PREVALENCE, VIRULENCE PROFILES, AND GENETIC RELATIONSHIP OF ATYPICAL ENTEROPATHOGENIC *ESCHERICHIA COLI* O145 FROM BEEF, SOUTHERN THAILAND

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Abstract. *Escherichia coli* O145 is a food-borne pathogen, which has a potential to cause outbreaks worldwide. From 100 beef samples collected from markets in southern Thailand during July 2016 to February 2017, 1,470 bacterial colonies were obtained, of which 10 were O145 from 3 beef samples. They were shown to be atypical enteropathogenic *E. coli* (aEPEC) carrying 2 pivotal genes, *fimH* (encoding type 1 fimbrial component) and *lpf* (encoding long polar fimbria), suggesting adherence ability to human intestine. PCR-based phylogenetic group analysis revealed that they were members of phylogenetic group D. Five integration sites of stx_2 phages were found intact, indicating absence of integrated prophages. Although aEPEC O145 strains were susceptible to 10 antimicrobial agents tested, the majority carried bla_{TEM}. Surprisingly, aEPEC O145 strains, although isolated from different samples at different occasions, exhibited identical DNA profile generated by BOX- and $(GTG)_5$-PCR, suggesting that they might be genetically very closely related, or even originating from the same bacterial clone. The presence of aEPEC in beef in southern Thailand indicates the possibility of outbreaks from these strains in this region of the country.

Keywords: *Escherichia coli* O145, atypical EPEC, beef, Thailand

INTRODUCTION

Enterohemorrhagic *Escherichia coli* (EHEC) causes several large gastroenteritis outbreaks worldwide (Nataro and Kaper, 1998). EHEC is defined by the presence of two kinds of virulence genes, namely, *stx* (stx_1 and stx_2) encoding Shiga toxin (Stx) responsible for hemolytic uremic syndrome (HUS), and *eae* encoding intimin responsible for bacterial adherence to host cells. As stx_2 is brought into *E. coli* by stx_2 phage, this gene can also be lost from EHEC either *in vitro* or in human intestine during infection by poorly understood processes (Bielaszewska *et al*, 2007; Serra-Moreno *et al*, 2007). EHEC that has been cured of *stx* and carries only *eae* is known as atypical enteropathogenic *E. coli* (aEPEC) (Mellmann *et al*, 2008).

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Within the EHEC group, EHEC serotype O157:H7 is well-known to be the most important serotype causing large outbreaks throughout the world (Rangel *et al*, 2005). However, other serotypes, namely, O26, O45, O103, O111, and O145, have the potential to cause gastroenteritis outbreaks (Perelle *et al*, 2007), in particular EHEC O145 that has a common lineage with *E. coli* O157:H7 (Cooper *et al*, 2014). Sources of infections and outbreaks by EHEC O145 were reported to include romaine lettuce (Taylor *et al*, 2013), ice cream (de Schrijver *et al*, 2008), and cattle, the pivotal reservoir host of EHEC (Padola *et al*, 2002; Heinikainen *et al*, 2007; Mainil *et al*, 2011; Oh *et al*, 2012). In southern Thailand, EHEC serotype O157:H7 was reported to be present at high rate in beef (Sukhumungoon *et al*, 2011a; Sirikaew *et al*, 2015), but the contamination rate of *E. coli* O145 in beef has not been described in this region.

In the course of our investigation of EHEC, we obtained 10 aEPEC isolates from beef. The occurrence of aEPEC in beef in Thailand has been rarely reported (Pannuch *et al*, 2014). Hence, this study investigated the prevalence, virulence characteristics, antimicrobial susceptibility profiles, and genetic relatedness of aEPEC O145 in raw beef. The information should be of importance to public health in southern Thailand.

MATERIALS AND METHODS

Sample collection and immunomagnetic separation (IMS)

E. coli O145 in beef was investigated in 100 beef samples collected from 8 open-markets throughout Hat Yai city, Songkhla Province, southern Thailand once a week during July 2016 to February 2017. Samples were processed within 2 hours after purchased. Isolation of *E. coli* O145 from raw meat samples was performed as described by Sirikaew *et al* (2015). In brief, 50 g of sample were mixed with 450 ml of tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD) and homogenized for 1 minute in a stomacher CIR-400 (Seward, West Sussex, UK). The liquid portion was incubated at 37ºC for 6 hours and 1 ml aliquot of the solution was mixed with O145-specific antibody-coated magnetic beads (Dynabeads, Life Technologies AS, Oslo, Norway). The mixture was incubated at ambient temperature for 30 minutes with occasional inversion of tube. Beads were washed, recovered following the manufacturer's instruction, streaked on eosin methylene blue (EMB) agar (Becton Dickinson) and incubated at 37ºC for 18 hours. Fifteen green metallic sheen colonies per sample were randomly selected and stored at -80ºC for further analysis.

PCR-based identification of O145

DNA template was prepared by a boiling method (Sirikaew *et al*, 2015). In short, an individual colony was inoculated into 3 ml of TSB (Becton Dickinson) and incubated at 37ºC for 3 hours with shaking. One ml aliquot of bacterial culture was boiled for 10 minutes, immediately immersed on ice for 5 minutes and centrifuged at 11,000*g* for 10 minutes. A 10-fold diluted supernatant in sterile deionized water was used as PCR template. PCR was performed in a 25-µl reaction consisting of 0.4 µM O145.6 and O145.B primers (Table 1), 0.1 mM dNTPs, 1X Go*Taq* DNA polymerase buffer, 0.5 U Go*Taq* DNA polymerase (Promega, Madison, WI) and 2 µl of DNA template. Thermocycling (performed in T100™ Thermal Cycler; Bio-Rad, Hercules, CA) conditions were as follows: 95ºC for 3 minutes; 35 cycles of 94ºC for 1 minute, 50ºC for 1 minute, and 72ºC for 1 minute; with a final step of 72ºC

for 5 minutes. Amplicons were analyzed by 1.0% agarose gel-electrophoresis and visualized by ethidium bromide staining in an WSE-5200 Printpraph 2M gel imaging system (ATTO, Tokyo, Japan).

E. coli pathotype classification and detection of other virulence genes

Pathotype classification of *E. coli* O145 was performed by PCR targeting pathotype-specific genes for six diarrheagenic *E. coli* (DEC) categories, namely, *stx+eae* for EHEC, *bfp+eae* for typical enteropathogenic *E. coli* (tEPEC), *eae* alone for atypical enteropathogenic *E. coli* (aEPEC); *est*/*elt* for enterotoxigenic *E. coli*, ETEC; *aggR* for EAEC, *ipaH* for enteroinvasive *E. coli* (EIEC), and *daaE* for diffusely adherent *E. coli* (DAEC) (Table 1). PCR was performed in a 25-µl reaction consisting of specific primer for each gene (Table 1), 0.1 mM dNTPs, 1X Go*Taq* DNA polymerase buffer, 0.5 U Go*Taq* DNA polymerase (Promega) and 2 µl of DNA template. Thermocycling conducted in T100™ Thermal Cycler, (Bio-Rad) with conditions as follows: 95°C for 3 minutes; 35 cycles of 94°C for 1 minute, 40°C (for *est*), 50°C (*elt*, *aggR*, stx_2), 55°C (stx_1, *eae*, *bfp*, *daaE*), or 60°C (*ipaH*) for 1 minute, and 72°C for 1 minute or 1.15 minutes (for *eae*); and final step of 72°C for 5 minutes. Amplicons were analyzed as described above. Other *E. coli* virulence genes were investigated using PCR with appropriate primer pairs (Table 1), reaction mixture and thermocycling conditions as described above except that the annealing temperature was 50°C for *astA* and *pet*, 55°C for *fimH* and *lpf*, 58°C for *cnf1* and *hlyA*, and 67°C for *agn43*. *E. coli* was identified by PCR targeting *uidA* (annealing temperature of 50°C) (Table 1).

Antimicrobial susceptibility assay

Antimicrobial susceptibility was performed by a disk diffusion method (CLSI, 2014) using 10 common antimicrobial agents: amikacin (30 µg), cephalothin (30 µg), chloramphenicol (30 µg), fosfomycin (200 µg), gentamicin (10 µg), imipenem (10 µg), kanamycin (30 µg), streptomycin (10 µg), tetracycline (30 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg) (Oxoid, Hamshire, UK). Diameter of clear zone was measured with a Vernier caliper.

Integrity of _E. coli_ integration sites for _stx_ phages assay

The intactness of five *E. coli* specific integration sites for stx_2 phages, namely, *sbcB*, *wrbA*, *yecE*, *yehV*, and Z2577, was examined by PCR (Sirikaew *et al*, 2015). In brief, PCR amplification of the insertion locus was carried out as described above using primers specific to each locus (Table 1) with the following annealing temperatures, 47°C for *wrbA*, 50°C for *sbcB* and *yehV*, 53°C for Z2577, and 60°C for *yecE*. Absence of amplicon indicates presence of phage integration at that locus.

Yeast agglutination assay

Adherence of *E. coli* to host tissues often is paralleled with ability to agglutinate yeast cells (Schembri *et al*, 2001). In brief, a 30 µl aliquot of an 18-hour bacterial culture in Luria Bertani (LB) (Becton Dickinson) broth was mixed with 5% *Saccharomyces cerevisiae* in normal saline solution on a glass slide. Agglutination was monitored by eye. Positive agglutination reaction was judged within 1 minute after mixing. A suspension of yeast cells in LB broth was used as negative control.

Phylogenetic group examination

PCR amplification of *chuA*, *yjaA*, and TspE4C2 fragment were carried out in a reaction mixture using specific primers (Table 1) as described above, but employing the following thermocycling conditions: 95°C for 3 minutes; 35 cycles of

Table 1
Oligonucleotide primers used in the study.

Gene	Virulence	Primer name	Sequence (5′ to 3′)	Amplicon size (bp)	Reference
wzxO145	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		
stx_1	Shiga toxin 1	EVT-1	CAACACTGGATGATCTCAG	350	Sukhumungoon *et al* (2011a)
		EVT-2	CCCCCTCAACTGCTAATA		
stx_2	Shiga toxin 2	EVS-1	ATCAGTCGTCACTCACTGGT	404	Sukhumungoon *et al* (2011a)
		EVS-2	CCAGTTATCTGACATTCTG		
aggR	Transcriptional activator of AAF/I	AggR-1	CAGAATACATCAGTACACTG	433	Tsukamoto (1996)
		AggR-2	GAAGCTTACAGCCGATATAT		
ipaH	Enteroinvasive mechanism	ipaIII	GTTCCTTGACCGCCTTTCCGATACCGTC	603, 619	Sethabutr *et al* (1993)
		ipaIV	GCCGGTCAGCCACCCTCTGAGAGTAC		
daaE	F1845 fimbriae	daaF-F	GAACGTTGGTTAATGTGGGGTAA	542	Vidal *et al* (2005)
		daaF-R	TATTCACCGGTCGGTTATCAGT		
elt	Heat-labile enterotoxin	TW20	GGCGACAGATTATACCGTGC	450	Stacy-Phipps *et al* (1995)
		JW11	CGGTCTCTATATTCCCTGTT		
est	Heat-stable enterotoxin	JW14	ATTTTTACTTTCTGTATTAGTCTT	190	Stacy-Phipps *et al* (1995)
		JW7	CACCCGGTACAAGGCAGGATT		
astA	EAST1	EAST11a	CCATCAACACAGTATATCCGA	111	Yamamoto and Echeverria (1996)
		EAST11b	GGTCGCGAGTGACGGCTTTGT		
agn43	Antigen 43	1-Kpn	GAACCTGTCGGTACCGATGCCCTCCC	≈900	Danese *et al* (2000)
		2-Bam	CGGGATCCGTTGCCACTGTACCGGGCTTGACGACC		
lpf	Long polar fimbriae	*lpfA1*-F	GGTCGTTTTTGCCTTAACCGC	≈500	Torres *et al* (2004)
		lpfA1-R	AGGTTGAAATCGACCTGCGC		
fimH	Type 1 fimbriae	fimH-F	TGCAGAACGGATAAGCCGTGG	508	Johnson and Stell (2000)
		fimH-R	GCAGTCACCTGCCCTCCGGTA		
wrbA	Quinone oxidoreductase	*wrb*A1	ATGGCTAAAGTTCTGGTG	600	Toth *et al* (2003)
		*wrb*A2	CTCCTGTTGAAGATTAGC		

Table 1 (Continued).

Gene	Virulence	Primer name	Sequence (5′ to 3′)	Amplicon size (bp)	Reference
yecE	Unknown	EC10	GCCAGCGCCGAGCAGCACAATA	400	DeGreve *et al* (2002)
		EC11	GGCAGGCAGTTGCAGCCAGTAT		
sbcB	Exonuclease I	sbcB1	CATGATCTGTTGCCACTCG	1,800	Ohnishi *et al* (2002)
		sbcB2	AGGTCTGTCCGTTTCCACTC		
yehV	Transcriptional regulator	Primer A	AAGTGGCGTTGCTTTGTGAT	340	Shaikh and Tarr (2003)
		Primer B	AACAGATGTGTGGTGAGTGTCTG		
Z2577	Oxidoreductase	Z2577F	AACCCCATTGATGCTCAGGCTC	909	Koch *et al* (2003)
		Z2577R	TTCCCATTTTACACTTCCTCCG		
chuA	Heme transport	chuA1	GACGAACCAACGGTCAGGAT	279	Clermont *et al*, 2000
		chuA2	TGCCGCCAGTACCAAAGACA		
yjaA	Unknown	yjaA1	TGAAGTGTCAGGAGACGCTG	211	Clermont *et al*, 2000
		yjaA2	ATGGAGAATGCGTTCCTCAAC		
TspE4.C2	Unknown	TspE4.C2-1	GAGTAATGTCGGGGCATTC A	152	Clermont *et al*, 2000
		TspE4.C2-2	CGCGCCAACAAAGTATTACG		
uidA	β-glucoronidase	uidA-F	ATCACCGTGGTGACGCATGTCGC	486	Heninger *et al*, 1999
		uidA-R	CACCACGATGCCATGTTCATCTGC		
bla_{SHV}	β-lactamase	bla-SHV.SE	ATGCGTTATATTCGCCTGTG	747	Paterson *et al*, 2003
		bla-SHV.AS	TGCTTTGTTATTCGGGCCAA		
bla_{TEM}	β-lactamase	TEM-164.SE	TCGCCGCATACACTATTCTCAGAATGA	445	Monstein *et al*, 2007
		TEM-164.AS	ACGCTCACCGGCTCCAGATTTAT		
bla_{CTX-M}	β-lactamase	CTX-M-U1	ATGTGCAGYACCAGTAARGTKATGGC	593	Boyd *et al*, 2004
		CTX-M-U2	TGGGTRAARTARGTSACCAGAAYCAGCGG		
pet	Plasmid encoded toxin	pet-F	ACTGGCGGACTCATTGCTGT	832	Vila *et al*, 2000
		pet-R	GCGTTTTTCCGTTCCCTATT		
cnf1	Cytotoxic necrotizing factor-1	cnf1-F	GGCGACAAATGCAGTATTGCTTGG	552	Yamamoto *et al*, 1995
		cnf1-R	GACGTTGGTTGCGGTAATTTTGGG		
hlyA	α-hemolysin	hly1	AACAAGGATAAGCACTGTTCTGGCT	1,177	Yamamoto *et al*, 1995
		hly2	ACCATATAAGCGGTCATTCCCGTCA		
	Repetitive sequences	$(GTG)_5$	GTGGTGGTGGTGGTG	Variable	Versalovic *et al*, 1991
	Repetitive sequences	BOXA1R	CTACGGCAAGGCGACGCTGACG	Variable	Versalovic *et al*, 1994

K = G or T; R = A or G; S = G or C; Y = C or T.

94ºC for 50 seconds, 54ºC for 50 seconds, and 72ºC for 30 seconds; with a final step of 72ºC for 5 minutes. Amplicons were analyzed as described above.

Typing of *E. coli* O145

DNA profiling of *E. coli* O145 strains was performed using two approaches, namely, BOX- and $(GTG)_5$-PCR to assess the genetic relatedness among O145 strains (Sirikaew *et al*, 2015; Sukkua *et al*, 2017). For BOX- and $(GTG)_5$-PCR, the 25-µl reaction mixture containing 0.2 µM BOXA1R or $(GTG)_5$ primer (Table 1), 0.2 mM dNTPs, 1X Go*Taq* DNA polymerase buffer, 3.0 mM $MgCl_2$, 1.25 U Go*Taq* DNA polymerase (Promega) and 50 ng of DNA template [prepared using glass fiber matrix spin column (Geneaid, Taipei, Taiwan)], was run in thermocycling machine, T100™ Thermal Cycler (Bio-Rad) as follows: 95ºC for 3 minutes; 30 cycles of 94ºC for 3 seconds, 92ºC for 30 seconds, 50ºC for BOXA1R or 40ºC for $(GTG)_5$ primer, for 1 minute, and 65ºC for 8 minutes. Amplicons were separated by 1.5% agarose gel-electrophoresis and recorded as described above.

RESULTS

Prevalence of *E. coli* O145 in raw beef and pathotype classification

Of 1,470 bacterial colonies from 100 beef samples, 3 samples (comprising 10 isolates) demonstrated the presence of both *wzx*O145 and *uidA* (for confirming *E. coli*). DEC pathotype examination by PCR revealed that they carried only *eae* (Table 2). Thus, all 10 *E. coli* O145 isolates were aEPEC O145.

Virulence genes and phylogenetic group

All 10 aEPEC O145 strains possessed the two pivotal virulence genes, *fimH* (encoding type 1 fimbrial tip) and *lpf* (encoding long polar fimbria) but not *astA*, *agn43*, *cnf1*, *pet*, and *hlyA* (Table 2). The aEPEC O145 strains did not agglutinate *S. cerevisiae* (Table 2). PCR-based phylogenetic group analysis using *chuA*, *yjaA*, and TspE4.C2 fragment revealed that all aEPEC O145 belonged to group D (Table 2).

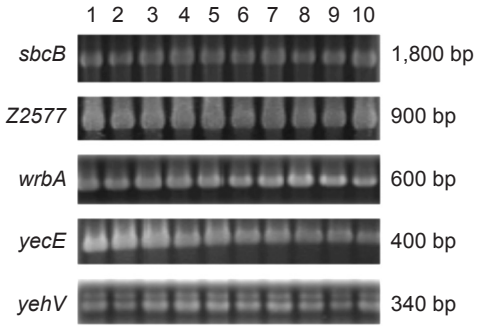

Fig 1–Intactness of five stx_2 phage insertion sites determined by PCR in 10 aEPEC isolated from beef during July 2016 to February 2017, southern Thailand. PCR was performed using primer pairs listed in Table 1 and analyzed by 1.0% agarose gel- electrophoresis. Lane 1-10, PJP-1 to -10, respectively.

Integrity of *E. coli* integration sites for stx_2 phages

In order to investigate the integrity of integration occupancy sites of stx_2 phages in *E. coli* O145 genome, five genes frequently occupied by stx_2 phages were examined. All five integration sites were still intact, indicating that no stx_2 phage had integrated into these sites (Fig 1).

Antimicrobial susceptibility and presence of ESBL genes

All 10 aEPEC O145 strains were susceptible to the 10 antimicrobial agents tested (Table 2). Eight aEPEC O145 strain carried bla_{TEM} and none carried $bla_{CTX\text{-}M}$ and bla_{SHV}.

Table 2
Characteristics of aEPEC O145 isolated from beef during July 2016 to February 2017, southern Thailand.

Sample ID (number of isolate)	Strain name	Virulence trait	Phylogenetic group	Yeast agglutination	Drug resistance	ESBL gene
12 (1)	PJP-1	*eae, fimH, lpf*	D	-	SA[a]	bla_{TEM}
96 (2)	PJP-2	*eae, fimH, lpf*	D	-	SA	-
	PJP-3	*eae, fimH, lpf*	D	-	SA	bla_{TEM}
99 (7)	PJP-4	*eae, fimH, lpf*	D	-	SA	bla_{TEM}
	PJP-5	*eae, fimH, lpf*	D	-	SA	bla_{TEM}
	PJP-6	*eae, fimH, lpf*	D	-	SA	bla_{TEM}
	PJP-7	*eae, fimH, lpf*	D	-	SA	bla_{TEM}
	PJP-8	*eae, fimH, lpf*	D	-	SA	-
	PJP-9	*eae, fimH, lpf*	D	-	SA	bla_{TEM}
	PJP-10	*eae, fimH, lpf*	D	-	SA	bla_{TEM}

[a]SA, susceptible to all 10 antimicrobial agents tested.

Genetic relationship among the 10 aEPEC O145 strains

Genetic relationship among the aEPEC O145 strains from 3 different meat samples at different occasions were conducted by BOX- and $(GTG)_5$-PCR. All 10 aEPEC O145 strains, surprisingly, exhibited identical DNA profiles (Fig 2).

DISCUSSION

Prevalence of *E. coli* O145 in raw meat in this study was extremely low. Immunomagnetic separation technique using O145 antibody-coated immunomagnetic beads was employed due to its high isolation efficiency (Chapman *et al*, 1994) and such immunomagnetic beads have been successfully employed to isolate *E. coli* O157 and *E. coli* O26 from raw meat samples (demonstrating 18% and 12%, respectively) (Sukhumungoon *et al*, 2011a; Sirikaew *et al*, 2015). Thus, the negative result for O145 detection in most of beef samples in this study reflects its low prevalence.

Padola *et al* (2002) in Argentina were the first to isolate EHEC O145:H^- during the course of investigating EHEC/STEC from 59 cattle rectal swabs. Nine EHEC O145:H^- carrying stx_1 or stx_2 with two different random amplified polymorphic DNA (RAPD) profiles corresponding to the possession of both *stx* types, were described. Heinikainen *et al* (2007) revealed an association between severe diarrhea and three STEC serotypes including O145:H28 from cattle in Finland. This O145:H28 was isolated from 5-year old boy and his mother and the causative strain was also isolated from the family's home farm. In addition, in USA multistate outbreaks of STEC O145 were reported in April and May, 2010 with 26 confirmed cases and 5 probable cases from Michigan, Ohio, New York, Tennessee, and Pennsylvania (Taylor *et al*, 2013). Among the cases, 45% were hospitalized and 11% developed HUS. The outbreak was shown to be associated with consumption of contaminated romaine lettuce. These reports affirm the

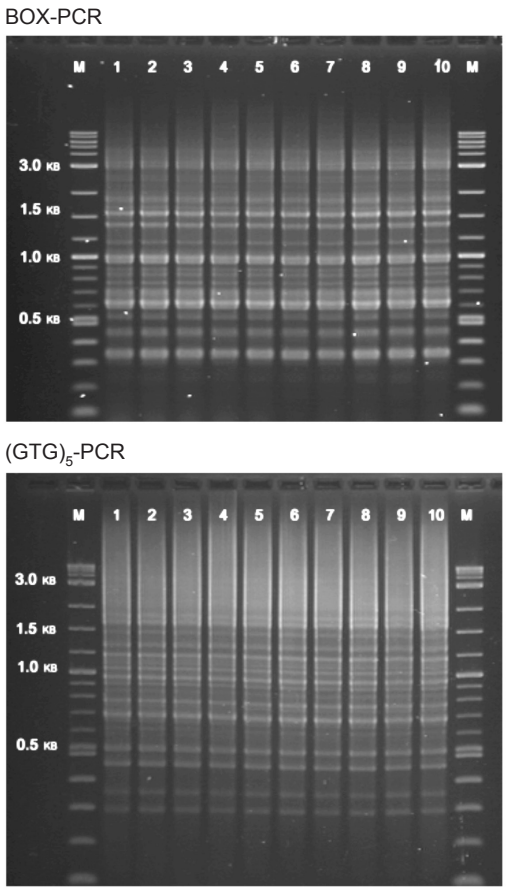

Fig 2–DNA profiles generated by BOX- and $(GTG)_5$-PCR of aEPEC O145 isolated from beef during July, 2016 to February, 2017, southern Thailand. PCR was performed using primers listed in Table 1 and analyzed by 1.5% agarose gel-electrophoresis. Lane M, DNA size markers; lane 1-10, PJP-1 to -10, respectively.

importance of *E. coli* O145 contamination in food.

Highly dynamic interchange between aEPEC and EHEC of serotype O26 has been demonstrated through the loss and gain of stx_2-encoding phages (Bielaszewska *et al*, 2007). The aEPEC strains can gain *stx* and become more virulent and contributing to possible outbreaks. stx_2 phage integration into *yecE* and *wrbA* is important in both *E. coli* O26 and *E. coli* O157: NM (non-motile) strains (Bielaszewska *et al*, 2007; Mellmann *et al*, 2008). In this study, we found no prophage integration into the five integration sites examined in the 10 aEPEC strains. This not only suggests that there is no stx_2-encoding phage integration, but also the possibility of aEPEC to obtain stx_2-encoding phages into it in the future.

Investigation of virulence genes carried by aEPEC O145 strains in this present study showed that they carried three virulence genes (*eae*, *fimH* and *lpf*) responsible for adherence. Intimin plays a key role in bacterial intimate adherence to its host, while type 1 fimbria confers adhesion and autoaggregation of bacteria leading to colonization of the host's intestine (Schembri *et al*, 2001). Lpf also is involved in *E. coli* O157:H7 and other pathogenic *E. coli* adherence both *in vitro* and *in vivo* (Sukkua *et al*, 2016). Therefore, aEPEC strains in this study were considered to have an effective adherent ability that could lead to pathogenicity of their host in some extent, although toxin genes such as *cnf1*, *pet*, and *hlyA,* were absent.

Agglutination to yeast cells or erythrocytes is a classical assay for monitoring type 1 fimbriae-mediated adhesion (Schembri *et al*, 2000). In this present study, however, the lack of correspondence between the presence of *fimH* and the lack of yeast cell agglutination was observed. One of the possible explanations is that mutation in *fimH*, which does not enhance the binding between bacteria and yeast cell but diminish agglutination capacity (Schembri *et al*, 2000). Switching from fimbriated to non-fimbriated state through affecting the expression of fimbrial major subunit gene might also be

one of the factors involved in the failure in agglutination (Knudsen and Klemm, 1998). Other possible explanations include defects in transport and assembly of FimH (Schembri *et al*, 2000).

Antimicrobial-resistant ability can emerged and be transferred among bacterial species, and the dissemination of this resistance capability has become a worldwide problem due to the overuse of antibiotics for therapy of infected humans and animals, including for prophylaxis and animal growth promotion (Rasheed *et al*, 2014). Antimicrobial-resistant rates of DEC strains isolated from beef in this area have varied from study to study throughout this decade (Sukhumungoon *et al*, 2011a,b; Sirikaew *et al*, 2014; Sirikaew *et al*, 2015; Sirikaew *et al*, 2016; Wameadesa *et al*, 2017). Nonetheless, some susceptible strains, namely, STEC O157 and *E. coli* O104, also could be detected (Sukhumungoon *et al*, 2011a,b; Wameadesa *et al*, 2017). Therefore, the presence of drug susceptible aEPEC O145 strain in this study is not surprising. These O145 strains are believed to be present in the Thai environment at low levels and are thought to have not intensively been exposed to antimicrobial agents.

Previous studies on DNA profiling of DEC strains from meat samples in this geographical area indicated that the repetitive sequence-PCR is an efficient tracking tool (Sirikaew *et al*, 2015; Sirikaew *et al*, 2016; Sukkua *et al*, 2017). BOX- and $(GTG)_5$-PCR are PCR-based DNA profiling methods targeting repetitive sequences that are spread throughout bacterial genome. In this current study, identical DNA profiles of aEPEC O145 isolated from different samples and on different occasions suggests the possibility that they share the same genome core as observed in the case of enteroaggregative *E. coli* (EAEC) O44 strains from different patients in which the EAEC O44 strains exhibit different virulence gene and antimicrobial susceptibility profiles but have identical DNA pattern generated by BOX- and ERIC2-PCR (Sukkua *et al*, 2015).

In summary, this study shows that aEPEC O145 did indeed exist in beef sold in southern Thai area albeit at low frequency and can be isolated only with the use of immunobead method. Identical DNA profile among the aEPEC O145 strains isolated from different samples at different occasions suggests that they are closely related clones or originated from the same clone circulating in the Thai environment. These aEPEC O145 strains may not be able to play a crucial role in pathogenesis at this moment but they retain the ability to become more virulent in the future by acquiring *stx* phages. The data regarding the prevalence and virulence characteristics of aEPEC O145 isolated from raw beef in southern Thailand should be of benefit to the public health of this region of the country.

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REFERENCES

Bielaszewska M, Prager R, Köck R, *et al*. Shiga toxin gene loss and transfer in vitro and in vivo during enterohemorrhagic *Escherichia coli* O26 infection in humans. *Appl Environ Microbiol* 2007; 73: 3144-50.

Boyd DA, Tyler S, Christianson S, *et al*. Complete nucleotide sequence of a 92-kilobase

plasmid habouring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. *Antimicrob Agents Chemother* 2004; 48: 3758-64.

Chapman PA, Wright DJ, Siddons CA. A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine faeces. *J Med Microbiol* 1994; 40: 424-7.

Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; 66: 4555-8.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; Twenty-fourth informational supplement M100-S24. Wayne: CLSI, 2014.

Cooper KK, Mandrell RE, Louie JW, *et al*. Comparative genomics of enterohemorrhagic *Escherichia coli* O145:H28 demonstrates a common evolutionary lineage with *Escherichia coli* O157:H7. *BMC Genomics* 2014; 15: 17.

Danese PN, Pratt LA, Dove SL, Kolter R. The outer membrane protein, Antigen 43, mediates cell-to-cell interactions within *Escherichia coli* biofilms. *Mol Microbiol* 2000; 37: 424-32.

de Schrijver K, Buvens G, Posse B, *et al*. Outbreak of verocytotoxin-producing *E. coli* O145 and O26 infections associated with the consumption of ice cream produced at a farm, Belgium, 2007. *Euro Surviell* 2008; 13: pii=8041.

DeGreve H, Qizhi C, Deboeck F, Hernalsteens JP. The Shiga toxin VT2-encoding bacteriophage varphi297 integrates at a distinct position in the *Escherichia coli* genome. *Biochim Biophys Acta* 2002; 1579: 196-202.

Gannon VPJ, Rashed M, King RK, Thomas EJG. Detection and characterization of the *eae* gene of Shiga-like toxin-producing *Escherichia coli* using polymerase chain reaction. *J Clin Microbiol* 1993; 31: 1268-74.

Gunzburg ST, Tornieporth NG, Riley LW. Identification of enteropathogenic *Escherichia coli* by PCR-based detection of the bundle-forming pilus gene. *J Clin Microbiol* 1995; 33: 1375-7.

Heinikainen S, Pohjanvirta T, Eklund M, Siitonen A, Pelkonen S. Tracing shigatoxigenic *Escherichia coli* O103, O145, and O174 infections from farm residents to cattle. *J Clin Microbiol* 2007; 45: 3817-20.

Heninger A, Binder M, Schmidt S, Unartl K, Botzenhart K, Doring G. PCR and blood culture of *Escherichia coli* bacteremia in rats. *Antimicrob Ag Chem* 1999; 37: 2479-82.

Johnson JR, Stell AL. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. *J Infect Dis* 2000; 181: 261-72.

Knudsen TB, Klemm P. Probing the receptor recognition site of the FimH adhesion by fimbriae-displayed FimH-FocH hybrids. *Microbiol* 1998; 144: 1919-29.

Koch C, Hertwig S, Appel B. Nucleotide sequence of the integration site of the temperate bacteriophage 6220, which carries the Shiga toxin gene *stx* (1ox3). *J Bacteriol* 2003; 185: 6463-6.

Mainil JG, Bardiau M, Ooka T, *et al*. Typing of O26 enterohaemorrhagic and enteropathogenic *Escherichia coli* isolated from humans and cattle with IS*621* multiplex PCR-based fingerprinting. *J Appl Microbiol* 2011; 111: 773-86.

Mellmann A, Lu S, Karch H, *et al*. Recycling of Shiga toxin 2 gene in sorbitol-fermenting enterohemorrhagic *Escherichia coli* O157:NM. *Appl Environ Microbiol* 2008; 74: 67-72.

Monday SR, Beisaw A, Feng PCH. Identification of Shiga toxigenic *Escherichia coli* seropathotypes A and B by multiplex PCR. *Mol Cell Probes* 2007; 21: 308-11.

Monstein HJ, Östholm-Balkhed A, Nilsson MV, Nilsson M, Dornbusch K, Nilsson LE. Multiplex PCR amplification assay for the detection of ^{bla}SHV, ^{bla}TEM and ^{bla}CTX-M

genes in enterobacteriaceae. *APMIS* 2007; 115: 1400-8.

Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 1998; 11: 142-201.

Oh JY, Kang MS, An BK, *et al*. Prevalence and characteristics of intimin-producing *Escherichia coli* strains isolated from healthy chickens in Korea. *Poultry Sci* 2012; 91: 2438-43.

Ohnishi M, Terajima J, Kurokawa K, *et al*. Genomic diversity of enterohemorrhagic *Escherichia coli* O157 revealed by whole genome PCR scanning. *PNAS* 2002; 99: 17043-8.

Padola NL, Sanz ME, Lucchesi PMA, *et al*. First isolation of the enterohaemorrhagic *Escherichia coli* O145:H- from cattle in feedlot in Argentina. *BMC Microbiol* 2002; 2: 6.

Pannuch M, Sirikaew S, Nakaguchi Y, Nishibuchi M, Sukhumungoon P. Quantification of enteropathogenic *Escherichia coli* from retailed meats. *Int Food Res J* 2014; 21: 547-51.

Paterson DL, Hujer KM, Hujer AM, *et al*. Extended-spectrum- β lactamases in *Klebsiella pneumoniae* bloodstream isolates from seven countries: dominance and widespread prevalence of SHV-and CTX-M-type β lactamases. *Antimicrob Agents Chemother* 2003; 47: 3553-60.

Perelle S, Dilasser F, Grout J, Fach P. Screening food raw materials for the presence of the world's most frequent clinical cases of Shiga toxin-encoding *Escherichia coli* O26, O103, O111, O145 and O157. *Int J Food Microbiol* 2007; 113: 284-8.

Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg Infect Dis* 2005; 11: 603-9.

Rasheed MU, Thajuddin N, Ahamed P, Teklemariam Z, Jamil K. Antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources. *Rev Inst Med Trop Sao Paulo* 2014; 56: 341-6.

Schembri MA, Christiansen G, Klemm P. FimH-mediated autoaggregation of *Escherichia coli*. *Mol Microbiol* 2001; 41: 1419-30.

Schembri MA, Sokurenko EV, Klemm P. Functional flexibility of the FimH adhesion: insights from a random mutant library. *Infect Immun* 2000; 68: 2638-46.

Serra-Moreno R, Jofre J, Muniesa M. Insertion site occupancy by stx_2 bacteriophages depends on the locus availability of the host strain chromosome. *J Bacteriol* 2007; 189: 6645-54.

Sethabutr O, Venkatesan M, Murphy GS, Eampokalap B, Hoge CW, Echeverria P. Detection of Shigellae and enteroinvasive *Escherichia coli* by amplification of the invasion plasmid antigen H DNA sequence in patients with dysentery. *J Infect Dis* 1993; 167: 458-61.

Shaikh N, Tarr PI. *Escherichia coli* O157:H7 Shiga toxin- encoding bacteriophages: integrations, excisions, truncations, and evolutionary implications. *J Bacteriol* 2003; 185: 3596-605.

Sirikaew S, Patungkaro W, Rattanachuay P, Sukkua K, Sukhumungoon P. Enterotoxigenic *Escherichia coli* O169:HUT from a diarrheal patient: phylogenetic group and antimicrobial susceptibility. *Southeast Asian J Trop Med Public Health* 2014; 45: 1376-84.

Sirikaew S, Rattanachuay P, Nakaguchi Y, Sukhumungoon P. Immuno-magnetic isolation, characterization and genetic relationship of *Escherichia coli* O26 from raw meats, Hat Yai city, Songkhla, Thailand. *Southeast Asian J Trop Med Public Health* 2015; 46: 241-53.

Sirikaew S, Sukkua K, Rattanachuay P, Khianngam S, Sukhumungoon P. 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* 2016; 47: 1008-19.

Stacy-Phipps S, Mecca JJ, Weiss JB. Multiplex PCR assay and simple preparation method for stool specimens detect enterotoxigenic *Escherichia coli* DNA during course of in-

fection. *J Clin Microbiol* 1995; 33: 1054-59.

Sukhumungoon P, Nakaguchi Y, Ingviya N, *et al*. Investigation of stx_2^+ eae^+ *Escherichia coli* O157:H7 in beef imported from Malaysia to Thailand. *Int Food Res J* 2011a; 18: 381-6.

Sukhumungoon P, Mittraparp-arthorn P, Pomwised R, Charernjiratragul W, Vuddhakul V. High concentration of Shiga toxin 1-producing *Escherichia coli* isolated from southern Thailand. *Int Food Res J* 2011b; 18: 683-8.

Sukkua K, Patungkaro W, Sukhumungoon P. Detection and molecular characterization of enteroaggregative *Escherichia coli* from diarrheal patients in tertiary hospitals, southern Thailand. *Southeast Asian J Trop Med Public Health* 2015; 46: 901-10.

Sukkua K, Rattanachuay P, Sukhumungoon P. *Ex vivo* adherence to murine ileal, biofilm formation ability and presence of adherence-associated of human and animal diarrheagenic *Escherichia coli*. *Southeast Asian J Trop Med Public Health* 2016; 47: 55-65.

Sukkua K, Pomwised R, Rattanachuay P, Khianngam S, Sukhumungoon P. Characterization of extraintestinal pathogenic *Escherichia coli* from meat in southern Thailand. *Southeast Asian J Trop Med Public Health* 2017; 48: 98-108.

Taylor EV, Nguyen TA, Machesky KD, *et al*. Multistate outbreak of *Escherichia coli* O145 infections associated with romaine lettuce consumption, 2010. *J Food Prot* 2013; 76: 939-44.

Torres AG, Kanack KJ, Tutt C, Popov V, Kaper JB. 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 Microbiol Lett* 2004; 238: 333-44.

Toth I, Schmidt H, Dow M, Malik A, Oswald E, Nagy B. 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* 2003; 69: 7242-7.

Tsukamoto T. [PCR methods for detection of enteropathogenic *Escherichia coli* (localzed adherence) and enteroaggregative *Escherichia coli*]. *Kansenshogazu Zasshi* 1996; 70: 569-73.

Versalovic J, Koeuth T, Lupski JP. Distribution of repetitive DNA-sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* 1991; 19: 6823-31.

Versalovic J, Schneider M, de Bruijn FJ, Lupski JR. Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. *Methods Mol Cell Biol* 1994; 5: 25-40.

Vidal M, Kruger E, Duran C, *et al*. Single multiplex PCR assay to identify simultaneously the six categories of diarrheagenic *Escherichia coli* associated with enteric infections. *J Clin Microbiol* 2005; 43: 5362-5.

Vila J, Vargas M, Henderson IR, Gascon J, Nataro JP. Enteroaggregative *Escherichia coli* virulence factors in traveler's diarrhea strains. *J Infect Dis* 2000; 182: 1780-3.

Wameadesa N, Sae-lim A, Hayeebilan F, Rattanachuay P, Sukhumungoon P. Enteroaggragative *Escherichia coli* O104 from Thai and imported Malaysian raw beef. *Southeast Asian J Trop Med Public Health* 2017; 48: 338-50.

Yamamoto S, Terai A, Yuri K, Kurazono H, Takeda Y, Yoshida O. Detection of urovirulence factors in *Escherichia coli* by multiplex polymerase chain reaction. *FEMS Immun Med Microbiol* 1995; 12: 85-90.

Yamamoto T, Echeverria P. Detection of the enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 gene sequences in enterotoxigenic *E. coli* strains pathogenic for humans. *Infect Immun* 1996; 64: 1441-5.