PREVALENCE, MOLECULAR CHARACTERIZATION AND GENETIC RELATEDNESS OF *ESCHERICHIA COLI* O103 FROM MEAT IN SOUTHERN THAILAND

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Abstract. *Escherichia coli* O103 causes gastroenteritis worldwide. Among 1,498 *E. coli* isolates obtained from 59 meat samples in southern Thailand during July 2016 to February, 2017, 125 isolates were identified as *E. coli* O103. Although these isolates did not belong to any of the six diarrheagenic *E. coli* categories, they possessed *E. coli* virulence genes, *fimH* (encoding type 1 fimbrial tip) (100%) and *astA* (encoding enteroaggregative heat-stable enterotoxin 1) (21%). Surprisingly, one isolate (*E. coli* O103 strain 103.10) was identified as an extraintestinal pathogenic *E. coli* carrying *iutA* (encoding aerobactin receptor) and *kpsMT*II (encoding capsular antigen). Phylogenetic group analysis revealed 3%, 5%, 30%, and 62% of the isolates belonged to group B2, D, B1, and A, respectively. Sixty-nine percent of *E. coli* O103 of strains carried prophage in *sbcB* integration sites. The O103 strains were resistant to cephalothin (78%), streptomycin (93%) and tetracycline (48%). Genetic relatedness of 68 *E. coli* O103 surrogate strains determined by BOX-PCR showed, at 80% similarity, 22 clusters. Some of them that demonstrated identical DNA profile were collected from different samples and different time, suggesting an origin from the same source. The high prevalence of *E. coli* O103 in raw meat highlights the public health importance of this food source as a potential vehicle for the transfer of this pathogenic bacterial type to consumers in southern Thailand.

Keywords: *Escherichia coli* O103, antibiogram, BOX-PCR, meat, southern Thailand

INTRODUCTION

Enterohemorrhagic *Escherichia coli* (EHEC) is highly virulent among the six diarrheagenic *E. coli* (DEC) categories (Nataro and Kaper, 1998). This EHEC group has emerged as a serious public health concern for several decades (Riley *et al*, 1983), especially in outbreaks caused by the serotype O157:H7 (Themphachana *et al*, 2014). EHEC carries *stx* (*stx*$_1$ or *stx*$_2$ or both) encoding Shiga toxins, which affect mainly to human renal cells, leading to hemolytic uremic syndrome (HUS) and mortality (Nataro and Kaper, 1998). However, non-O157 group also causes sporadic and gastroenteritis outbreaks worldwide (Perelle *et al*, 2007; Brown *et al*, 2012).

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Within the non-O157 group, *E. coli* O103 is one of the most important serotypes as it causes either sporadic disease or outbreak in both western and eastern hemispheres (Ogura *et al*, 2007; L' Abée-Lund *et al*, 2012). In Norway during 2006, a severe food-borne outbreak occurred by stx_2-positive EHEC O103:H25 infection with seventeen cases, with 10 HUS and 1 fatality; the source was traced to contaminated mutton sausages. In France, the infection was caused by stx_1-carrying *E. coli* O103:H2 (Mariani-Kurkdjian *et al*, 1993), and in Japan, the infection in 2001 was caused by *E. coli* O103:H2 carrying stx_1 and stx_2 (Ogura *et al*, 2007). The *stx*-negative *E. coli* O103 also plays a role in pathogenesis (L' Abée-Lund *et al*, 2012). Natural host reservoirs of highly virulent *E. coli* O103 are mainly ruminants especially sheep and cattle where the bacteria reside in the gut allowing readily transfer to humans through number of common routes, *eg* bacterial shedding from animal gut to vegetables in the farm or raw meat contamination during the slaughtering processing (Pannuch *et al*, 2014).

Although many studies on *E. coli* O103 have been conducted in many areas of the world (Karama *et al*, 2008; Schimmer *et al*, 2008), information regarding *E. coli* O103 from animal sources in Thailand is sparse (Kayali *et al*, 2015). A high prevalence of *stx*-negative *E. coli* O103 exists in raw meat, an important vehicle of EHEC (Mainil *et al*, 2011). In this study, from raw meat samples in southern Thailand more than 100 *E. coli* O103 strains obtained using an immune-magnetic separation method, were characterized for their virulence, antibiogram, and DNA profile. This study provides information on bacterial distribution and their characteristics of importance to control measures of food-borne diseases in this part of Thailand.

MATERIALS AND METHODS

Sample collection

E. coli O103 from 140 raw meat samples (54 beef, 43 chicken and 43 pork) were collected from eight open markets throughout Hat Yai city, Songkhla Province, southern Thailand between July, 2016 and February, 2017. All samples were processed within two hours after procurement.

Immuno-magnetic separation (IMS)

IMS to isolate *E. coli* O103 from raw meat samples was performed as previously described (Sirikaew *et al*, 2015). In brief, 50 g of raw meat were mixed with 450 ml of tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD), homogenized and the liquid fraction was incubated at 37ºC for six hours. One ml aliquot of culture was mixed with O103-specific magnetic beads (Dynabeads, Thermo Fisher Scientific, Waltham, MA) and incubated at ambient temperature for 30 minutes. The magnetic beads were washed and recovered according to the manufacturer's instructions. Beads were 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 until used.

E. coli O103 identification

Identification of *E. coli* O103 was performed by PCR using primers specific to *wzx*O103 (Monday *et al*, 2007) (Table 1). DNA template was prepared by boiling method as described previously (Phetkhajorn *et al*, 2014). In short, an individual colony was inoculated into TSB, incubated at 37ºC for three hours with aeration and an aliquot of bacterial culture was boiled for ten minutes, immersed on ice, centrifuged at 11,000*g* for 10 minutes and

supernatant used as DNA template. PCR was performed in a 25-µl reaction mixture comprising of 0.4 µM primer pair, 0.1 mM dNTPs, 1X Go*Taq* DNA polymerase buffer (Promega, Madison, WI), 0.5 U Go*Taq* DNA polymerase, and 2 µl of DNA template. Thermocycling condition (conducted in T100™ Thermal Cycler; Bio-Rad, Hercules, CA) was as follows: 95ºC for 3 minutes; followed by 35 cycles of 94ºC for 1 minute, 50ºC for 1 minute, and 72ºC for 1 minute; with a final step at 72ºC for 5 minutes. Amplicons were analyzed by 1.0% agarose gel-electrophoresis, stained with ethidium bromide and image captured by an ATTO WSE-5200 Printpraph 2M gel imaging system (ATTO, Tokyo, Japan). *E. coli* identification was performed by PCR targeting *uidA* (annealing temperature of 59ºC) (Table 1).

DEC *E. coli* pathotype classification, extraintestinal pathogenic *E. coli* (ExPEC) identification and other *E. coli* O103 virulence genes detection

DEC pathotype classification of *E. coli* O103 was performed by PCR targeting pathotype-specific genes of all six DEC categories, *eg*, *stx+eae* for EHEC, *bfp+eae* for typical enteropathogenic *E. coli* (tEPEC), *eae* alone for atypical enteropathogenic *E. coli* (aEPEC); *est*/*elt* for ETEC, *aggR* for EAEC, *ipaH* for enteroinvasive *E. coli* (EIEC), and *daaE* for diffusely adherent *E. coli* (DAEC) (Table 1). PCR was carried out in a 25-µl reaction mixture consisting of 0.4 µM specific primers for each gene, 0.1 mM dNTPs, 1X Go*Taq* DNA polymerase buffer (Promega), 0.5 U Go*Taq* DNA polymerase and 2 µl of DNA template. Thermocycling was conducted in T100™ Thermal Cycler, (Bio-Rad) with the following conditions: 95ºC for 3 minutes; 35 cycles of 94ºC for 1 minute, 40ºC (for *est*), 50ºC (*elt*, *aggR*, stx_2), 55ºC (stx_1, *eae*, *bfp*, *daaE*), or 60ºC (*ipaH*) for 1 minute, and 72ºC for 1 minute or 1.15 minutes (for *eae*); and a final step at 72ºC for 5 minutes.

ExPEC is defined as *E. coli* carrying two or more of the following virulence genes: *afa*, *iutA*, *kpsMT*II, *papA* and/or *papC*, and *sfaDE* (Johnson *et al*, 2003). PCR amplification of these genes (Sukkua *et al*, 2015) was performed in a 25-µl reaction mixture composed of 0.4 µM each specific primer pair (Table 1), 0.1 mM dNTPs, 1X Go*Taq* DNA polymerase buffer (Promega), 3.0 mM $MgCl_2$, 0.5 U Go*Taq* DNA polymerase, and 2 µl of DNA template. Thermocycling was carried out in a thermal cycler (T100™, Bio-rad) for 35 cycles of denaturation at 94ºC for 1 minute, 60ºC (*afa*, *papA*, *papC*), 58ºC (*iutA*, *sfaDE*), 52ºC (*kpsMTII*), or 50ºC (*kpsMT*K1) for 1 minute, and 72ºC for 1 minute; and a final step of 72ºC 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, thermocycling conditions (except that the annealing temperature, 50ºC for *astA*, 55ºC for *fimH* and *lpf*, 58ºC for *cnf1* and *hlyA*, and 67ºC for *agn43*), and amplicon analysis were as described above.

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

As stx_2 phages can integrate into specific genes and may lead to an increase in bacterial virulence, the intactness of five *E. coli* specific integration sites for stx_2 phages was investigated by PCR (Saelim *et al*, 2017). PCR amplification of the insertion locus was performed in a 25-µl of reaction mixture consisting of the same components as for *E. coli* O103 identification except the use of specific primers for each locus (Table 1) and the following annealing temperatures: 47ºC for *wrbA*,

Table 1
Primers used in the study.

Gene	Encoded factor	Primer name	Sequence (5′ to 3′)	Amplicon size (bp)	Reference
*wzx*O103	O103 antigen	5′O103	TATCCTTCATAGCCTGTTGTT	320	Monday *et al* (2007)
		3′O103	AATAGTAATAAGCCAGACACCTG		
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* (2011)
		EVT-2	CCCCCTCAACTGCTAATA		
stx2	Shiga toxin 2	EVS-1	ATCAGTCGTCACTCACTGGT	404	Sukhumungoon *et al* (2011)
		EVS-2	CCAGTTATCTGACATTCTG		
aggR	AggR, a 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		
fimH	Type 1 fimbrial tip	fimH-F	TGCAGAACGGATAAGCCGTGG	508	Johnson and Stell, 2000
		fimH-R	GCAGTCACCTGCCCTCCGGTA		
hlyA	α-hemolysin	hly1	AACAAGGATAAGCACTGTTCTGGCT	1,177	Yamamoto *et al* (1995)
		hly2	ACCATATAAGCGGTCATTCCCGTCA		
cnf1	Cytotoxic necrotizing factor-1	cnf1-F	GGCGACAAATGCAGTATTGCTTGG	552	Yamamoto *et al* (1995)
		cnf1-R	GACGTTGGTTGCGGTAATTTTGGG		
lpf	Long polar fimbriae	*lpfA*1-F	GGTCGTTTTTGCCTTAACCGC	≈500	Torres *et al* (2004)
		*lpfA*1-R	AGGTTGAAATCGACCTGCGC		

Table 1 (Continued).

Gene	Encoded factor	Primer name	Sequence (5′ to 3′)	Amplicon size (bp)	Reference
agn43	Antigen 43	1-Kpn	GAACCTGTCGGTACCGATGCCCTCCC	≈900	Danese *et al* (2000)
		2-Bam	CGGGATCCGTTGCCACTGTACCGGGCTTGACGACC		
papA	P fimbriae subunit	papA f	ATGGCAGTGGTGTCTTTTGGTG	720	Johnson and Stell (2000)
		papA r	CGTCCCACCATACGTGCTCTTC		
papC	Outer membrane usher protein	papC f	GTGGCAGTATGAGTAATGACCGTTA	200	Johnson and Stell (2000)
		papC r	ATATCCTTTCTGCAGGGATGCAATA		
sfaDE	S fimbriae	sfaDE-F	CTCCGGAGAACTGGGTGCATCTTAC	408	Le Bouguénec *et al* (1992)
		sfaDE-R	CGGAGGAGTAATTACAAACCTGGCA		
afa	Afa adhesin	afa1	GCTGGGCAGCAAACTGATAACTCTC	750	Le Bouguénec *et al* (1992)
		afa2	CATCAAGCTGTTTGTTCGTCCGCCG		
kpsMTII	Capsular antigen	kpsII f	GCGCATTTGCTGATACTGTTG	272	Johnson and Stell (2000)
		kpsII r	CATCCAGACGATAAGCATGAGCA		
iutA	Aerobactin	AerJ f	GGCTGGACATCATGGGAACTGG	300	Johnson and Brown (1998)
		AerJ r	CGTCGGGAACGGGTAGAATCG		
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		
uidA	β-glucoronidase	uidA-F	ATCACCGTGGTGACGCATGTCGC	486	Heninger *et al* (1999)
		uidA-R	CACCACGATGCCATGTTCATCTGC		
boxA	N/A	BOXA1R	CTACGGCAAGGCGACGCTGACG	Variable	Versalovic *et al* (1994)

N/A, not applicable.

Table 2
Prevalence of *E. coli* O103 in raw meat samples collected during July 2016 to February 2017, Hat Yai, Songkhla, Thailand.

Source	Number of positive samples/number of samples (%)	Number of O103 isolates/total number isolates (%)
Chicken	21/43 (49)	40*/430 (9)
Pork	16/43 (37)	40/430 (9)
Beef	22/54 (41)	45/638 (7)
Total	59/140 (42)	125/1,498 (8)

*One isolate is ExPEC O103 strain 103.10.

50ºC for *sbcB* and *yehV*, 53ºC for Z2577, and 60ºC for *yecE*. Absence of amplicon indicates the presence of phage integration at the locus of interest.

Phylogenetic group analysis

PCR targeting three genes were employed for investigation of phylogenetic group, namely, *chuA*, *yjaA*, and TspE4. C2 fragment (Clermont *et al*, 2000). Each reaction was carried out as a singleplex PCR using specific primers (Table 1) in a 25-µl solution comprising of 0.4 µM primer pair, 0.1 mM dNTPs, 1X Go*Taq* DNA polymerase buffer (Promega), 0.5 U Go*Taq* DNA polymerase, and 2 µl of DNA template. Thermocycling (in T100™ Thermal Cycler; Bio-Rad) conditions for those three reactions were as follows: 95ºC for 3 minutes; followed by 35 cycles of 94ºC for 50 seconds, 54ºC for 50 seconds, and 72ºC for 30 seconds; and a final step at 72ºC for 5 minutes. Amplicons were analyzed as described above.

Antibiogram determination

Antimicrobial susceptibility of *E. coli* O103 was performed using a disk diffusion method (CLSI, 2014) against 10 common antimicrobial agents, namely, 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 (25 µg) (Oxoid, Hampshire, UK).

DNA profiling of *E. coli* O103

For the construct of DNA profiles, for each meat sample, a strain from each genotype detected was selected to be a surrogate for that genotype. DNA profiling of *E. coli* O103 was carried out using BOX-PCR (Versalovic *et al*, 1994). In short, PCR was performed in a 25-µl reaction mixture consisting of 0.2 µM each specific primer (Table 1), 0.2 mM dNTPs, 1X Go*Taq* DNA polymerase buffer (Promega), 3.0 mM $MgCl_2$, 1.25 U Go*Taq* DNA polymerase and 10 ng of DNA template [prepared by glass fiber matrix spin column (Geneaid, Taipei, Taiwan)]. Thermocycling conditions conducted in a thermal cycler (T100™, Bio-rad), were as follows: 95ºC for 3 minutes, followed by 35 cycles of 94ºC for 3 seconds, 92ºC for 30 seconds, 50ºC for 1 minute, and 65ºC for 8 minutes. Amplicons were analyzed using 1.5 % agarose gel-electrophoresis at 90 V for 2 hours and recorded as described above. Dendrogram was constructed using unweighted pair-group method of arithmetic average (UPGMA) (BioProfile

software; Vilber Lourmat, Torey, France).

Statistical analysis

Data were analyzed using SPSS for Windows version 11.0 (SPSS, Chicago, IL). One-way ANOVA was used to analyze significant difference in *E. coli* O103 prevalence among meat types. Significance is accepted at $p < 0.05$.

RESULTS

Prevalence of *E. coli* O103 in raw meat samples

A total of 1,498 colonies isolated from 140 raw meat samples were investigated for the presence of *E. coli* O103, which was found in 59 (42%) samples, comprising 125 (8%) isolates (Table 2). The prevalence of *E. coli* O103 in chicken (49%), beef (41%) and pork (37%) samples were not statistically different.

DEC *E. coli* pathotype classification, ExPEC identification and detection of other *E. coli* O103 virulence genes

All but one *E. coli* O103 strains did not belong to DEC and ExPEC groups, one strain (*E. coli* O103 strain 103.10 from chicken) was an ExPEC possessing *iutA* and *kpsMTII* (data not shown). Interestingly, all *E. coli* O103 strains carried *fimH* (encoding type 1 fimbrial tip), conferring bacterial adherence and auto-aggregation, 26 (21%) carried *astA* [encoding enteroaggregative heat-stable enterotoxin 1 (EAST-1)], responsible for diarrhea but no strain carried *agn43*, *cnf1*, *hlyA*, and *lpf* (Fig 1A).

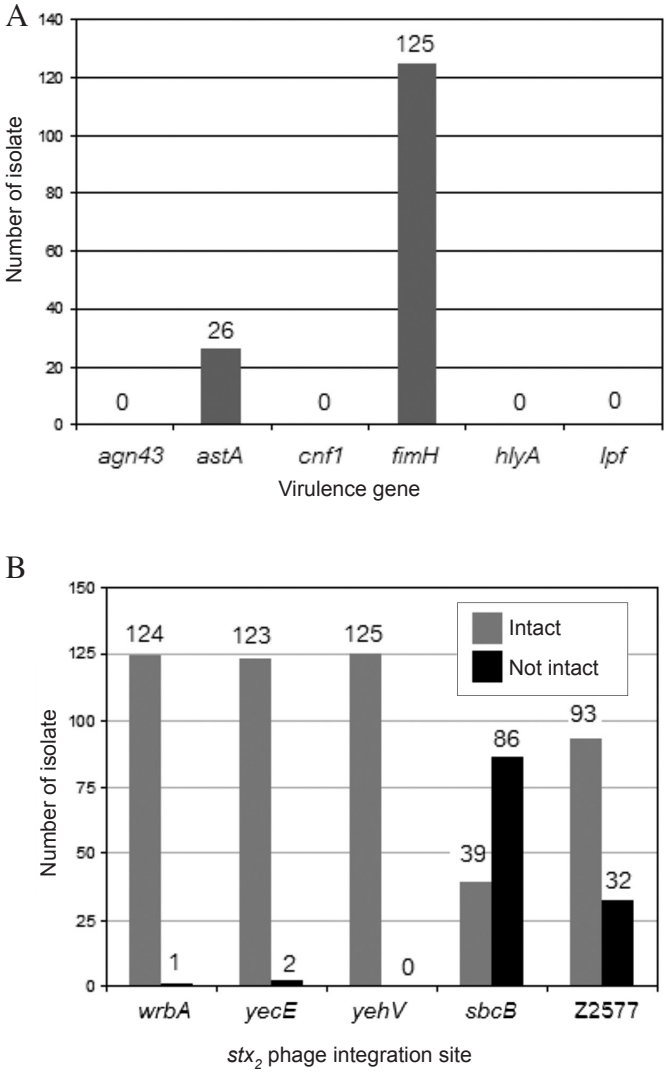

Fig 1–*E. coli*-associated virulence genes (A) and intactness of stx_2 phage insertion-associated genes (B) in 125 *E. coli* O103 isolates from raw meat samples collected during July 2016 to February 2017, Hat Yai, Songkhla, Thailand. Genes were PCR amplifies using specific primer pairs listed in Table 1.

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

In order to investigate the integrity

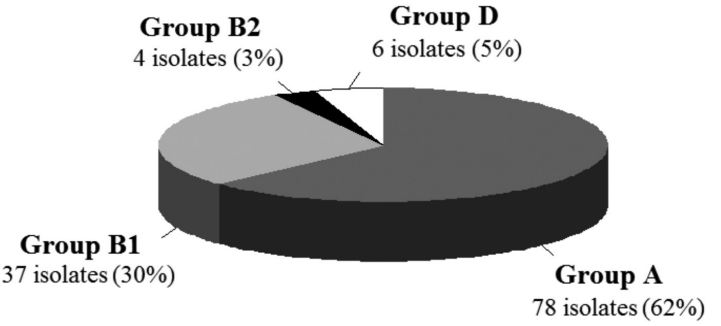

Fig 2–Phylogenetic group of 125 *E. coli* O103 strains isolated from meat samples collected during July 2016 to February 2017, Hat Yai, Songkhla, Thailand. Phylogenetic group analysis was based on PCR amplification of *chuA*, *yjaA*, and TspE4. C2 fragment.

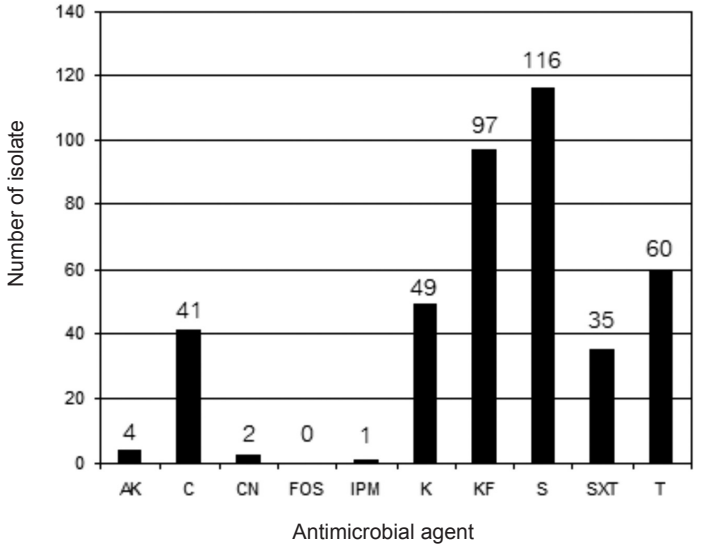

Fig 3–Antimicrobial susceptibility by disc diffusion assay of 125 *E. coli* O103 strains isolated from meat samples collected during July 2016 to February 2017, Hat Yai, Songkhla, Thailand. Column represents number of resistant strains. AK, amikacin (30 μg); C, chloramphenical (30 μg); CN, gentamicin (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).

of integration sites of stx_2 phages in *E. coli* O103 genome, five genes (*wrbA*, *sbcB*, *yehV*, *yecE*, and Z2577) frequently occupied by stx_2 phages were examined for their intactness, which revealed *yehV* was not integrated by stx_2 phage in all 125 *E. coli* O103 strains while *sbcB* was integrated at the highest (86 strains, 69%) (Fig 1B).

Phylogenetic group evaluation

PCR-based phylogenetic group analysis performed on *chuA*, *yjaA*, and TspE4.C2 fragment showed 3%, 5%, 30%, and 62% of *E. coli* O103 strains belonged to group B2, D, B1, and A, respectively (Fig 2).

Antibiogram profile

The majority of *E. coli* O103 strains were resistant to streptomycin (93%), followed by cephalothin (78%), and tetracycline (48%) (Fig 3). All strains were still susceptible to fosfomycin. Nevertheless, one *E. coli* O103 strain demonstrated resistance to imipenem, suggesting the possibility of this strain as a carbapenem-resistant *E. coli*.

DNA profiling

DNA profiles generated by BOX-PCR demonstrated, at 80% similarity,

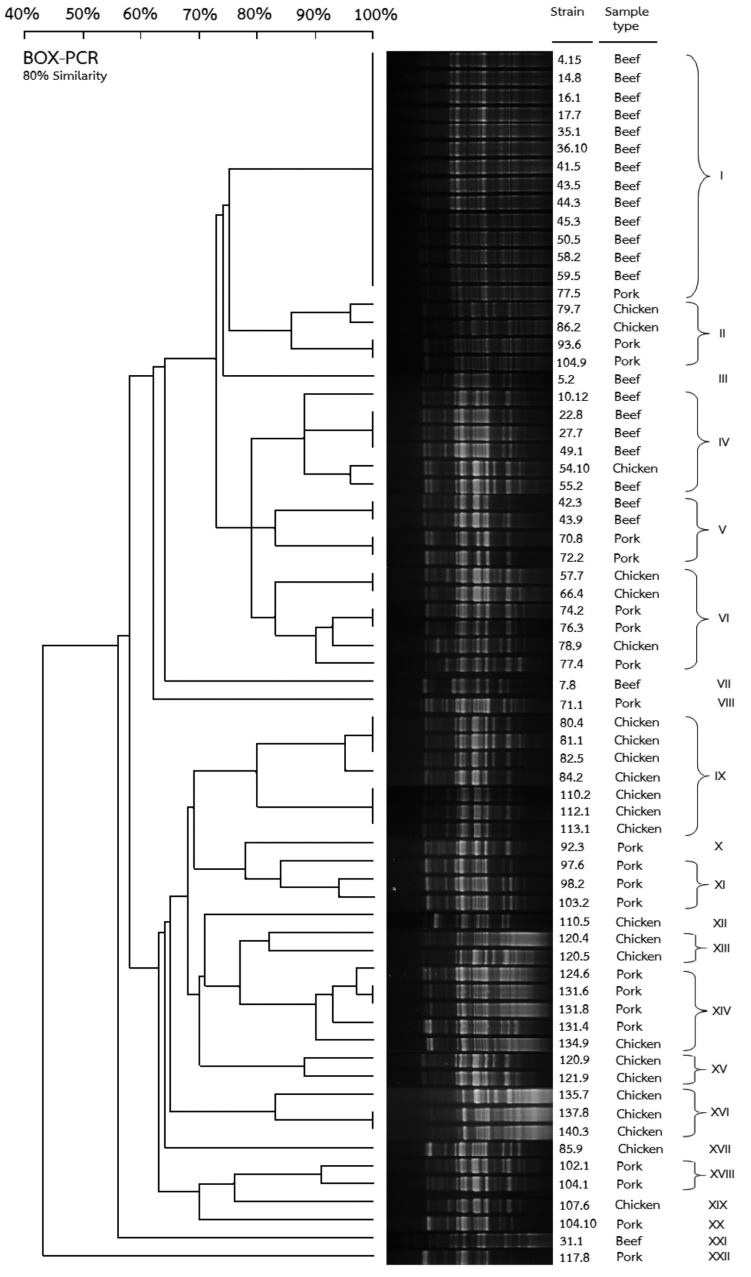

Fig 4–BOX-PCR-based dendrogram of 68 surrogate *E. coli* O103 strains from raw meat samples collected during July 2016 to February 2017, Hat Yai, Songkhla, Thailand. DNA profiles were generated from PCR amplification of *boxA*. The dendrogram was constructed using unweighted pair-group method of arithmetic average (UPGMA) (BioProfile software; Vilber Lourmat, Torey, France) and profiles were placed into distinct groups based on 80% similarity.

a dendrogram constructed from 68 surrogate *E. coli* O103 strains, which categorized them into 22 distinguishable patterns (cluster I-XXII) (Fig 4). Among 11 identical DNA patterns, 10 were from strains isolated from the same type of meat. Cluster I contained strains obtained from beef and pork. Those identical DNA profiles were observed in *E. coli* O103 strains isolated from different meat samples and at different times, demonstrated that there were probably generated from the same source and there were several O103 clones circulated in Thai environment.

DISCUSSION

Subsequent to the first report in 1988 of STEC O103 in Spain (Blanco *et al*, 1988), this *E. coli* O103 serotype has emerged as an important food-borne pathogen worldwide (Karama *et al*, 2008). Among the DEC serotypes, prevalence of *E. coli* O103 in this study was high (42%). Sukhumungoon *et al* (2011) reported an 18% prevalence of EHEC O157 from beef samples in southern Thailand,

and comparable prevalence of *E. coli* O26 (12%) and EAEC O104 (17%) were observed from meat samples in the same area in 2013-2014 and 2017, respectively (Sirikaew *et al*, 2015; Wameadesa *et al*, 2017), while very low (3%) prevalence of aEPEC O145 was found in 2017 (Sae-lim *et al*, 2017). Our results on *E. coli* O103 corresponds to the previous study of Sekse *et al* (2013) in Norway, where *E. coli* O103 is positive in 31.5% of sheep (n = 585). The existence and distribution of *E. coli* O103 in various geographical locations indicated that the problem is not only restricted to Thailand.

In the nationwide investigation of Norwegian sheep during 2006-2007, Sekse *et al* (2013) demonstrated that *eae*-negative, *stx*-negative *E. coli* O103 was common (27.5%), consistent with our results demonstrating that *E. coli* O103 strains from meat in this southern area of Thailand are *stx*-negative *E. coli* O103. Focusing on their infection capability, even though they are *stx*-negative, it is worth noting that they are considered pathogenic to some extent as they carry the virulence genes, *fimH* and *astA*, and perhaps others. L' Abée-Lund *et al* (2012) have also reported food-borne infection caused by *stx*-negative O103.

Integration of prophage plays a key role in *E. coli* O157:H7 evolution (Ooka *et al*, 2009) and the increase in its pathogenesis (Sukhumungoon and Nakaguchi, 2013). Integration of phage occurs through site-specific recombination or transposition and phage integration sites are located within housekeeping genes or in regions in close proximity to tRNA genes (Schmidt, 2001). The preference sites for *stx* phage integration are *yehV* for *E. coli* O157:H7 from Spain (Serra-Moreno *et al*, 2007) and *yecE* for sorbitol-fermenting *E. coli* O157:non-motile (NM) (Mellmann *et al*, 2008). In this study, 69% of *E. coli* O103 have prophages integrated into *sbcB*, consistent not only with data showing *E. coli* O157:H7 from beef collected between 1998-2012 in Thailand with 98% *sbcB* occupancy (Sukhumungoon and Nakaguchi, 2013) but also with those of *E. coli* O26 (Sirikaew *et al*, 2015) and EAEC O104 from the same area (Wameadesa *et al*, 2017) demonstrating 22% and 39% occupancy, respectively. Thus, *sbcB* might be the preferred site for prophage integration in both *E. coli* O157 and non-O157 in this area. Worrisomely, the highly dynamic interchange between aEPEC and EHEC through the loss and gain of stx_2-encoding phage was demonstrated (Bielaszewska *et al*, 2007). Thus, there is a possibility that these *E. coli* O103 strains can obtain stx_2 phage in the future, resulting in a serious public health problem.

Antimicrobial resistant profiles of *E. coli* O103 to cephalothin, streptomycin, and tetracycline are consistent with other studies in the same geographical area. In 2015, *E. coli* O26 from meats is 22%, 93%, and 56% resistant to cephalothin, streptomycin, and tetracycline, respectively (Sirikaew *et al*, 2015). Moreover, all four *stx*-negative *E. coli* O157 strains isolated in the same year are resistant to cephalothin and streptomycin, and one strain is resistant also to tetracycline (Sirikaew *et al*, 2016). This indicates the ineffectiveness of these antimicrobial agents for treatment of possible infection by DEC from meat.

BOX-PCR has proven to be a robust method for investigating genetic relatedness of bacteria. It is easy to perform and the resulting DNA profile is reproducible and reliable as *boxA*, the target of BOX-PCR, is highly conserved among bacterial species (Versalovic *et al*, 1994). In the current study, although some *E. coli* O103 strains were shown to be distantly related

but some O103 strains from different samples exhibited identical DNA profiles, suggesting they probably originated from the same source, and certain *E. coli* O103 strains may continuously and widely be distributed in the environment.

In summary, this study demonstrates that the prevalence of *E. coli* O103 in meat in this area is concerningly high. The bacteria show a capability to cause disease and problems in therapy. The identical DNA profiles among the *E. coli* O103 strains isolated from different samples and at different times suggest that there are certain clones circulating in southern Thailand, which may subsequently be introduced into cattle, resulting in contamination of feces in animal carcasses during the meat slaughtering processes. Thus, the high distribution of *E. coli* O103 in meat in this region of the country should be concerned to public agencies involved in protecting public health and safety.

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