IMMUNO-MAGNETIC ISOLATION, CHARACTERIZATION AND GENETIC RELATIONSHIP OF *ESCHERICHIA COLI* O26 FROM RAW MEATS, HAT YAI CITY, SONGKHLA, THAILAND

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Abstract. *Escherichia coli* O26 is the most important serotype in non-O157 group, which plays a significant role in gastrointestinal illnesses. However, information regarding the prevalence and its characteristics are lacking in Thailand. As raw meat is frequently a source of diarrheagenic *E. coli*, a total of 1,279 *E. coli* colonies were obtained from 157 raw meat samples obtained from retail markets in Hat Yai City, Songkhla Province, Thailand and *E. coli* O26 isolated using an immune-magnetic separation technique. Twenty-seven *E. coli* O26 strains were isolated from 18 samples of raw beef, chicken and pork meats. These *E. coli* O26 strains could not be classified into the six diarrheagenic *E. coli* categories and did not harbor virulence genes, except 5 strains carrying *escV*, encoding type III secretion system component. Phylogenetic group examination demonstrated that 26 strains belonged to phylogenetic group A, and one to group D. Antimicrobial susceptibility test revealed that the *E. coli* O26 strains were the multi-drug resistant strains. Genetic relatedness employing $(GTG)_5$-PCR and ERIC2-PCR showed that some of O26 which isolated from different samples and different time intervals revealed the identical fingerprint pattern, suggesting that they were derived from the same clone. Examination of five stx_2-containing phage integration sites showed that 6 strains had prophage occupancy at *sbcB*, suggesting that these isolates have the potential in horizontal gene transfer of virulence trait. Moreover, the intactness of *yecE* and *wrbA*, the important integration sites in *E. coli* O26, indicated the possibility of stx_2-phage lysogenization in the future.

Keywords: *Escherichia coli* O26, diarrhea, phylogenetic group, raw meat, $(GTG)_5$-PCR, ERIC2-PCR, Thailand

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INTRODUCTION

Diarrheagenic *Escherichia coli* plays an important role in severe illnesses in humans. Among the six diarrheagenic *E. coli* categories, enterohemorrhagic *E. coli* (EHEC) including Shiga toxin-producing *E. coli* (STEC) cause the most devastating pathological effects (Nataro and Kaper, 1998). Shiga toxin (Stx) can lead to renal failure and death. Since the first outbreak of *E. coli* O157 in 1982 (Riley *et al*, 1983), this serotype has become the most important serotype to date. In Europe, besides infections caused by *E. coli* O157, *E. coli* O26 constitutes the most common non-O157 EHEC group, resulting in hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (EFSA, 2007, 2009; Bugarel *et al*, 2011) and several outbreaks caused by *E. coli* O26 through food consumption, have been reported to date (Ethelberg *et al*, 2009; Brown *et al*, 2012; CDC, 2012).

Raw meats have been reported to be sources of pathogenic *E. coli* worldwide (Mainil *et al*, 2011; Oh *et al*, 2012), including southern Thailand (Vuddhakul *et al*, 2000; Sukhumungoon *et al*, 2011; Pannuch *et al*, 2014). EHEC serotype O157:H7 was detected at a high proportion, but contamination of non-O157 group in raw meat has as yet not been detected in southern Thailand.

This study investigates the prevalence of *E. coli* O26, the most important serotype in the non-O157 group, in raw meat sold at retail stores in a city in southern Thailand. Pathotype classification, detection of virulence genes and antimicrobial resistance, and investigation of genetic relatedness were conducted.

MATERIALS AND METHODS

Sample collection

A total of 157 raw meat samples were collected from fresh markets in Hat Yai city, Songkhla Province, southern Thailand between October 2013 and May 2014 and processed within 2 hours.

Immuno-magnetic separation (IMS) of *E. coli* O26

E. coli O26 was isolated from raw meat samples using an IMS procedure as previously described (Sukhumungoon *et al*, 2011). In brief, 50 g of raw meat were mixed with 450 ml of tryptic soy broth (TSB) (Becton, Dickinson, Franklin Lakes, NJ) in a sterile plastic bag, homogenized for 1 minute in a Stomacher CIR-400 (Seward, West Sussex, UK) and supernatant was manually obtained by rinsing into a sterile bottle aseptically. Supernatant was incubated at 37ºC for 6 hours, and then 1 ml aliquot was mixed with immune-O26-specific magnetic beads (Dynabeads, Invitrogen, Carlsbad, CA) and incubated at ambient temperature with occasional gentle inversion of the incubation tube for 30 minutes. Magnetic beads were washed and isolated following the manufacturer's instructions, and then streaked on eosin methylene blue (EMB) agar (Becton, Dickinson, Franklin Lakes, NJ) and incubated at 37ºC for 18 hours. Ten green metallic sheen colonies from each meat sample were kept as stock in 10% glycerol at -80ºC for further analysis.

Agglutination and PCR-based detection of *E. coli* O26

For PCR-based detection of *E. coli* O26, DNA was extracted using a boiling method (Pannuch *et al*, 2014). In short, an individual bacterial colony was inoculated into 3 ml of TSB and incubated at 37ºC for 6 hours with aeration and 1 ml aliquot of the culture was boiled for 10 minutes and immediately immersed on ice for 5 minutes. Supernatant portion was obtained by centrifugation at 11,000*g* for 10 minutes. A 10-fold dilution of the

supernatant was carried out in sterile deionized water and used as PCR template. PCR using primers specific to *E. coli* O26 gene (Durso *et al*, 2005) (Table 1) was performed in a 25-µl reaction containing 0.4 µM each primer, 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; followed by 35 cycles of 94ºC for 1 minute, 48ºC for 1 minute, and 72ºC for 50 seconds; with a final step at 72ºC for 5 minutes. Amplicon was analyzed by 0.8% agarose gel-electrophoresis, stained with ethidium bromide and recorded using WSE-5200 Printpraph 2M gel imaging system (Tokyo, Japan). Confirmation of the presence of *E. coli* O26 was carried out by an agglutination assay using *E. coli* O26-specific antiserum (Denka Seiken, Tokyo, Japan).

E. coli pathotype classification and detection of other virulence genes

PCR was employed for the classification of *E. coli* O26 isolates into six pathotypes (*stx* + *eae* for EHEC; *bfp* + *eae* for enteropathogenic *E. coli* (EPEC); *est*/*elt* for enterotoxigenic *E. coli* (ETEC); *aggR* for enteroaggregative *E. coli* (EAEC); *ipaH* for enteroinvasive *E. coli* (EIEC); *daaE* for diffusely adherent *E. coli* (DAEC)) and detection of virulence genes, *ibeA* (a gene associated with avian pathogenic *E. coli* responsible for human neonatal meningitis), *astA* (coding for enteroaggregative heat-stable enterotoxin 1, EAST1), and *escV* [encoding Type III secretion system (T3SS), a component in the locus of enterocyte effacement, LEE, which is frequently found in EHEC and EPEC], using primers shown in Table 1. *E. coli* identification was performed by amplifying *uidA* (Table 1). PCR mixtures were as described above and thermocycling conditions were as follows: 95ºC for 3 minutes; followed by 35 cycles of 94ºC for 1 minute, 40ºC (for *est*), 50ºC (for *elt*, *aggR* and stx_2), 55ºC (for stx_1, *eae*, *bfp* and *daaE*), or 60ºC (for *ipaH*) for 1 minute, and 72ºC for 1 minute (except 1.15 minutes for *eae*); with a final step at 72ºC for 5 minutes. Amplicons were analyzed as described above.

Antimicrobial susceptibility test

Antimicrobial susceptibility of *E. coli* O26 was performed using a disk diffusion method (CLSI, 2014) using 9 common antimicrobial agents, namely, cephalothin (30 µg), chloramphenical (30 µg), fosfomycin (200 µg), gentamicin (10 µg), kanamycin (30 µg), penicillin G (10 µg), streptomycin (10 µg), tetracycline (30 µg) and vancomycin (30 µg) (Oxiod, Hamshire, UK). Vancomycin susceptibility was judged according to the criterion of CLSI (2007).

Identification of integrity of _E. coli_ integration sites for stx_2 phages

As stx_2 phages are able to integrate into specific genes, leading to increase in bacterial virulence, the intactness of 5 *E. coli* specific integration sites for stx_2 phages was examined by a PCR-based approach, based on that if a stx_2 phage integrates into a particular locus, PCR amplification does not occur due to the large inserted stx_2 phage genome (Bielaszewska *et al*, 2007). In brief, PCR amplifications of the insertion loci were carried out as described above for that of virulence genes but using primers specific to each integration locus (Table 1).

Phylogenetic group determination

Three PCR amplifications were carried out for determination of phylogenetic groups, namely, that of *chuA*, *yjaA* and TspE4.C2 fragment (Clermont *et al*, 2000). The reactions were performed using a reaction mixture as described above and

Table 1
Primers used in the study.

Gene	Virulence factor	Primer name	Sequence (5′ to 3′)	Amplicon size (bp)	Reference
wzx-wzyO26	O26 antigen	wzx-wzyO26f	AAATTAGAAGCGCGTTCATC	596	Durso *et al*, 2005
		wzx-wzyO26r	CCCAGCAAGCCAATTATCACT		
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		
stx1	Shiga toxin 1	EVT-1	CAACACTGGATGATCTCAG	350	Sukhumungoon *et al*,
		EVT-2	CCCCCTCAACTGCTAATA		2011
stx2	Shiga toxin 2	EVS-1	ATCAGTCGTCACTCACTGGT	404	Sukhumungoon *et al*,
		EVS-2	CCAGTTATCTGACATTCTG		2011
aggR	AggR, a transcriptional	AggR-1	CAGAATACATCAGTACACTG	433	Tsukamoto, 1996
	activator of AAF/I	AggR-2	GAAGCTTACAGCCGATATAT		
ipaH	Enteroinvasive	ipaIII	GTTCCTTGACCGCCTTTCCGATACCGTC	603,619	Sethabutr *et al*, 1993
	mechanism	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
		EAST11b	GGTCGCGAGTGACGGCTTTGT		Echeverria , 1996
ibeA	IbeA	ibeA-5F	TATTAGCATGATGTTGCTTG	1,500	Germon *et al*, 2005
		ibeA-5R	TGCCAACAACCAACACGATC		

Table 1 (Continued).

Gene	Virulence factor	Primer name	Sequence (5′ to 3′)	Amplicon size (bp)	Reference
wrbA	Quinone oxidoreductase	*wrb*A1	ATGGCTAAAGTTCTGGTG	600	Toth *et al*, 2003
		*wrb*A2	CTCCTGTTGAAGATTAGC		
yecE	Unknown	EC10	GCCAGCGCCGAGCAGCACAATA	400	DeGreve *et al*, 2002
		EC11	GGCAGGCAGTTGCAGCCAGTAT		
sbcB	Exonuclease I	*sbc*B1	CATGATCTGTTGCCACTCG	1,800	Ohnishi *et al*, 2002
		*sbc*B2	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		
escV	LEE	escV-F	GGCTCTCTTCTTCTTTATGGCTG	534	Müller *et al*, 2006
		escV-R	CCTTTTACAAACTTCATCGCC		
uidA	β-Glucoronidase	uidA-F	ATCACCGTGGTGACGCATGTCGC	486	Heninger *et al*, 1999
		uidA-R	CACCACGATGCCATGTTCATCTGC		
		(GTG)5[a]	GTGGTGGTGGTGGTG	Variable	Versalovic *et al*, 1991
		ERIC2[a]	AAGTAAGTGACTGGGGTGAGCG	Variable	Versalovic *et al*, 1991

[a]Repetitive sequence primers.

Table 2
Contamination of *E. coli* O26 in raw meat samples.

Source	No. of positive samples/ no. of samples (%)	No. of O26 positive isolates/ no. of isolates (%)
Chicken	11/74 (15)	15/674 (2)
Pork	1/28 (4)	1/181 (1)
Beef	6/55 (11)	11/424 (3)
Total	18/157 (12)	27/1,279 (2)

Samples were collected from retail markets, Hat Yai City, Songkhla Province, southern Thailand during October 2013 to May 2014.

primers listed in Table 1. Thermocycling conditions were as follows: 95ºC for 3 minutes; 35 cycles of 94ºC for 50 seconds, 54ºC for 50 seconds, and 72ºC for 30 seconds; and a final step of 72ºC for 5 minutes. Amplicons were analyzed as described above.

Typing of *E. coli* O26 strains

Typing of *E. coli* O26 strains was performed using two approaches, namely, polytrinucleotide $(GTG)_5$-PCR and enterobacterial repetitive intergenic consensus (ERIC) 2-PCR. Both $(GTG)_5$-PCR and ERIC2-PCR were carried out in a 25 μl reaction mixture containing 0.2 μM $(GTG)_5$ or ERIC2 primers (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 a glass fiber matrix spin column (Geneaid, New Taipei City, Taiwan)]. Thermocycling conditions were as follows: 95ºC for 3 minutes; followed by 30 cycles of 94ºC for 3 seconds, 92ºC for 30 seconds, 50ºC for 1 minute, and 65ºC for 8 minutes. Amplicons were analyzed by 1.5% agarose gel-electrophoresis for 2.5 hours at 100 V, and stained with ethidium bromide. Dendrograms then were constructed using unweighted pair-group method of arithmetic average (UPGMA) (BioProfile software, USA).

Statistical analysis

Data were analyzed using SPSS for Windows software, version 11.0 (SPSS, Chicago, IL). Pearson chi-square method was used to compare the relationship between presence of *E. coli* O26 and type of meat. Significant level was set at $p < 0.05$.

RESULTS

Prevalence of *E. coli* O26 in raw meat samples

From a total of 1,279 green metallic sheen bacterial colonies from 157 raw meat samples, *E. coli* O26 was found in 18 (12%) samples comprising 27 (2%) *E. coli* O26 isolates (Table 2). There are no statistically significant differences in percent *E. coli* O26 contamination among the three kinds of raw meat.

***E. coli* O26 pathotype and presence of virulence genes**

All 27 *E. coli* O26 strains exhibited the lack of amplicons specific to six *E. coli* categories. In addition, 5 strains (PSU102-PSU106 isolated from beef) possessed *escV* and no strains carried *ibeA* and *astA* genes responsible for diarrhea and severe illnesses (Table 3).

Phylogenetic group

Phylogenetic group identification

Table 3
Characteristics of *E. coli* O26 isolates from in raw meat samples.

Sample		Strain name	Virulence trait	Phylogenetic group[a]	Antimicrobial resistance[b]
Source	Sample no. (no. of isolates)				
Beef	12 (5)	PSU102	*escV*	A	FOS, P, S, Va
		PSU103	*escV*	A	FOS, P, S, Va
		PSU104	*escV*	A	FOS, P, S, Va
		PSU105	*escV*	A	FOS, P, S, Va
		PSU106	*escV*	A	FOS, P, S, Va
	24 (1)	PSU107	-	A	P, S, Va
	36 (1)	PSU108	-	A	P, S, Va
	37 (1)	PSU110	-	A	C, FOS, K, P, TE, S, Va
	38 (2)	PSU111	-	A	P, Va
		PSU112	-	A	P, Va
	41 (1)	PSU113	-	A	C, P, S, Va
Chicken	49 (1)	PSU114	-	A	K, P, S, TE, Va
	59 (1)	PSU115	-	A	KF, P, S, TE, Va
	64 (1)	PSU116	-	A	P, S, TE, Va
	87 (1)	PSU200	-	A	P, S, TE, Va
	92 (3)	PSU202	-	A	P, S, TE, Va
		PSU203	-	A	KF, P, S, TE, Va
		PSU204	-	A	P, S, TE, Va
	101 (1)	PSU205	-	A	P, S, Va
	103 (1)	PSU206	-	A	C, K, KF, P, S, TE, Va
	105 (3)	PSU207	-	A	C, K, P, S, TE, Va
		PSU208	-	D	KF, P, S, Va
		PSU209	-	A	C, K, P, S, TE, Va
	108 (1)	PSU210	-	A	P, S, TE, Va
	113 (1)	PSU211	-	A	K, KF, P, S, TE, Va
	137 (1)	PSU213	-	A	C, K, P, S, TE, Va
Pork	115 (1)	PSU212	-	A	C, KF, P, S, TE, Va

Samples were collected from retail markets, Hat-Yai city, Songkhla Province, southern Thailand during October 2013 to May 2014. [a]group A (*chuA* and TspE4.C2 were negative); group D (*chuA* was negative but *yjaA* was positive). [b]C, chloramphenicol (30 μg); FOS, fosfomycin (200 μg); K, kanamycin (30 μg); KF, cephalothin (30 μg); P, penicillin G (10 μg); S, streptomycin (10 μg); TE, tetracycline (30 μg); Va, vancomycin (30 μg).

based on PCR detection of *chuA*, *yjaA*, and TspE4.C2 fragment revealed that 26/27 *E. coli* O26 strains belonged to phylogenetic group A (*chuA* and TspE4.C2 negative) while one (PSU208) to group D (*chuA* negative and *yjaA* positive) (Table 3).

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

In order to investigate the integration site occupancy by stx_2 phage in *E. coli* O26, the integrity of five genes (*yecE*, *sbcB*, Z2577, *yehV*, and *wrbA*) frequently

reported to be occupied by stx_2 phages (DeGreve *et al*, 2002; Ohnishi *et al*, 2002; Koch *et al*, 2003; Shaikh and Tarr, 2003; Toth *et al*, 2003), were examined by PCR. Only 6/27 (22%) of *E. coli* O26 strains (PSU108, PSU112, PSU113, PSU200, PSU208, and PSU211) (Fig 1, lane 7, 10, 11, 15, 22, and 24, respectively) demonstrated phage occupation in *sbcB* gene. The remaining 4 genes in these 6 strains and all 5 genes in the remaining 21 strains were intact.

Antimicrobial susceptibility test

The disk diffusion method demonstrated that PSU110 from beef and PSU206 from chicken were resistant to 7/9 antimicrobial agents with the same resistant pattern except that PSU110 was resistant to fosfomycin and PSU206 to cephalothin (Table 3). Five strains (PSU207, PSU209, PSU211-213) were resistant to 6/9 antimicrobial agents, 3 (PSU114, PSU115, PSU203) to 5/9 agents, and all strains to penicillin G and vancomycin.

Genetic relationship among the *E. coli* O26 strains

Dendrograms generated from amplicon size varieties produced by ERIC2-PCR and $(GTG)_5$-PCR revealed that at 80% similarity the 27 *E. coli* O26 could be categorized into 13 and 11 distinct clusters, respectively (Fig 2). From the former dendogram, PSU102-PSU106 (cluster I) isolated from the same beef sample displayed the identical amplicon size profile, as did PSU111 and PSU112 (cluster III) from the same beef sample and PSU207 and PSU209 (cluster XI) from the same beef sample. Importantly, in clusters VII (PSU116, PSU200 and PSU202-PSU205) and VIII (PSU210 and PSU211), these strains were obtained from different meat samples and at different times. As for $(GTG)_5$-PCR-based dendrogram, although this approach showed lower discriminatory power than ERIC2-PCR, similar results were obtained, except for PSU200 from chicken, which represented identical amplicon size profile with PSU202-PSU205 in ERIC2-PCR (cluster VII) but was located distantly related (less than 40% similarity) by $(GTG)_5$-PCR (cluster III) (Fig 2).

DISCUSSION

IMS using magnetic beads coated with antibodies specific to somatic O antigen of bacterial cell wall increases isolation efficiency by 100-folds (Chapman *et al*, 1994). In this study, IMS was applied to isolate *E. coli* O26 from three common raw meats (beef, chicken and pork) from retail markets in Hat Yai City, Songkhla Province, southern Thailand. *E. coli* O26 from clinical source has been frequently shown to be a member of EHEC/STEC or atypical enteropathogenic *E. coli* (aEPEC) (Bielaszewska *et al*, 2007; Mainil *et al*, 2011). In addition, strains from environmental source possessing *stx* or *eae* are classified in EHEC or aEPEC pathotype (O'Hanlon *et al*, 2004; Mainil *et al*, 2011; Oh *et al*, 2012). Surprisingly, this current study found that almost all (22/27) *E. coli* O26 isolated lacked virulence genes and could not be classified into any of the six reported *E. coli* pathotypes. One sample from beef (containing PSU102-PSU106) carried *escV*. Thus, the majority of *E. coli* O26 contaminating raw meats sold in markets of Hat Yai City could be considered avirulent, there still exists strains containing *escV* (19%) capable of causing illness.

The majority of the putative avirulent *E. coli* O26 in this present study belonged to *E. coli* phylogenetic group A, a group with low pathogenicity. However, strain PSU208, belonging to group D, a high pathogenicity group, lacked the virulence genes investigated. This may be due to a

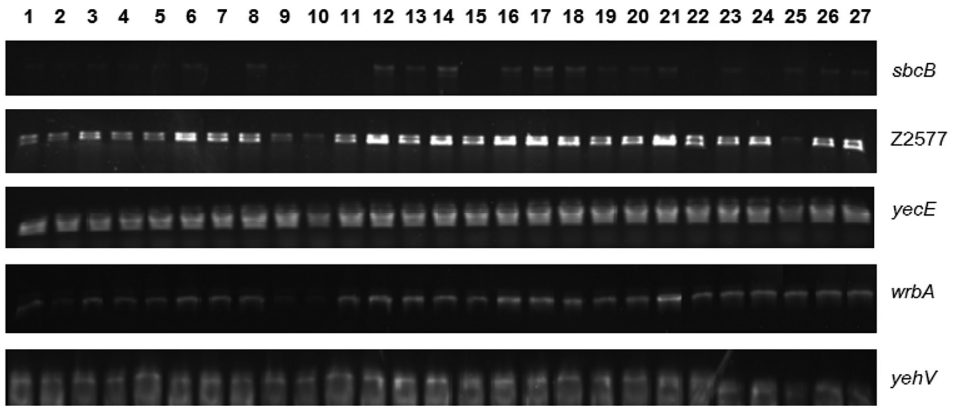

Fig 1–PCR amplification of stx_2-phage insertion-associated genes in 27 *E. coli* O26 isolated from raw meats. PCR conditions are described in Materials and Methods and primers listed in Table 1. Lanes 1-7 are PSU102-108, lanes 8-14 are PSU110-116; lane 15 is PSU200; lanes 16-23 are PSU202-PSU209; lanes 24-25 are PSU211-PSU212; lanes 26 and 27 are PSU 210 and PSU213.

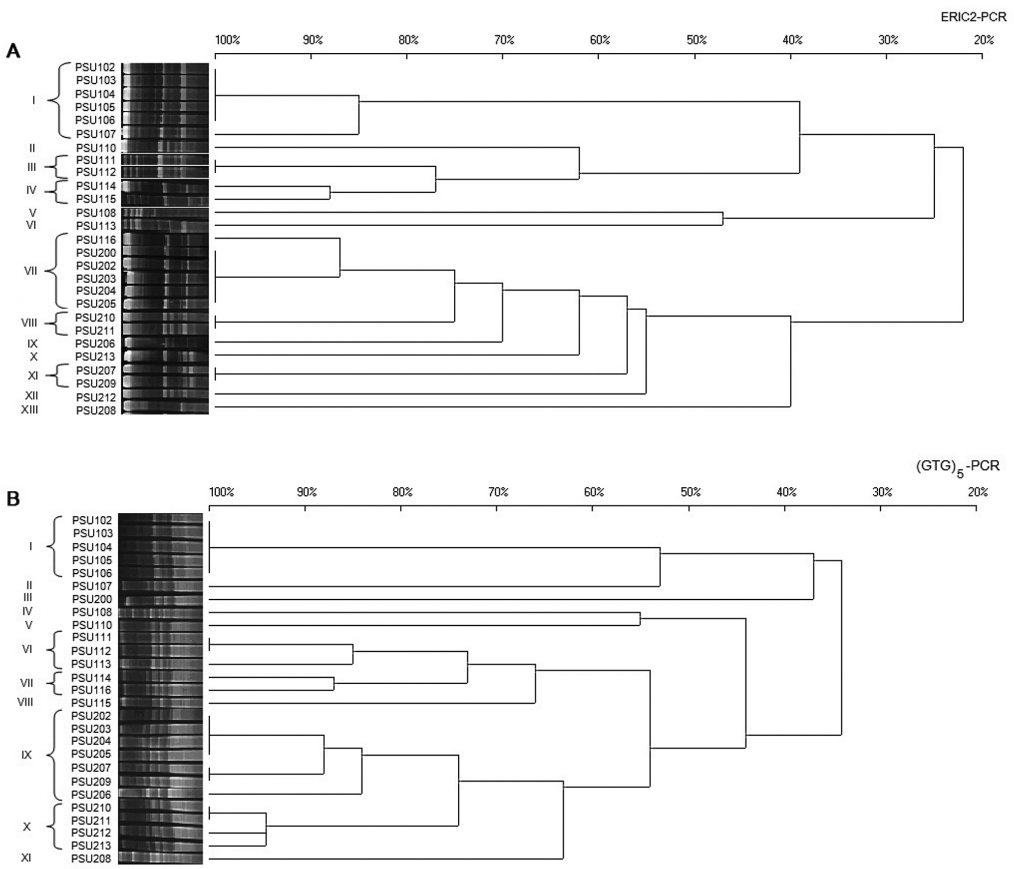

Fig 2–Dendrograms of 27 *E. coli* O26 isolated from raw meats based on (A) ERIC2-PCR and (B) $(GTG)_5$-PCR. PCR conditions are described in Materials and Methods and primers listed in Table 1. Classification into distinct clusters is based on 80% similarity of the repetitive sequence profiles.

presence of a DNA region homologous to that of group D signature sequence (Clermont *et al*, 2000), or a group D *E. coli* O26 that has lost its virulence traits. The acquisition of LEE pathogenicity island may explain strains PSU102-PSU106 possessing *escV* but belonging to phylogenetic group A.

$(GTG)_5$-PCR has been shown to be a promising source-tracking tool with higher discriminatory power compared to ERIC-PCR and other rep-PCR for *E. coli* (Mohapatra *et al*, 2007). Nonetheless, Adiguzel *et al* (2012) described the genetic relationship of *E. coli* O157:H7 isolated from beef, which demonstrates comparable efficiency between ERIC-PCR and $(GTG)_5$-PCR. Rasschaert *et al* (2005) also found similar results for *Salmonella enterica*. In addition, there are studies showing the superior efficiency of using ERIC-PCR employing $(GTG)_5$-PCR for identifying *Bacillus* spp (Freitas *et al*, 2008) and *Yersinia ruckeri* (Huang *et al*, 2013). Thus, we suggest that the discriminatory power among repetitive sequences-PCR may vary from laboratory to laboratory depending on such factors as bacterial species and the type of functional analysis used. This study found that ERIC2-PCR and $(GTG)_5$-PCR are both reliable tools for *E. coli* typing.

Importantly, ERIC2-PCR revealed that 8 *E. coli* O26 strains (6 from cluster VII and 2 from cluster VIII) have the same amplicon size profile although they were obtained from different samples and at different times, suggesting the possibility that these *E. coli* O26 strains have originated from the same clone, suggesting that there may be present certain clones circulating in the environment, which are subsequently introduced into animals resulting in their infection and spread to other animals in the same farm.

Although the majority of *E. coli* O26 strains exhibit non-pathogenic characteristics, however, in the future these strains may pose a possible threat to human health by gaining virulence genes, such as stx_2, through integration of virulence gene-containing bacteriophage into specific gene (Bielaszewska *et al*, 2007). Integration into *yecE* or *wrbA* allows stx_2-encoding phage lysogenization of not only *E. coli* O26 but also *E. coli* O157:NM (non motile) (Mellmann *et al*, 2008). In this current study, it was found that 6/27 *E. coli* O26 isolates contained prophage occupation at *sbcB*, and these strains are possible sources of future horizontal gene transfer. More importantly, *yecE* and *wrbA*, important integration sites in *E. coli* O26 are still intact, suggesting a high possibility of bacteriophage integration into these sites in the future.

Antimicrobial drug resistance in *E. coli* was observed to have increased in the last 5 decades in USA, particularly to ampicillin, sulfonamide and tetracycline (Tadesse *et al*, 2012). Studies from Vietnam demonstrated that tetracycline resistance is the most frequent in raw meats (Van *et al*, 2008), which corresponds to our current work of 56% tetracycline resistant strains in raw meats. The existence of multi-drug resistance in *E. coli*, defined as resistance to at least 3 different classes of antibiotics, is not surprising in this region of the world where non-prescription drugs are readily available as was reported in the present study. Although the majority of *E. coli* O26 strains in this study did not carry virulence genes, their potential to obtain such genes may occur and this will be a public health problem in the future.

In summary, we have shown that *E. coli* O26 exists in raw meats sold in Hat Yai City, Songkhla Province, southern Thailand, although at low prevalence and

with the majority having non-pathogenic characteristics. Typing analysis revealed that a number of strains, collected from different localities and at different times, originated from the same clone. As these *E. coli* O26 strains contain intact sites for integration of virulence gene-carrying phages, they retain an ability to become more virulent in the future. In addition, the multi-drug resistant characteristic of these strains pose problems in therapeutic intervention. To the best of our knowledge, this is the first report conducted in Thailand on the monitoring of the prevalence and characterization of *E. coli* O26 from raw meats.

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