ANTIMICROBIAL RESISTANCE, INTEGRON, VIRULENCE GENE, AND MULTILOCUS SEQUENCE TYPING OF *ESCHERICHIA COLI* ISOLATES FROM POSTWEANING PIGLETS WITH AND WITHOUT DIARRHEA

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Abstract. Pathogenic *Escherichia coli* is a major cause of diarrhea in postweaning piglets. Virulence genes, antimicrobial resistance, integrons, and genetic diversity of *E. coli* were determined in 100 rectal swab samples collected from postweaning piglets with and without diarrhea (5-7 weeks of age) in a farm in a central province of Thailand. Of 246 *E. coli* isolates, 141 were positive for at least one virulence gene determined by multiplex PCR, the most commonly found from both groups of piglets being *astA*, while *lt, F4, F18*, and *F41* only from diarrheal piglets. More than 80% of *E. coli* isolates were resistant to 7 of 12 antimicrobial agents. One hundred and fifty-seven *E. coli* isolates carried class 1 and/or 2 integron(s). Integron-positive isolates are significantly associated with strains resistant to kanamycin, oxytetracycline, streptomycin, sulfamethoxazole/trimethoprim and tetracycline. Phylogenetic analysis by multilocus sequence typing revealed that the 31 representative *E. coli* isolates were genetically diverse, especially those from diarrheal piglets suggesting that *E. coli* from postweaning piglets were not derived from a single clone. Sequence type (ST)10, ST641 and ST1114 were most commonly found in both groups of piglets. No correlation was observed among ST, presence of integron and antimicrobial resistance. The study suggests that swines in a farm could be a reservoir and possible spread of diarrheagenic *E. coli* including strains with antimicrobial resistance genes.

Keywords: *Escherichia coli*, diarrhea, multilocus sequence typing, postweaning piglet, virulence gene

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INTRODUCTION

Postweaning phase of piglets is a critical period for bacterial infection because many changes including physiological, environmental and social occur when weaned from a sow (Campbell *et al*,

2013). Diarrhea in the weaning period accounts for economic loss due to mortality, morbidity, decreased growth rate, and cost of medication. Enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), and Shiga toxin producing *E. coli* (STEC), and enteroaggregative *E. coli* (EAEC) are the main causes of diarrhea in weaning piglets (Nataro and Kaper, 1998). In addition, virulence factors of ETEC are species-specific fimbrial adhesions, *eg*, *F4* (K88), *F5* (K99), *F6* (987P), *F18* and *F41*, found only in animals and two enterotoxins, namely, heat-stable toxin *sth* (specific to humans) and *stp* (specific to porcine), and heat-labile toxin *lt* (specific to humans and swine) (Ray and Bhunia, 2008). Furthermore, STEC or EHEC producing Shiga toxin 2e (encoded by *stx2e*) is recognized as a cause of edema in piglets (Fratamico *et al*, 2008).

EAEC isolates harboring *astA* [encoding heat stable enterotoxin 1 (EAST1)] were detected in suckling and weaning piglets at the highest percentage (Toledo *et al*, 2012). Interestingly, *astA* was also found in other *E. coli* pathotypes, *eg*, EHEC, ETEC, enteropathogenic *E. coli* (EPEC), and diffuse-adhering *E. coli* (DAEC) (Yamamoto and Echeverria, 1996), but at a low percentage. However, the detection rate of other virulence factors of *E. coli* in postweaning piglets, with and without diarrhea, in a farm swine population has only a few published (Chapman *et al*, 2006; Lay *et al*, 2012).

Antimicrobials are regularly used as growth promoters in swine production as prophylactics, for metaphylactic treatment to prevent diseases and for therapeutic purposes (Barton, 2014). On the other hand, the negative effects of using antibiotics in agriculture might be partly responsible for the emergence of drug-resistant microorganisms (Marshall and Levy, 2011).

In Thailand, antimicrobial agents are used as feed supplement to control diarrhea in weaning piglets (Prapasarakul *et al*, 2010). Prevalence of antimicrobial resistant *E. coli* strains in weaning piglets is significantly higher than those in farrow-to-finish pigs (Akwar *et al*, 2008). However, information regarding antimicrobial resistant *E. coli* isolated from weaning piglets both with and without diarrhea are scarce in Thailand (Prapasarakul *et al*, 2010).

Dissemination of antibiotic resistance genes by horizontal gene transfer has led to the rapid emergence of antibiotic resistance among bacteria (Barlow *et al*, 2004). Integrons have been shown to play an important role in the evolution and dissemination of multidrug resistance in gram-negative bacteria (Naas *et al*, 2001). There are 3 main classes of integrons associated with antimicrobial resistance in the clinical samples (Naas *et al*, 2001). Class 1 integrons were detected in high percentage of *E. coli* isolates obtained from healthy and diarrheal swine, swine farmers and non-farmers in Thailand (Phongpaichit *et al*, 2007). However, the distribution of antimicrobial resistance determinants and integrons of *E. coli* in postweaning piglets with and without diarrhea have not yet been determined in the country.

Molecular typing techniques have been applied in epidemiological investigations and in determining the primary sources of infection, information important to improve public health (Foley *et al*, 2009). Multilocus sequence typing (MLST) is one tool for understanding diversity, population structure and dynamics of bacterial pathogens worldwide (Perez-Losada *et al*, 2013). The reproducibility of MLST is excellent and the results are easily shared electronically among laboratories

(Foley *et al*, 2009).

Hence, this study used multiplex PCR and MLST to investigate molecular epidemiology of *E. coli* and their predominant sequence types (STs) in a swine farm with particular emphasis on virulence genes and relationship between antimicrobial resistance and integrons from weaning piglets with and without diarrhea in a farm in Thailand.

MATERIALS AND METHODS

Sample collection

One hundred of rectal swabs were collected from postweaning piglets (5-7 weeks of age) with and without diarrhea in equal number (50 each) in a swine farm in central province of Thailand. Rectal swab samples were dispersed in Cary-Blair transport medium (Ningbo MFLab Medical Instruments, Ningbo, China), kept on ice and immediately transferred to the laboratory within 24 hours.

Animal ethical clearance was reviewed and approved by the Institutional Animal Care and Use Committee, Thammasat University (IACUC-TU), Protocol no. 008/2558.

Bacterial isolation and identification

Each rectal swab sample was inoculated onto MacConkey agar (Difco, Detroit, MI) and incubated at 37ºC for 24 hours. Colonies positive for lactose fermentation were picked and confirmed by the triple sugar/iron/indole, methyl red, Voges-Proskauer, and citrate (IMViC) tests (Adams and Moss, 2000). The identified *E. coli* isolates were kept as stocks in 20% glycerol and stored at -80°C until used.

Determination of virulence genes

E. coli isolates were cultured in Luria-Bertani broth (LB Broth; Difco, Detroit, MI) at 37ºC for 15-18 hours and DNA was extracted using Qiagen's QIAamp® DNA mini kit (Qiagen, Hilden, Germany). All *E. coli* isolates were evaluated for the presence of virulence genes *lt, sth, stp, stx1A, stx2A, aggRks* and *pCVD*432 using multiplex PCR (Taniguchi, unpublished data). The PCR mixture (25 µl) consisted of 1X Green Go*Taq* reaction buffer, 0.2 mM dNTPs, 0.5 µl of each primer (0.5 mM) (Table 1), 1 U Go*Taq* DNA polymerase (Promega, Fitchburg, WI), and 1 µl of DNA template. Thermocycling was performed in MyCycler™ instrument (BIO-RAD, Hercules, CA) as follows: 95ºC for 5 minutes; 35 cycles of 95ºC for 1 minute, 52ºC for 1 minute and 72ºC for 1 minute; and a final step at 72ºC for 5 minutes. The other virulence genes, *F4, F5, F6, F18, F41, astA* and *stx2e*, were carried out as previously described (Lee *et al*, 2008). All 14 primer sets for amplification of the virulence genes used in the study together with amplicon sizes are listed in Table 1. Amplicons were analyzed by 2% agarose gel-electrophoresis, stained with ethidium bromide and visualized under ultraviolet light using a gel documenting system (BIS 303 PC, Jerusalem, Israel).

Antimicrobial susceptibility testing

Antimicrobial susceptibility to 12 antimicrobial agents, namely, amoxicillin/clavulanic acid (AMC), ampicillin (AMP), ceftazidime (CAZ), cefotaxime (CTX), doxycycline (DO), enrofloxacin (ENR), gentamicin (GM), kanamycin (K), oxytetracycline (OTC), streptomycin (S), sulfamethoxazole/trimethoprim (SXT), tetracycline (TE) (Oxoid, Basingstoke, UK) was carried out using a disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI, 2013). *E. coli* ATCC 25922 was used as control.

Detection of class 1 and 2 integrons

The presence of class 1 and 2 integrons on all isolates was determined by

multiplex PCR (Su *et al*, 2006). PCR mixture (25 μl) contained 1X buffer solution (Promega), 2 mM $MgCl_2$, 0.2 mM dNTP s, 0.4 μM of each primer (Table 1), 1 U *Taq* polymerase (Promega), and 2 μl of DNA template. Thermocycling was performed in MyCycler™ instrument (BIORAD) as follows: 94ºC for 5 minutes; 30 cycles of 94ºC for 30 seconds, 52ºC for 30 seconds and 72ºC for 2 minutes; and a final step at 72ºC for 7 minutes. Amplicons were analyzed as described above.

MLST assay

MLST was performed as described previously (Wirth *et al*, 2006). Seven housekeeping genes of *E. coli* were amplified by PCR using 7 primer pairs targeting *adk*, *fumC*, *gyrB*, *icd*, *purA*, *mdh*, and *recA* (Table 1). Gel-purified amplicons were sequenced by 3130 Genetic Analyzer (Applied BioSystems, Foster City, CA). Sequences were analyzed using BioEdit version 7.1.9. Allele numbers and ST of each *E. coli* isolate were obtained by comparing with MLST *E. coli* database (http://mlst.warwick.ac.uk/mlst). New sequence types were deposited in PubMLST database (http://mlst.warwick.ac.uk/mlst).

Statistical analysis

Data were analyzed by using SPSS software (version 18.0, IBM, Armonk NY) and *p*-value was calculated using chi-square test, with *p*-value <0.05 considered statistically significant. For phylogenetic analysis, minimum-likelihood tree for in-frame concatenated sequence of each isolates (3,423 bp) was constructed by MEGA 5.2 software, with a bootstrap test of 1,000 replicates.

RESULTS

Bacterial isolation and presence of virulence genes in postweaning piglets

Of 246 *E. coli* isolates obtained from postweaning piglets (5-7 weeks of age) with and without diarrhea (50 samples each), 130 and 116 isolates were obtained from piglets with and without diarrhea, respectively. Virulence genes were detected in 96 (39%) isolates, comprising *astA* (92; 96%) (in both with and without diarrhea), *F18* (27; 28%), *lt* (13; 13%), *F4* (8; 8%) and *F41* (1; 1%) (latter four only in the diarrheal piglets) (Table 2). Piglets hosted *E. coli* with 5 different combinations of virulence genes: three types of double and two types of triple combinations (Table 2).

Antibiogram and relationship with presence of class 1 and 2 integrons

All *E. coli* isolates were resistant to at least one of twelve antimicrobial agents tested, with over 90% resistant to ampicillin, enrofloxacin, gentamicin, oxytetracycline, and tetracycline (Table 3). More than 80% of *E. coli* isolates from diarrheal piglets were resistant to 6/12 antimicrobials, and, surprisingly, >80% from nondiarrheal piglets were resistant to 7/12 antimicrobials.

Among 157 (64% of total isolates) integron-positive isolates, 153 (97%) carried class 1 integron, 3 (2%) class 2 integron, and 1 (<1%) both class 1 and 2 integrons (Table 3). Of these integron-positive isolates, 93 (59%) were detected in *E. coli* isolated from piglets with diarrhea and 64 (41%) from those without diarrhea. *E. coli* isolates resistant to kanamycin, streptomycin, oxytetracycline, sulfamethoxazole/trimethoprim and tetracycline are significantly more common in isolates carrying integrons ($p<0.05$).

Phylogenetic analysis of postweaning piglets with and without diarrhea

Sequence type (ST) and allelic profile of each tested isolate revealed 31 representative *E. coli* STs, 25 known STs (http://mlst.warwick.ac.uk/mlst) and 6 novel

Table 1
Primers used for PCR amplification of target genes.

Virulence gene	Sequence (5´- 3´)	Size (bp)	Reference
lt	ATGACGGATATGTTTCCACTTCTC AACCTTGTGGTGCATGATGAATCC	393	Taniguchi *et al* (unpublished data)
sth	TTCACCTTTCGCTCAGGATGCTA CACCCGGTACAAGCAGGATT	168	Taniguchi *et al* (unpublished data)
stp	TTAATAACATCCAGCACAGGCAGG TCCCCTCTTTTAGTCAGTCAACTG	176	Taniguchi *et al* (unpublished data)
stx1A	TCTGCAATAGGTACTCCATTACAG CCGGACACATAGAAGGAAAC	724	Taniguchi *et al* (unpublished data)
stx2A	TTGACCATCTTCGTCTGATTATTG CTGATGATGGCAATTCAGTATAAC	541	Taniguchi *et al* (unpublished data)
aggR	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	254	Taniguchi *et al* (unpublished data)
pCVD432	CTCTGGCGAAAGACTGTATC CATCTCTACATCAAGAGCAG	463	Taniguchi *et al* (unpublished data)
F4	GCCTGGATGACTGGTGATTT TCTGACCGTTTGCAATACCC	706	Lee *et al* (2008)
F5	TTGGGCAGGCTGCTATTAGT TAGCACCACCAGACCCATTT	222	Lee *et al* (2008)
F6	GCGTGCATCGAAATGAGTT GGTGGTTCCGATGTATGCTT	589	Lee *et al* (2008)
F18	CTTTCACATTGCGTGTGGAG ATTCGACGCCTTAACCTCCT	441	Lee *et al* (2008)
F41	GGAGCGGGTCATATTGGTAA CTGCAGAAACACCAGATCCA	941	Lee *et al* (2008)
stx2e	TGGTGTCAGAGTGGGGAGAA TACCTTTAGCACAATCCGCC	351	Lee *et al* (2008)
astA	CCATCAACACAGTATATCCGA GGTCGCGAGTGACGGCTTTGT	111	Chapman *et al* (2006)
int1	ACGAGCGCAAGGTTTCGGT GAAAGGTCTGGTCATACATG	565	Su *et al* (2006)
int2	GTGCAACGCATTTTGCAGG 5´CAACGGAGTCATGCAGATG	403	Su *et al* (2006)
int3	´CATTTGTGTTGTGGACGGC 5´GACAGATACGTGTTTGGCAA	717	Su *et al* (2006)
adk	ATTCTGCTTGGCGCTCCGGG CCGTCAACTTTCGCGTATTT	583	Wirth *et al* (2006)
fumC	TCACAGGTCGCCAGCGCTTC GTACGCAGCGAAAAAGATTC	806	Wirth *et al* (2006)
gyrB	TCGGCGACACGGATGACGGC ATCAGGCCTTCACGCGCATC	911	Wirth *et al* (2006)
icdF	ATGGAAAGTAAAGTAGTTGTTCCGGCACA GGACGCAGCAGGATCTGTT	878	Wirth *et al* (2006)
mdh	ATGAAAGTCGCAGTCCTCGGCGCTGCTGGCGG TTAACGAACTCCTGCCCCAGAGCGATATCTTTCTT	932	Wirth *et al* (2006)
purA	CGCGCTGATGAAAGAGATGA CATACGGTAAGCCACGCAGA	816	Wirth *et al* (2006)
recA	CGCATTCGCTTTACCCTGACC TCGTCGAAATCTACGGACCGGA	780	Wirth *et al* (2006)

Table 2
Virulence gene profiles of *Escherichia coli* isolated from weaning piglets with and without diarrhea.

Virulence gene profile	Weaning piglet with diarrhea Number (%)	Weaning piglet without diarrhea Number (%)	Total Number (%)
Single virulence gene			
astA	21 (16)	35 (30)	56 (23)
lt	3 (2)	.	3 (1)
F18	1 (1)	.	1 (<1)
Double virulence genes			
astA,F18	25 (19)	.	25 (10)
astA,F41	1 (1)	.	1 (<1)
astA,lt	1 (1)	.	1 (<1)
Triple virulence genes			
astA,lt,F4	8 (6)	.	8 (3)
astA,lt,F18	1 (1)	.	1 (<1)
None detected	69 (53)	81 (70)	150 (61)
Total	130	116	246

STs, namely, ST5218, ST5541, ST5706, ST5708, ST5709 (from diarrheal piglets) and ST5707 (from nondiarrheal piglets) (Table 4). The majority (12.9%) of isolates were assigned to ST10, with 14 STs from the piglets with diarrhea and 11 STs from nondiarrheal piglets. Additionally, ST10, ST641 and ST1114 were detected in *E. coli* isolates from both diarrheal and nondiarrheal piglets.

A phylogenetic tree was constructed using maximum likelihood (ML) based on concatenated sequences of seven housekeeping genes loci (3,423 bp) of *E. coli* isolates obtained from piglets demonstrated that ST3057 from piglets with diarrhea was located distant from other STs (Fig 1). Associations among STs and virulence profiles were observed for ST10, ST88, ST641, ST1114 and ST5218, with ST10 and ST641 from nonpathogenic *E. coli*) (negative for the virulence genes studied) and the remaining from pathogenic *E. coli* [positive for at least one virulence gene (ST88, lt^+; ST11114, $astA^+$; and ST5218, $F18^+$+$astA^+$)]. Of note, there were no obvious relationships among STs, antibiogram profiles and presence of integrons.

DISCUSSION

Pathogenic *E. coli* is a common agent responsible for diarrhea and edema in pigs (Martins *et al*, 2000; Vu-Khac *et al*, 2007). However, little is known concerning the genetic diversity of *E. coli* in postweaning piglets. To the best of the authors' knowledge, the present work is the first report of the prevalence of *E. coli* with different virulence genes, antimicrobial resistance genes and their transferring factors, and the phylogeny of the bacteria in postweaning piglets.

The highest proportion of virulence gene in postweaning piglets was *astA* (encoding EAST1 toxin). Although the role of EAST1 toxin in swine colibacillosis has not been demonstrated, this gene is commonly found in *E. coli* from

Table 3
Relationship between antibiogram profile and presence of integron in 246 *Escherichia coli* isolates from postweaning piglets.

Antimicrobial agent	Weaning piglet				Number			Association with integron*
	With diarrhea		Without diarrhea		Integron positive Number (%) (total = 157)	Integron negative Number (%) (total = 89)	Antimicrobial resistance Number (%) (total = 246)	
	Integron positive Number (%) (total = 93)	Integron negative Number (%) (total = 37)	Integron positive Number (%) (total = 48)	Integron negative Number (%) (total = 52)				
AMP	93 (100)	37 (100)	64 (100)	52 (100)	157 (100.0)	89 (100)	246 (100)	-
AMC	15 (16)	4 (11)	8 (12)	6 (11)	23 (14.6)	10 (11)	33 (13)	0.45
CTX	76 (82)	28 (76)	63 (98)	50 (96)	139 (88.5)	78 (88)	217 (88)	0.83
CAZ	41 (44)	16 (43)	22 (34)	28 (52)	63 (40.1)	44 (49)	107 (43)	0.16
KAN	45 (48)	8 (22)	28 (44)	12 (23)	73 (46.5)	20 (22)	93 (38)	0.00
STR	69 (74)	19 (51)	53 (83)	35 (67)	122 (77.7)	54 (61)	176 (71)	0.00
GEN	80 (86)	33 (89)	63 (98)	48 (92)	143 (91.1)	81 (91)	224 (91)	0.99
TET	92 (99)	36 (97)	63 (98)	46 (88)	155 (98.7)	82 (92)	237 (96)	0.01
DO	69 (74)	26 (70)	62 (97)	45 (86)	131 (83.4)	71 (80)	202 (82)	0.47
OTC	91 (98)	36 (97)	63 (98)	45 (86)	154 (98.1)	81 (91)	235 (95)	0.02
ENR	85 (91)	34 (92)	60 (94)	50 (96)	145 (92.4)	84 (94)	229 (93)	0.55
SXT	84 (90)	14 (38)	57 (89)	11 (21)	141 (89.8)	25 (28)	166 (67)	0.00

AMP, ampicillin; AMC, amoxicillin/clavulanic acid; CTX, cefotaxime; CAZ, ceftazidime; KAN, kanamycin; STR, streptomycin; GEN, gentamicin; TET, tetracycline; DO, doxycycline; OTC, oxytetracycline; ENR, enrofloxacin; SXT, sulfamethoxazole/trimethoprim. *p-value (significance at <0.05).

Table 4
Sequence types (STs) and allele profiles of thirty-one representative *Escherichia coli* isolates from weaning piglets with and without diarrhea.

No.	Isolate code	Weaning piglet	ST	Multilocus ST profile						
				adk	*fumC*	*gyrB*	*icd*	*mdh*	*purA*	*recA*
1	EC-R1	With diarrhea	5218	6	19	3	16	9	8	341
2	EC-R5	With diarrhea	3057	290	54	55	324	35	40	223
3	EC-R8	With diarrhea	1114	10	27	5	10	12	1	2
4	EC-R9	With diarrhea	5706	6	4	12	1	411	12	7
5	EC-R13	With diarrhea	165	10	27	5	10	12	8	2
6	EC-R18	With diarrhea	5541	6	19	3	16	9	9	341
7	EC-R29	With diarrhea	100	10	27	5	10	12	9	2
8	EC-R43	With diarrhea	5708	10	4	5	8	8	8	2
9	EC-R44	With diarrhea	1421	8	7	1	8	8	8	2
10	EC-R58	With diarrhea	100	10	27	5	10	12	9	2
11	EC-R65	With diarrhea	88	6	4	12	1	20	12	7
12	EC-R68	With diarrhea	5218	6	19	3	16	9	8	341
13	EC-R78	With diarrhea	542	112	11	5	12	8	8	86
14	EC-R83	With diarrhea	10	10	11	4	8	8	8	2
15	EC-R98	With diarrhea	88	6	4	12	1	20	12	7
16	EC-R104	With diarrhea	641	9	6	33	131	24	8	7
17	EC-R113	With diarrhea	5709	10	11	5	8	12	1	2
18	EC-R183	Without diarrhea	1112	10	11	5	10	8	1	2
19	EC-R184	Without diarrhea	10	10	11	4	8	8	8	2
20	EC-R187	Without diarrhea	101	43	41	15	18	11	7	6
21	EC-R203	Without diarrhea	10	10	11	4	8	8	8	2
22	EC-R210	Without diarrhea	641	9	6	33	131	24	8	7
23	EC-R215	Without diarrhea	10	10	11	4	8	8	8	2
24	EC-R216	Without diarrhea	34	10	11	4	1	8	8	2
25	EC-R219	Without diarrhea	5176	427	636	188	1	8	18	6
26	EC-R226	Without diarrhea	29	6	4	12	16	9	7	7
27	EC-R239	Without diarrhea	5229	43	41	15	18	11	7	44
28	EC-R241	Without diarrhea	1114	10	27	5	10	12	1	2
29	EC-R253	Without diarrhea	1114	10	27	5	10	12	1	2
30	EC-R275	Without diarrhea	206	6	7	5	1	8	18	2
31	EC-R283	Without diarrhea	5707	10	736	5	10	12	1	2

diarrheal weaning pigs (Choi *et al*, 2001; Osek, 2003). In our study, the prevalence of postweaning piglets with diarrhea is significantly higher than those without diarrhea, in agreement with a previous study reporting 33.3% of isolates found in weaned pigs with diarrhea and/or edema (Choi *et al*, 2001). The presence of *astA* is not restricted to EAEC but also is carried by other pathotypes, *eg*, EHEC, ETEC and EPEC, from both humans and animals (Yamamoto and Echeverria, 1996). Also, it was shown that *astA* is possibly incorporated together with *lt*, *F18*, *F41* and/or *lt*/

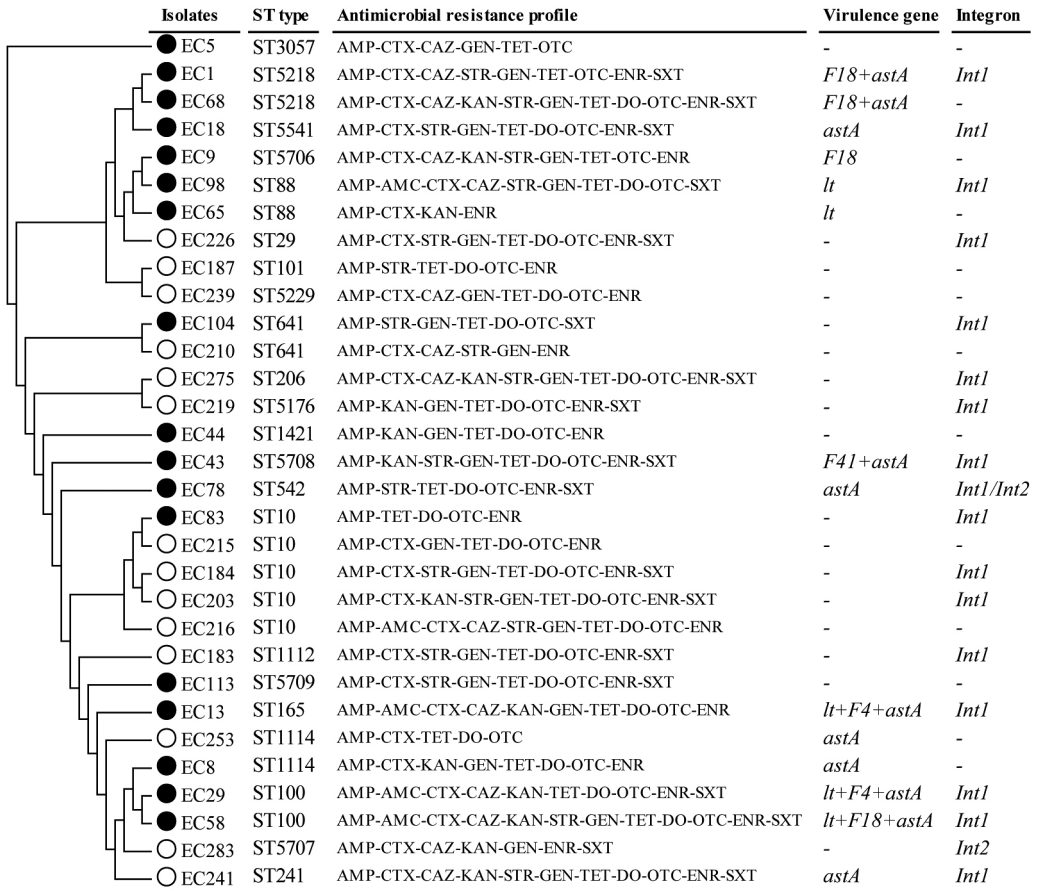

Fig 1–Maximum likelihood phylogenetic tree of 31 representative *Escherichia coli* isolates from weaning piglets with and without diarrhea. The tree was constructed using MEGA 7.0 program based on concatenated sequences of seven housekeeping enzyme genes loci (3,423 bp), 1,000 bootstraps. Solid circle, *E. coli* isolate from weaning piglet with diarrhea; open circle, *E. coli* isolate from weaning piglet without diarrhea.

F18 of ETEC (Osek, 2003). Thus, our study confirms that ETEC is the most common pathotype among porcine isolates. The high prevalence of *astA* may also suggest that this gene carried by transposon can be transferred to other *E. coli* pathotypes (Yamamoto and Echeverria, 1996). However, further studies are required to define the role of *astA* in the pathogenesis of diarrhea in pigs.

In order to determine the virulence determinants of *E. coli*, selected numbers of fimbriae genes were analyzed, showing *F18* was present with the highest frequency, followed by *F4* and *F41* in *E. coli* isolated from diarrheal postweaning piglets, while all tested fimbriae genes (*F4*, *F5*, *F6*, *F18*, and *F41*) were not detected in nondiarrheal piglets, similar to a previous report from Denmark showing *F4* and *F18* are significant important virulence factors in weaning piglets (Frydendahl, 2002). The presence of *F5*, *F6*, *F18*, and *F41* fimbriae were employed in differentiating

between clinical and commensal isolates in swines (Chapman *et al*, 2006).

The growing problem of antibiotic resistance has become a significant public health concern (Shiekh *et al*, 2012). Antimicrobial agents have frequently been used in swine farms for growth promotion, prevention of disease and therapeutic purposes (Barton, 2014). In this study, >80% of *E. coli* isolates from postweaning piglets both with and without diarrhea were resistant to half of 12 antimicrobials tested. The detection rates of resistant strains of *E. coli* were not different between diarrheal and nondiarrheal piglets might be due to the use of antibiotics in animal feed targeting other types of pathogenic bacteria. However, these compounds also affect commensal organisms that make up the major part of the gastrointestinal flora and thereby resulting in generating resistance to these drugs. These commensal bacteria may, therefore, function as a reservoir of resistance genes that can be transferred to pathogenic bacteria (Salyers *et al*, 2004).

Generally, integrons are able to disseminate antibiotic resistance genes by horizontal or vertical transfer and have been shown to play a key role in the evolution and dissemination of multidrug resistance in gram-negative bacteria (Deng *et al*, 2015). Class 1 integrons of *E. coli* are commonly found in clinical and commensal isolates from livestock, and companion and rare animals (Goldstein *et al*, 2001). We found over 50% integron-positive isolates, and class 1 integron was the predominant class, Xu *et al* (2015) reported that *E. coli* isolates from postweaning piglets with diarrhea in China are positive for class 1 integron while class 2 and 3 integrons are not detected. Phongpaichit *et al* (2007) noted that *E. coli* isolates positive for class 1 integron are detected in healthy (36.1%), diarrheal pigs (16%), pig farmers (3.1%), and non-farmers (8.3%) in southern Thailand. The high detection rate of integrons in postweaning piglets both with and without diarrhea in this study might be due to the farm size and amount or frequency of antimicrobials used in the farms (Phongpaichit *et al*, 2007).

Association between the presence of integrons and multidrug resistance in clinical Enterobacteriaceae isolated from humans has been described in previous studies (White *et al*, 2001; Leverstein-van Hall *et al*, 2003). Our study demonstrates that resistances to kanamicin, oxytetracycline, streptomycin, tetracycline, and trimethoprim/sulfamethoxazole are significantly more common in isolates carrying class 1 integron. Changkaew *et al* (2015) reported that integron-positive *E. coli* isolates in healthy pigs carried gene cassettes resistant to aminoglycosides, lincosamide and trimethoprim. Likewise, Phongpaichit *et al* (2007) earlier reported similar percent association between antimicrobial resistance and integron-positive as well as integron-negative isolates from swines and pig farmers. Although integrons play a major role in the spread of antimicrobial resistance gene(s), resistance is not only spread disseminated via integrons but also through other exchange mechanisms, such as plasmid, transposon and bacteriophage (Kang *et al*, 2005). Thus, gene cassettes encoding resistance to class 1 integron in the isolates of the present study should be further investigated.

MLST has been widely used to study molecular epidemiology and to analyze evolutionary relationships among *E. coli* isolated from different sources (Croxall *et al*, 2011; Abraham *et al*, 2014; Herrero-Fresno *et al*, 2015; Cadona *et al*, 2016). We found ST10 and ST1114 were the most

common STs in isolates from postweaning piglets, and ST10 and ST641 were found in non-pathogenic *E. coli* in postweaning piglets. ST10 is commonly associated with emerging pathogenic strains (Manges and Johnson, 2012). ST10 has been detected in other *E. coli* pathotypes, such as EAEC, ETEC, and ExPEC, and also in commensal *E. coli* (Olesen *et al*, 2012). In Denmark ST10 was found in commensal *E. coli* from nursery pigs (Herrero-Fresno *et al*, 2015). ST10, ST641 and ST1114 were detected from postweaning piglets both with and without diarrhea. Such results have been interpreted as providing evidence of a genetic link amomg the organs causing frequent and continuous inter-compartment transmission between diarrheal and nondiarrheal piglets in farms (Hu *et al*, 2013). However, 6/25 STs in this study were novel. The majority of these isolates have their origins from diarrheal piglets, indicating high genetic diversity among *E. coli* isolated from diarrheal piglets. Thus, the diarrheal piglet intestinal tract also serves as a potential reservoir of unique *E. coli* isolates that are not commonly found among nondiarrheal piglet isolates.

The absence of relationships among ST, antibiogram profile and integrin presence agrees with the results in Denmark of Herrero-Fresno *et al* (2015), who reported no correlation among REP profile, ST, antimicrobial resistance profile, and virulence patterns of *E. coli* from piglets in nursery farms. The finding that STs were not correlated with antimicrobial resistance patterns and presence of integron might be due to horizontal gene transfer in the bacteria community.

In summary, this is the first study to analyze the genetic diversity of *E. coli* from postweaning piglets in a swine farm. *E. coli* isolates were genetically diverse especially those from the diarrheal piglets suggesting that *E. coli* in the postweaning piglets were not derived from a single clone. ST10, ST641, and ST1114 were the more common STs in strains from both diarrheal and nondiarrheal piglets. There were no correlation among ST, presence of integron and antibiogram profile of these bacteria. Our study suggests that farm swines (food-producing animals) could serve as a reservoir of *E. coli* haborimg antimicrobial resistance genes resulting in antimicrobial-resistant pathogenic bacteria posing risk to consumer health.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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