim*H	*hly*A	*lpf*		*wrbA*	*yecE*	*yehV*	*sbcB*	Z2577	
Beef	**48.5**	-	-	-	+	-	-	B1	+	+	+	-	+	KF
	48.20	-	-	-	+	-	-	B1	+	+	+	-	+	KF
Chicken	**57.1**	-	-	-	+	-	+	D	+	+	+	-	-	CN, KF
	57.2	-	-	-	+	-	+	D	+	+	+	-	+	CN, KF
	57.7	-	-	-	+	-	+	D	+	+	+	-	+	CN, KF
	57.9	-	-	-	+	-	+	D	+	+	+	+	+	CN, KF
	57.13	-	-	-	+	-	+	D	+	+	+	-	+	CN
Chicken	**58.18**	-	+	-	+	-	-	D	+	+	+	-	+	C, CN, KF, S, SXT, TE
Pork	**60.5**	-	+	-	+	-	-	A	+	+	+	-	+	KF, S, TE
Pork	**63..5**	-	+	-	+	-	-	A	+	+	+	-	+	C, SXT, TE
Chicken	**64.5**	-	+	-	+	-	-	D	+	+	+	-	+	KF, S, SXT
	64.6	-	-	-	+	-	+	D	+	+	+	-	+	CN, K, KF
	64.7	-	-	-	+	-	+	D	+	+	+	-	+	CN, KF
	64.8	-	-	-	+	-	+	D	+	+	+	+	+	CN, KF
	64.9	-	-	-	+	-	+	D	+	+	+	-	+	CN, K, KF
	64.10	-	-	-	+	-	+	D	+	+	+	-	+	CN, KF
	64.12	-	-	-	+	-	+	D	+	+	+	-	+	CN, KF
	64.15	-	+	-	+	-	-	D	+	+	+	-	+	KF, S, SXT
	64.18	-	-	-	+	-	+	D	+	+	+	-	+	CN, KF
	64.19	-	-	-	+	-	+	D	+	+	+	-	+	CN, KF
	64.20	-	-	-	+	-	+	D	+	+	+	-	+	CN, FOS, KF, S

APPENDIX C. Further characterization of 49 *E. coli* O45 strains (continued)

Sample source	Sample ID	Other *E. coli* virulence genes						Phylogenetic group	*stx*2 phage integration site					Antimicrobial resistance
		agn43	*ast*A	cnf*1*	*fim*H	*hly*A	*lpf*		*wrbA*	*yecE*	*yehV*	*sbcB*	Z2577	
Chicken	**71.6**	-	+	-	+	-	-	D	+	+	+	-	+	KF, S, SXT
	71.19	-	+	-	+	-	-	D	+	+	+	-	+	KF, S, SXT
Chicken	**72.7**	-	+	-	+	-	-	D	+	+	+	-	+	KF, S, SXT
	72.8	-	+	-	+	-	-	D	+	+	+	-	+	KF, S, SXT
	72.14	-	+	-	+	-	-	D	+	+	+	-	+	KF, S, SXT
	72.16	-	+	-	+	-	-	D	+	+	+	-	+	S, TE
	72.19	-	+	-	+	-	-	D	+	-	+	-	+	KF, S, SXT
Pork	**76.4**	-	-	-	+	-	-	A	+	+	+	-	+	KF, S, TE
	76.20	-	+	-	+	-	-	A	+	+	+	-	+	K, KF, S, TE
Chicken	**78.6**	-	+	-	+	-	-	D	+	+	+	-	-	C, S, SXT, TE
	78.10	-	+	-	+	-	-	D	+	+	+	-	+	C, KF, S, SXT, TE
	78.16	-	-	-	+	-	+	D	+	+	+	-	+	CN, KF
Chicken	**80.1**	-	+	-	+	-	-	D	-	+	+	-	-	SA
	80.2	-	+	-	+	-	-	D	-	+	+	-	-	C, KF, S, SXT, TE
	80.3	-	+	-	+	-	-	D	-	+	+	-	-	S
	80.4	-	+	-	+	-	-	D	+	+	+	-	+	C, KF, S, SXT, TE
	80.6	-	+	-	+	-	-	D	+	+	+	-	+	KF
	80.8	-	+	-	+	-	-	D	-	+	+	-	-	SA
	80.10	-	+	-	+	-	-	D	-	+	+	-	-	KF
	80.11	-	+	-	+	-	-	D	+	+	+	-	+	C, KF, S, SXT, TE
	80.12	-	+	-	+	-	-	D	-	+	+	-	-	SA

APPENDIX C. Further characterization of 49 *E. coli* O45 strains (continued)

Sample source	Sample ID	Other *E. coli* virulence genes						Phylogenetic group	*stx*2 phage integration site					Antimicrobial resistance
		agn43	*ast*A	cnf*1*	*fim*H	*hly*A	*lpf*		*wrbA*	*yecE*	*yehV*	*sbcB*	Z2577	
	80.14	-	+	-	+	-	-	D	+	+	+	-	+	C, KF, S, SXT, TE
	80.15	-	+	-	+	-	-	D	-	+	+	-	+	S
	80.16	-	+	-	+	-	-	D	-	+	+	-	-	SA
	80.18	-	+	-	+	-	-	D	+	+	+	-	-	SA
Chicken	**87.1**	-	+	-	+	-	-	D	+	+	+	-	-	C, KF, S, SXT, TE
Pork	**95.1**	-	+	-	+	-	-	D	-	+	+	-	-	C, KF, S, SXT, TE
	95.9	-	+	-	+	-	-	D	-	+	+	-	-	C, KF, S, SXT, TE

Number of isolates (bold) = surrogated strains for BOX-PCR analysis

C, chloramphenicol; CN, gentamicin; FOS, fosfomycin; K, kanamycin; KF, cephalothin; S, streptomycin; SXT, cotrimoxazole; TE, tetracycline; SA, susceptible to all agents

APPENDIX D. Characterization of 125 *E. coli* O103 strains

Sample source	Sample ID	*wzx*O103	*uidA*	DEC indicator gene									ExPEC indicator gene					
													Gr. 1		Gr. 2	Gr. 3	Gr. 4	Gr. 5
				stx₁	*stx₂*	*eae*	*bfp*A	*elt*	*est*	*agg*R	*ipa*H	*daa*E	*pap*A	*pap*C	*sfa*DE	*afa*	*Kps*MTII	*iut*A
Beef	**4.15**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	**5.2**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	5.9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	**7.8**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	**10.12**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	14.5	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	14.8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	14.10	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	**16.1**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	16.4	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	17.2	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	17.7	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	17.10	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Beef	**22.8**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Beef	**27.7**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Beef	**31.1**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	31.2	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	**35.1**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	35.3	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	35.6	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	35.9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

APPENDIX D. Characterization of 125 *E. coli* O103 strains (continued)

Sample source	Sample ID	*wzx*O103	*uidA*	DEC indicator gene									ExPEC indicator gene					
													Gr. 1		Gr. 2	Gr. 3	Gr. 4	Gr. 5
				stx₁	*stx₂*	*eae*	*bfp*A	*elt*	*est*	*agg*R	*ipa*H	*daa*E	*pap*A	*pap*C	*sfa*DE	*afa*	*Kps*MTII	*iut*A
Beef	**36.10**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	**41.5**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	41.6	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	41.9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	**42.3**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	42.9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	**43.5**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	43.9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	**44.3**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	44.4	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	44.5	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	44.6	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	44.7	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	44.8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	**45.3**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	45.4	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	45.6	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	**49.1**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	**50.5**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	50.7	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**54.10**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

APPENDIX D. Characterization of 125 *E. coli* O103 strains (continued)

| Sample source | Sample ID | *wzx*O103 | *uidA* | DEC indicator gene | | | | | | | | | ExPEC indicator gene | | | | | |
| | | | | | | | | | | | | | Gr. 1 | | Gr. 2 | Gr. 3 | Gr. 4 | Gr. 5 |
				*stx*₁	*stx*₂	*eae*	*bfp*A	*elt*	*est*	*agg*R	*ipa*H	*daa*E	*pap*A	*pap*C	*sfa*DE	*afa*	*Kps*MTII	*iut*A
Beef	**55.2**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**57.7**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Beef	**58.2**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	58.4	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beef	**59.5**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**66.4**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pork	**70.8**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	70.9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pork	**71.1**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	71.8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pork	**72.2**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	72.3	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	72.4	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	72.6	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Pork	**74.2**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	74.7	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	74.9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Pork	**76.3**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	76.4	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	76.7	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	76.8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

APPENDIX D. Characterization of 125 *E. coli* O103 strains (continued)

Sample source	Sample ID	*wzx*O103	*uidA*	DEC indicator gene									ExPEC indicator gene					
													Gr. 1		Gr. 2	Gr. 3	Gr. 4	Gr. 5
				*stx*₁	*stx*₂	*eae*	*bfp*A	*elt*	*est*	*agg*R	*ipa*H	*daa*E	*pap*A	*pap*C	*sfa*DE	*afa*	*Kps*MTII	*iut*A
Pork	**77.4**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	77.5	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**78.9**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**79.7**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	79.9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**80.4**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	80.5	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	80.6	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	80.7	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	80.8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	80.9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	80.10	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**81.1**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	81.3	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	81.5	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	81.6	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	81.7	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	81.8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	81.9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**82.5**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**84.2**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

APPENDIX D. Characterization of 125 *E. coli* O103 strains (continued)

Sample source	Sample ID	*wzx*O103	*uidA*	*stx*₁	*stx*₂	*eae*	*bfp*A	*elt*	*est*	*agg*R	*ipa*H	*daa*E	*pap*A	*pap*C	*sfa*DE	*afa*	*Kps*MTII	*iut*A
						DEC indicator gene							ExPEC indicator gene					
													Gr. 1		Gr. 2	Gr. 3	Gr. 4	Gr. 5
Chicken	**85.9**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**86.2**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pork	**92.3**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	92.4	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	92.9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pork	**93.6**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	93.7	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	93.9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	93.10	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Pork	**97.6**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Pork	**98.2**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Pork	**102.1**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pork	**103.2**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	103.7	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	103.8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	103.10*	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Pork	**104.1**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	104.9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	104.10	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**107.6**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Chicken	**110.2**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

APPENDIX D. Characterization of 125 *E. coli* O103 strains (continued)

Sample source	Sample ID	*wzx*O103	*uidA*	DEC indicator gene									ExPEC indicator gene					
													Gr. 1		Gr. 2	Gr. 3	Gr. 4	Gr. 5
				stx₁	*stx₂*	*eae*	*bfp*A	*elt*	*est*	*agg*R	*ipa*H	*daa*E	*pap*A	*pap*C	*sfa*DE	*afa*	*Kps*MTII	*iut*A
	110.5	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**112.1**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Chicken	**113.1**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	113.10	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pork	**117.8**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**120.4**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	120.5	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	120.7	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	120.8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	120.9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**121.9**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pork	**124.6**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pork	**131.4**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	131.6	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	131.8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	131.10	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**134.9**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**135.7**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken	**137.8**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Chicken	**140.3**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

Number of isolates (bold) = surrogated strains for BOX-PCR analysis

Strain number 103.10* = an EXPEC carrying *KpsMTII* and *iutA*

APPENDIX D. Further characterization of 125 *E. coli* O103 strains

Sample source	Sample ID	Other *E. coli* virulence genes						Phylogenetic group	*stx*2 phage integration site					Antimicrobial resistance
		agn43	*ast*A	cnf*1*	*fim*H	*hly*A	*lpf*		*wrbA*	*yecE*	*yehV*	*sbcB*	Z2577	
Beef	**4.15**	-	-	-	+	-	-	A	+	+	+	-	-	K, KF, S
Beef	**5.2**	-	-	-	+	-	-	A	+	+	+	-	-	K, KF, S
	5.9	-	-	-	+	-	-	A	+	+	+	-	-	K, KF, S
Beef	**7.8**	-	-	-	+	-	-	B1	+	+	+	-	+	KF, S
Beef	**10.12**	-	-	-	+	-	-	B1	+	+	+	-	-	KF, S
Beef	14.5	-	-	-	+	-	-	A	+	+	+	-	-	KF, S
	14.8	-	-	-	+	-	-	A	+	+	+	-	-	S
	14.10	-	-	-	+	-	-	A	+	+	+	-	-	K, KF, S, TE
Beef	**16.1**	-	-	-	+	-	-	A	+	+	+	-	-	KF, S
	16.4	-	-	-	+	-	-	A	+	+	+	-	-	AK, K, KF, S
Beef	17.2	-	-	-	+	-	-	A	+	+	+	+	+	S
	17.7	-	-	-	+	-	-	A	+	+	+	-	-	S
	17.10	-	-	-	+	-	-	A	+	+	+	-	-	S
Beef	**22.8**	-	-	-	+	-	-	A	+	+	+	+	-	SA
Beef	**27.7**	-	-	-	+	-	-	A	+	+	+	-	+	KF, S
Beef	**31.1**	-	-	-	+	-	-	A	+	-	+	-	-	S, SXT
	31.2	-	-	-	+	-	-	A	+	-	+	-	-	C, KF, S, SXT, TE
Beef	**35.1**	-	-	-	+	-	-	A	+	+	+	-	-	C, KF, S, TE
	35.3	-	-	-	+	-	-	A	+	+	+	-	+	TE
	35.6	-	-	-	+	-	-	A	+	+	+	-	+	K, S
	35.9	-	-	-	+	-	-	A	+	+	+	-	-	S
Beef	**36.10**	-	-	-	+	-	-	A	+	+	+	-	-	S

APPENDIX D. Further characterization of 125 *E. coli* O103 strains (continued)

Sample source	Sample ID	Other *E. coli* virulence genes						Phylogenetic group	*stx*2 phage integration site					Antimicrobial resistance
		agn43	*ast*A	cnf*1*	*fim*H	*hly*A	*lpf*		*wrbA*	*yecE*	*yehV*	*sbcB*	Z2577	
Beef	**41.5**	-	-	-	+	-	-	A	+	+	+	-	-	S
	41.6	-	-	-	+	-	-	A	+	+	+	-	-	KF, S
	41.9	-	-	-	+	-	-	A	+	+	+	-	-	K, S
Beef	**42.3**	-	-	-	+	-	-	B1	+	+	+	-	+	KF, S
	42.9	-	-	-	+	-	-	B1	+	+	+	+	+	KF, S
Beef	**43.5**	-	-	-	+	-	-	A	+	+	+	-	-	K, S
	43.9	-	-	-	+	-	-	B1	+	+	+	+	+	S
Beef	**44.3**	-	-	-	+	-	-	A	+	+	+	-	-	S
	44.4	-	-	-	+	-	-	A	+	+	+	-	-	S
	44.5	-	-	-	+	-	-	A	+	+	+	-	-	KF, S
	44.6	-	-	-	+	-	-	A	+	+	+	-	-	S
	44.7	-	-	-	+	-	-	A	+	+	+	-	-	KF, S
	44.8	-	-	-	+	-	-	A	+	+	+	-	-	KF, S
Beef	**45.3**	-	-	-	+	-	-	A	+	+	+	-	-	KF, S
	45.4	-	-	-	+	-	-	A	+	+	+	-	-	KF, S
	45.6	-	-	-	+	-	-	A	+	+	+	-	-	S
Beef	**49.1**	-	-	-	+	-	-	A	+	+	+	-	+	KF, S
Beef	**50.5**	-	-	-	+	-	-	A	+	+	+	-	-	KF, S
	50.7	-	-	-	+	-	-	A	+	+	+	-	+	KF, S
Chicken	**54.10**	-	-	-	+	-	-	A	+	+	+	-	+	C, KF, S, SXT, TE
Beef	**55.2**	-	-	-	+	-	-	A	+	+	+	-	+	C, KF, S, SXT, TE
Chicken	**57.7**	-	-	-	+	-	-	B1	+	+	+	-	+	CN, KF, S, SXT

APPENDIX D. Further characterization of 125 *E. coli* O103 strains (continued)

Sample source	Sample ID	Other *E. coli* virulence genes						Phylogenetic group	*stx*2 phage integration site					Antimicrobial resistance
		agn43	*ast*A	cnf*1*	*fim*H	*hly*A	*lpf*		*wrbA*	*yecE*	*yehV*	*sbcB*	Z2577	
Beef	**58.2**	-	-	-	+	-	-	A	+	+	+	-	+	S
	58.4	-	-	-	+	-	-	A	+	+	+	-	+	KF, S
Beef	**59.5**	-	-	-	+	-	-	A	+	+	+	-	+	KF, S
Chicken	**66.4**	-	-	-	+	-	-	B1	+	+	+	-	+	C, KF, S, TE
Pork	**70.8**	-	-	-	+	-	-	B1	+	+	+	-	+	KF, S, SXT, TE
	70.9	-	-	-	+	-	-	B1	+	+	+	-	+	KF
Pork	**71.1**	-	-	-	+	-	-	A	+	+	+	-	+	K, S
	71.8	-	+	-	+	-	-	B1	+	+	+	-	+	KF, S
Pork	**72.2**	-	-	-	+	-	-	B1	+	+	+	-	+	C, S, TE
	72.3	-	-	-	+	-	-	B1	+	+	+	-	+	KF, S, SXT, TE
	72.4	-	-	-	+	-	-	B1	+	+	+	+	+	C, KF, S, TE
	72.6	-	-	-	+	-	-	A	+	+	+	+	+	C, S, TE
Pork	**74.2**	-	-	-	+	-	-	A	+	+	+	+	+	C, KF, S, TE
	74.7	-	-	-	+	-	-	A	+	+	+	+	+	C, S, TE
	74.9	-	-	-	+	-	-	A	+	+	+	+	+	C, K, KF, S, TE
Pork	**76.3**	-	-	-	+	-	-	A	+	+	+	-	+	C, KF, S, TE
	76.4	-	-	-	+	-	-	A	+	+	+	+	+	C, K, KF, S, TE
	76.7	-	-	-	+	-	-	A	+	+	+	-	+	C, IPM, KF, S, TE
	76.8	-	-	-	+	-	-	A	+	+	+	+	+	C, KF, S, TE
Pork	**77.4**	-	+	-	+	-	-	B1	+	+	+	-	+	KF, S
	77.5	-	-	-	+	-	-	A	+	+	+	-	+	K, KF, S
Chicken	**78.9**	-	-	-	+	-	-	B1	+	+	+	-	+	KF, TE

APPENDIX D. Further characterization of 125 *E. coli* O103 strains (continued)

Sample source	Sample ID	Other *E. coli* virulence genes						Phylogenetic group	*stx*2 phage integration site					Antimicrobial resistance
		agn43	*ast*A	cnf*1*	*fim*H	*hly*A	*lpf*		*wrbA*	*yecE*	*yehV*	*sbcB*	Z2577	
Chicken	**79.7**	-	-	-	+	-	-	A	+	+	+	-	+	C, KF, S, SXT, TE
	79.9	-	-	-	+	-	-	A	+	+	+	-	+	C, K, KF, S, SXT, TE
Chicken	**80.4**	-	+	-	+	-	-	A	+	+	+	-	+	C, K, KF, S, SXT, TE
	80.5	-	+	-	+	-	-	A	+	+	+	-	+	C, K, KF, S, SXT, TE
	80.6	-	+	-	+	-	-	A	+	+	+	-	+	C, K, KF, S, SXT, TE
	80.7	-	+	-	+	-	-	A	+	+	+	-	+	C, K, KF, S, SXT, TE
	80.8	-	+	-	+	-	-	A	+	+	+	-	+	C, K, KF, S, SXT, TE
	80.9	-	+	-	+	-	-	A	+	+	+	-	+	C, K, KF, S, SXT, TE
	80.10	-	+	-	+	-	-	A	+	+	+	-	+	C, K, KF, S, SXT, TE
Chicken	**81.1**	-	+	-	+	-	-	A	+	+	+	-	+	C, K, KF, S, SXT, TE
	81.3	-	+	-	+	-	-	A	+	+	+	-	+	C, K, KF, S, SXT, TE
	81.5	-	+	-	+	-	-	A	+	+	+	-	+	C, K, KF, S, SXT, TE
	81.6	-	+	-	+	-	-	A	+	+	+	-	+	C, K, KF, S, SXT, TE
	81.7	-	+	-	+	-	-	A	+	+	+	-	+	C, K, KF, S, SXT, TE
	81.8	-	+	-	+	-	-	A	+	+	+	-	+	C, K, KF, S, SXT, TE
	81.9	-	+	-	+	-	-	A	+	+	+	+	+	C, K, KF, S, SXT, TE
Chicken	**82.5**	-	+	-	+	-	-	A	+	+	+	+	+	C, K, KF, S, SXT, TE
Chicken	**84.2**	-	-	-	+	-	-	B1	+	+	+	+	+	KF, S, TE
Chicken	**85.9**	-	-	-	+	-	-	B2	+	+	+	+	+	C, K, KF, S, SXT, TE
Chicken	**86.2**	-	-	-	+	-	-	A	+	+	+	+	+	KF, S, TE
Pork	**92.3**	-	+	-	+	-	-	A	+	+	+	-	+	KF
	92.4	-	+	-	+	-	-	A	+	+	+	-	+	KF

APPENDIX D. Further characterization of 125 *E. coli* O103 strains (continued)

Sample source	Sample ID	Other *E. coli* virulence genes						Phylogenetic group	*stx*2 phage integration site					Antimicrobial resistance
		agn43	*ast*A	cnf*1*	*fim*H	*hly*A	*lpf*		*wrbA*	*yecE*	*yehV*	*sbcB*	Z2577	
	92.9	-	+	-	+	-	-	B1	+	+	+	+	+	KF
Pork	**93.6**	-	-	-	+	-	-	A	+	+	+	-	+	KF, S, TE
	93.7	-	-	-	+	-	-	B1	+	+	+	-	+	C, K, KF, S, TE
	93.9	-	-	-	+	-	-	B1	+	+	+	+	+	C, KF, S, TE
	93.10	-	-	-	+	-	-	B1	-	+	+	-	-	C, KF, S, TE
Pork	**97.6**	-	+	-	+	-	-	B1	+	+	+	+	+	KF, S, TE
Pork	**98.2**	-	+	-	+	-	-	B1	+	+	+	+	+	AK, K, KF, S, TE
Pork	**102.1**	-	-	-	+	-	-	B1	+	+	+	+	+	K, KF, S
Pork	**103.2**	-	+	-	+	-	-	B1	+	+	+	+	+	AK, K, KF, S, TE
	103.7	-	-	-	+	-	-	D	+	+	+	-	+	AK, C, K, KF, S, TE
	103.8	-	+	-	+	-	-	A	+	+	+	+	+	K, KF, S, TE
	103.10*	-	+	-	+	-	-	B2	+	+	+	+	+	KF, S, TE
Pork	**104.1**	-	-	-	+	-	-	D	+	+	+	-	+	KF, S, SXT, TE
	104.9	-	-	-	+	-	-	A	+	+	+	+	+	C, K, KF, S, SXT, TE
	104.10	-	-	-	+	-	-	B2	+	+	+	+	+	C, K, KF, S, SXT, TE
Chicken	**107.6**	-	-	-	+	-	-	B1	+	+	+	+	+	KF, S, SXT
Chicken	**110.2**	-	-	-	+	-	-	B1	+	+	+	+	+	KF, S, SXT
	110.5	-	-	-	+	-	-	A	+	+	+	+	+	K, KF
Chicken	**112.1**	-	-	-	+	-	-	B1	+	+	+	+	+	KF, S, SXT
Chicken	**113.1**	-	-	-	+	-	-	B1	+	+	+	+	+	KF, S, SXT
	113.10	-	-	-	+	-	-	B1	+	+	+	-	+	K, KF, S, SXT, TE
Pork	**117.8**	-	-	-	+	-	-	B1	+	+	+	+	+	K, KF, S, TE

APPENDIX D. Further characterization of 125 *E. coli* O103 strains (continued)

Sample source	Sample ID	Other *E. coli* virulence genes						Phylogenetic group	*stx*2 phage integration site					Antimicrobial resistance
		agn43	*ast*A	cnf*1*	*fim*H	*hly*A	*lpf*		*wrbA*	*yecE*	*yehV*	*sbcB*	Z2577	
Chicken	**120.4**	-	-	-	+	-	-	D	+	+	+	-	+	K, KF, S
	120.5	-	-	-	+	-	-	B1	+	+	+	+	+	K, KF, S
	120.7	-	-	-	+	-	-	B1	+	+	+	+	+	K, KF, S
	120.8	-	-	-	+	-	-	D	+	+	+	+	+	K, KF, S
	120.9	-	-	-	+	-	-	A	+	+	+	+	+	K, KF, S
Chicken	**121.9**	-	-	-	+	-	-	B1	+	+	+	+	+	KF, S, TE
Pork	**124.6**	-	-	-	+	-	-	B1	+	+	+	-	+	SA
Pork	**131.4**	-	-	-	+	-	-	B2	+	+	+	+	+	S
	131.6	-	-	-	+	-	-	A	+	+	+	-	+	CN, S
	131.8	-	-	-	+	-	-	B1	+	+	+	-	+	S
	131.10	-	-	-	+	-	-	A	+	+	+	-	+	K, S
Chicken	**134.9**	-	-	-	+	-	-	B1	+	+	+	-	+	C, K, KF, S, SXT, TE
Chicken	**135.7**	-	+	-	+	-	-	B1	+	+	+	+	+	C, K, KF, S, SXT, TE
Chicken	**137.8**	-	-	-	+	-	-	D	+	+	+	-	+	K, KF, S, TE
Chicken	**140.3**	-	-	-	+	-	-	D	+	+	+	-	+	KF, S, TE

Number of isolates (bold) = surrogated strains for BOX-PCR analysis

Strain number 103.10* = an EXPEC carrying *KpsMTII* and *iutA*

C, chloramphenicol; CN, gentamicin; IPM, imipenem; K, kanamycin; KF, cephalothin; S, streptomycin; SXT, cotrimoxazole; TE, tetracycline; SA, susceptible to all agents

APPENDIX E. Characterization of 10 *E. coli* O145 strains

Sample source (Sample ID)	Strain name	*wzx*O145	*uidA*	DEC indicator gene								
				stx$_1$	*stx*$_2$	*eae*	*bfp*A	*elt*	*est*	*agg*R	*ipa*H	*daa*E
Beef (12)	PJP1	+	+	-	-	+	-	-	-	-	-	-
Beef (96)	PJP2	+	+	-	-	+	-	-	-	-	-	-
Beef (99)	PJP3	+	+	-	-	+	-	-	-	-	-	-
	PJP4	+	+	-	-	+	-	-	-	-	-	-
	PJP5	+	+	-	-	+	-	-	-	-	-	-
	PJP6	+	+	-	-	+	-	-	-	-	-	-
	PJP7	+	+	-	-	+	-	-	-	-	-	-
	PJP8	+	+	-	-	+	-	-	-	-	-	-
	PJP9	+	+	-	-	+	-	-	-	-	-	-
	PJP10	+	+	-	-	+	-	-	-	-	-	-

APPENDIX E. Further characterization of 10 *E. coli* O145 strains

Sample source (Sample ID)	Strain name	Other *E. coli* virulence genes						Phylogenetic group	*stx*2 phage integration site					Antimicrobial resistance
		agn43	*ast*A	cnf*1*	*fim*H	*hly*A	*lpf*		*wrbA*	*yecE*	*yehV*	*sbcB*	Z2577	
Beef (12)	PJP1	-	-	-	+	-	+	D	+	+	+	+	+	SA
Beef (96)	PJP2	-	-	-	+	-	+	D	+	+	+	+	+	SA
Beef (99)	PJP3	-	-	-	+	-	+	D	+	+	+	+	+	SA
	PJP4	-	-	-	+	-	+	D	+	+	+	+	+	SA
	PJP5	-	-	-	+	-	+	D	+	+	+	+	+	SA
	PJP6	-	-	-	+	-	+	D	+	+	+	+	+	SA
	PJP7	-	-	-	+	-	+	D	+	+	+	+	+	SA
	PJP8	-	-	-	+	-	+	D	+	+	+	+	+	SA
	PJP9	-	-	-	+	-	+	D	+	+	+	+	+	SA
	PJP10	-	-	-	+	-	+	D	+	+	+	+	+	SA

SA, susceptible to all agents

VITAE

Name   Aphisara Sae-lim

Student ID   6010220069

Educational Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science (Microbiology)	Prince of Songkla University	2016

Scholarship Awards during Enrolment

2017   Faculty of Science Research fund, Prince of Songkla University (contract no. 1-2560-02-002)

List of Publication and Proceeding

Sae-lim, A., Jearanai, P., Rattanachuay, P., and Sukhumungoon, P. 2017. Prevalence, virulence profiles, and genetic relationship of atypical enteropathogenic *Escherichia coli* O145 from beef, southern Thailand. Southeast Asian Journal of tropical medicine and public health. 48(6): 1248-1259.

Wameadesa, N., **Sae-lim, A.**, Hayeebilan F., Rattanachuay, P., and Sukhumungoon, P. 2017. Enteroaggregative *Escherichia coli* O104 from Thai and imported Malaysian raw beef. Southeast Asian Journal of tropical medicine and public health. 48(2): 338-350.

Sae-lim, A., Wameadesa, N., Rattanachuay, P., and Sukhumungoon, P. 2018. Prevalence, Molecular characterization and genetic relatedness of *Escherichia coli* O103 from meat in southern Thailand. Southeast Asian Journal of tropical medicine and public health. 49(4): 646-659.

Sukhumungoon, P., Bunnueang, N., Butsabong, N., **Sae-lim, A.**, and Rattanachuay, P. 2018. Characterization of *Staphylococcus* spp from meat and ready-to eat food, Hat-Yai city, Songkhla, Thailand. Southeast Asian Journal of tropical medicine and public health. 49(1): 96-107.

Sae-lim, A., Wameadesa, N., Jearanai, P., Rattanachuay, P., and Sukhumungoon, P. 2019. Investigation of *Escherichia coli* 0103, 0111, and 0145 from meats, southern Thailand. U.S.-Japan Cooperative Medical Sciences Program's 21st International Conferecne on Emerging Infectious Diseases in the Pacific Rim. (Abstract)