MOLECULAR CHARACTERIZATION OF EXTRAINTESTINAL PATHOGENIC *ESCHERICHIA COLI* FROM HUMANS IN SOUTHERN THAILAND

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Abstract. Extraintestinal pathogenic *Escherichia coli* (ExPEC) is an important pathogen causing diverse damages to extraintestinal organs. Thirty-one percent of *E. coli* isolates from healthy volunteer fecal samples (n = 100) were identified as ExPEC, with 94% carrying *kpsMT*II, 87% *iutA* and 10% *afa*. Fifty-two percent of ExPEC strains belonged to phylogenetic group B2, 29% to group D, 16% to group A, and 3% to group B1. ExPEC-associated genes carried by ExPEC strains were *cnf1* (encoding cytotoxic necrotizing factor 1) (6%), *fimH* (encoding type 1 fimbrial tip) (94%), *kpsMT*K1 (encoding K1 capsular antigen, responsible for neonatal meningitis) (23%), and *traT* (encoding conjugal transfer surface exclusion protein) (58%). All 31 ExPEC strains belonged to 4 serogroups, namely, O1 (7 strains), O6 (2 strains), O25 (5 strains), and O164 (3 strains), with the remaining being untypeable O serogroup. Antibiogram profile showed 42% of ExPEC strains were multi-drug resistant. ESBL gene detection showed that 29% of ExPEC carried bla_{TEM} and 3% carrying both $bla_{\text{CTX-M}}$ and bla_{TEM}. BOX-PCR profiles demonstrated ExPEC O6 and O164 strains within each serogroup were identical in their fingerprint, while ExPEC O1 and O25 strains displayed two profiles each, with 78% and 98% genetic similarity, respectively. These findings provide information on existence and virulence of commensal ExPEC strains in healthy human intestinal microbiota in southern Thailand, which should be of benefit to an underearning of potential public health problems in this region of the country.

Keywords: extraintestinal pathogenic *Escherichia coli*, human, phylogenetic group, seroprevalence, Thailand

INTRODUCTION

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is a group of *E. coli*, which plays a role in diseases outside human intestinal tract, *viz*. sepsis, neonatal meningitis and pneumonia (Russo and Johnson, 2003). ExPEC causing infections in humans can be divided into three

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groups, namely, neonatal meningitis *E. coli* (NMEC), sepsis *E. coli* (SEPEC) and uropathogenic *E. coli* (UPEC) (Mellata, 2013).

Urinary tract infection (UTI) is very common in women, among whom approximately 10-20% experience UTI once in their lives (Johnson and Stamm, 1989). Bacterial source of UTI in healthy people is from contamination of feces to urethra (Jung *et al*, 2012). Upon infection, different extents of severity are presented based on organ infected, such as bacteriuria, cystitis, and pyelonephritis, all of which can result in complicated UTI (Foxman, 2003).

Neonatal meningitis is one of the important illnesses for newborns, particularly in developing countries with approximately 10 times higher number of cases than in developed countries (Smith *et al*, 2007). Contamination of bacteria from mother's gastrointestinal tract to infant may occur during passage through the birth canal resulting in early-onset infection while environmental contacts result in late-onset infection (Raymond *et al*, 2008). Newborns surviving infection have sequelae such as hydrocephalus and mental retardation (Klinger *et al*, 2000). Thus, infections caused by ExPEC are considered with great concern both in undeveloped and developing countries.

Presence in healthy people of ExPEC carrying pivotal virulence genes is believed to be prognostic of UTI infections in women and neonatal meningitis in newborns (Mellata, 2013). Hence, this study investigated the presence of ExPEC in human intestines to characterize its degree of virulence, serotype, antibiogram profile, and genetic relatedness among strains. The data should be of benefit in developing public health programs for prevention and control of UTI in women and neonatal meningitis in newborns in Thailand.

MATERIALS AND METHODS

Bacteria collection

Rectal swab samples were collected from healthy female ($n = 79$) and male ($n = 21$) participants ranging from 20 to 22 years of age in Hat Yai city, 2017. Samples were cultured on eosin methylene blue (EMB) agar (Becton Dickinson, Sparks, MD) and incubated at 37ºC for 18 hours. An individual green metallic sheen colony from each sample was randomly isolated as a surrogate *E. coli*. Bacterial isolates were stored at -80ºC until used.

The research protocol was approved by the ethics committee, Faculty of Medicine, Prince of Songkla University (EC code no. 58-234-19-2). Prior written consent was obtained from every participant.

ExPEC identification

ExPEC identification was carried out using PCR targeting six genes within 5 groups (*afa*, *iutA*, *kpsMT*II, *papA* and/or *papC* and *sfaDE*, encoding Afa adhesion, aerobactin, capsular antigen, fimbriae subunit/outer membrane usher protein and S fimbriae, respectively. An *E. coli* isolate carrying ≥ 2 groups of gene is defined as ExPEC (Johnson *et al*, 2003). Bacterial DNA was prepared as previously described (Sukkua *et al*, 2017). In brief, an individual colony was cultured in tryptic soy broth (TSB) (Becton Dickinson) at 37ºC for 6 hours with shaking. One ml aliquot of bacterial culture was centrifuged 11,000*g* for 1 minute and bacteria pellet was washed with phosphate buffer saline pH 7.4 (PBS), re-suspended in 1 ml of PBS, placed in boiling water for 10 minutes, immediately placed on ice for 5 minutes and centrifuged (11,000*g* for 10 minutes). A 10-fold dilution of supernatant in sterile deionized water was used as PCR template. Reaction mixture (25 μl) comprised 1X Go*Taq* DNA polymerase buffer (Promega, Madison, WI), 0.4 μM of each specific primer pair (Table 1), 0.1 mM dNTPs, 3.0 mM $MgCl_2$, 0.5 U Go*Taq* DNA polymerase (Promega) and 2 μl of DNA solution. Amplification reaction was performed in a thermalcycler (T100^{TM}; Bio-Rad, Hercules, CA) as follows: 95ºC for 3 minutes; 35 cycles of 94ºC for 1 minute, annealing at appropriate temperature for each gene (Table 1) for 1 minute and 72ºC for 1 minute; and a final step of 72ºC for 5 minutes. Amplicons were separated by 1.0% agarose gel-electrophoresis, stained with ethidium bromide, and images stored in a WSE5200 Printgraph 2M gel imaging system (ATTO, Tokyo, Japan). *E. coli* identification was confirmed by presence of *uidA* (Table 1).

ExPEC-associated genes investigation

Investigations of ExPEC-associated genes (*cnf1*, *fimH*, *hlyA*, *kpsMT*K1, and *traT*) and *E. coli*-associated genes (*agn43* and *lpf*) were performed using PCR as previously described (Sukkua *et al*, 2017). A 25-μl reaction mixture containing the same components as described above but including an appropriate primer pair (Table 1) was subjected to PCR amplification and amplicon analyzed using 1.0% agarose gel-electrophoresis as described above.

Serotype determination

O-antigen serotyping was carried out using an agglutination assay employing *E. coli* specific antisera (set1) (Denka Seiken, Tokyo, Japan) (Sukkua *et al*, 2017). In short, a single colony of each bacterial isolate was grown in three ml of TSB at 37ºC for 6 hours with shaking. Bacterial cells were harvested, suspended in normal saline solution (NSS) and heated at 121ºC for 15 minutes. Following centrifugation at 900*g* for 20 minutes, bacteria pellet was suspended in 500 μl of NSS, mixed with antisera on a glass slide and agglutination observed by eyes.

Phylogenetic group assay

Phylogenetic group assay was performed by PCR targeting three genes, namely, *chuA*, TspE4.C2 fragment and *yjaA* (Clermont *et al*, 2000). In brief, a 25-μl reaction mixture comprising the same PCR components as described above except for the relevant primer pair (Table 1) was subjected to amplification as follows: 95ºC for 3 minutes; 35 cycles of 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 using 1.0% agarose gel-electrophoresis as described above.

Antibiogram assay

Antibiogram profile of ExPEC was conducted by a disk diffusion method according to CLSI (2014) guidelines using 11 common antimicrobial agents: amikacin (30 μg), ceftriaxone (30 μg), cephalothin (30 μg), ciprofloxacin (5 μg), chloramphenical (30 μg), cotrimoxazole (25 μg), fosfomycin (200 μg), gentamicin (10 μg), imipenem (10 μg), streptomycin (10 μg), and tetracycline (30 μg) (Oxoid, Hampshire, UK).

ESBL gene investigation

Presence of ESBL genes, *bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$ and *bla*$_{\text{TEM}}$, were determined by PCR (Monstein *et al*, 2007) as described above, except using the pertinent primer pairs (Table 1) and amplification conducted for 35 cycles at 94ºC for 1 minute, 55ºC (for *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{TEM}}$) or 50ºC (for *bla*$_{\text{SHV}}$) for 1 minute and 72ºC for 1 minute.

BOX-PCR profiling of ExPEC strains

BOX-PCR was performed in a 25-μl reaction mixtures comprising 0.2 μM BOXA1R primer (Table 1) (Versalovic *et al*, 1994), 0.2 mM dNTPs, 1X Go*Taq* DNA

Table 1
Primers used in the study.

Gene	Virulence factor	Primer name	Sequence (5′→3′)	Annealing temperature (°C)	Amplicon size (bp)	Reference
papA	P fimbriae subunit	papA f papA r	ATGGCAGTGGTGTCTTTTGGTG CGTCCCACCATACGTGCTCTTC	60	720	Johnson and Stell (2000)
papC	Outer membrane usher protein	papC f papC r	GTGGCAGTATGAGTAATGACCGTTA ATATCCTTTCTGCAGGGATGCAATA	60	200	Johnson and Stell (2000)
sfaDE	S fimbriae	sfaDE-F sfaDE-R	CTCCGGAGAACTGGGTGCATCTTAC CGGAGGAGTAATTACAAACCTGGCA	58	408	Le Bouguénec *et al* (1992)
afa	Afa adhesin	afa1 afa2	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	60	750	Le Bouguénec *et al* (1992)
kpsMTII	Capsular antigen	kpsII f kpsII r	GCGCATTTGCTGATACTGTTG CATCCAGACGATAAGCATGAGCA	52	272	Johnson and Stell (2000)
iutA	Aerobactin	AerJ f AerJ r	GGCTGGACATCATGGGAACTGG CGTCGGGAACGGGTAGAATCG	58	300	Johnson and Brown (1998)
fimH	Type 1 fimbrial tip	fimH-F fimH-R	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	55	508	Johnson and Stell (2000)
traT	Conjugal transfer surface exclusion protein	traT f traT r	GGTGTGGTGCGATGAGCACAG CACGGTTCAGCCATCCCTGAG	58	290	Johnson and Stell (2000)
hlyA	α-hemolysin	hly1 hly2	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	58	1,177	Yamamoto *et al* (1995)
cnf1	Cytotoxic necrotizing factor-1	cnf1-F cnf1-R	GGCGACAAATGCAGTATTGCTTGG GACGTTGGTTGCGGTAATTTTGGG	58	552	Yamamoto *et al* (1995)
kpsMTK1	K1 capsular antigen	K1-f kpsII r	TAGCAAACGTTCTATTGGTGC CATCCAGACGATAAGCATGAGCA	50	153	Johnson and Stell (2000)
lpf	Long polar fimbriae	*lpfA1*-F *lpfA1*-R	GGTCGTTTTTGCCTTAACCGC AGGTTGAAATCGACCTGCGC	55	≈500	Torres *et al* (2004)

Table 1 (Continued)

Gene	Virulence factor	Primer name	Sequence (5′→3′)	Annealing temperature (°C)	Amplicon size (bp)	Reference
agn43	Antigen 43	1-Kpn 2-Bam	GAACCTGTCGGTACCGATGCCCTCCC CGGGATCCGTTGCCACTGTACCGGGCTTGACGACC	67	≈900	Danese *et al* (2000)
chuA	Heme transport	chuA1 chuA2	GACGAACCAACGGTCAGGAT TGCCGCCAGTACCAAAGACA	54	279	Clermont *et al* (2000)
yjaA	Unknown	yjaA1 yjaA2	TGAAGTGTCAGGAGACGCTG ATGGAGAATGCGTTCCTCAAC	54	211	Clermont *et al* (2000)
TSPE4.C2	Unknown	TspE4.C2-1 TspE4.C2-2	GAGTAATGTCGGGGCATTCA CGCGCCAACAAAGTATTACG	54	152	Clermont *et al* (2000)
bla$_{\text{SHV}}$	β -lactamase	bla-SHV.SE bla-SHV.AS	ATGCGTTATATTCGCCTGTG TGCTTTGTTATTCGGGCCAA	50	747	Paterson *et al* (2003)
bla$_{\text{CTX-M}}$	β -lactamase	CTX-M-U1 CTX-M-U2	ATGTGCAGYACCAGTAARGTKATGGC TGGGTRAARTARGTSACCAGAAYCAGCGG	55	593	Boyd *et al* (2004)
bla$_{\text{TEM}}$	β -lactamase	TEM-164.SE TEM-164.AS	TCGCCGCATACACTATTCTCAGAATGA ACGCTCACCGGCTCCAGATTTAT	55	445	Monstein *et al* (2007)
uidA	β-glucoronidase	uidA-F uidA-R	ATCACCGTGGTGACGCATGTCGC CACCACGATGCCATGTTCATCTGC	59	486	Heninger *et al* (1999)
boxA	-	BOXA1R	CTACGGCAAGGCGACGCTGACG	50	variable	Versalovic *et al* (1994)

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

polymerase buffer (Promega), 3.0 mM $MgCl_2$, 1.25 U Go*Taq* DNA polymerase (Promega) and 10 ng of DNA template [prepared by glass fiber matrix spin column (Geneaid, Taipei, Taiwan)]. Thermocycling (T100^{TM} instrument; Bio-Rad) was conducted as follows: 95ºC for 3 minutes; 35 cycles of 94ºC for 3 seconds, 92ºC for 30 seconds, 50ºC for 1 minute, and 65ºC for 8 minutes. Amplicons were resolved by 1.5% agarose gel-electrophoresis at 90 V for 1.5 hours, stained and image recorded as described above. Dendrogram was constructed using unweighted-pair group method of arithmetic average (UPGMA) (BioProfile Software; Vilber Lourmat, Torey, France).

RESULTS

ExPEC identification and phylogenetic group analysis

Of 100 *E. coli* isolates, 31 (31%) were shown to be ExPEC (Table 2), with 29 (94%) carrying *kpsMT*II, followed by *iutA* (27, 87%) while *afa* was found in only 3 (10%) ExPEC isolates. Phylogenetic group assay showed 16 (52%) ExPEC strains belonged to group B2, 9 (29%) to group D, 5 (16%) to group A, and 1 (3%) to group B1 (Table 2).

ExPEC-associated genes

In addition to the indicator genes used for ExPEC identification, these strains may also carry other virulence factors. The most frequent associated gene (29/31, 94%) was *fimH* (encoding type 1 fimbrial tip), followed by *traT* (encoding conjugal transfer surface exclusion protein) (18, 58%), while cytotoxic necrotizing factor 1 gene, *cnf1*, was present in 2 (6%) strains (Table 2). Surprisingly, *kpsMT*K1 (encoding K1 capsular antigen, responsible for neonatal meningitis) was detected in 7 (23%) strains as was Antigen 43 gene (responsible for autoagglutination and biofilm formation) while *lpf* (encoding long polar fimbriae) was not detected.

Seroprevalence of ExPEC

ExPEC strains isolated belonged to four serogroups: seven strains to O1 serogroup (KSP1, KSP2, KSP3, KSP4, KSP6, KSP14, and KSP16), two to O6 (KSP20 and KSP24), five to O25 (KSP5, KSP7, KSP8, KSP13, and KSP21), three to O164 (KSP10, KSP19 and KSP28), and the remaining (19) to untypeable group (OUT) (Table 2).

Antibiogram profile and ESBL genes

Thirteen (42%) ExPEC strains were multidrug-resistant (resistant to ≥ 3 classes of antimicrobial agents) with ExPEC O25 strain KSP7 resistant to seven antibiotics, four (O1 strain KSP14, O25 strains KSP5 and KSP8, and OUT strain KSP26) were resistant to six agents and two (O1 strain KSP4 and O25 strain KSP13) to five (Table 2). Highest number, 18 (58%), of ExPEC strains were resistant to cephalothin, followed by 17 (55%) to streptomycin, 16 (52%) tetracycline, and 14 (45%) to cotrimoxazole. One (3%) strain (O1 KPS3), was resistant to imipenem indicating the propensity to be carbapenem resistance. Among ESBL genes examined, nine strains (O1 KSP2, KSP6 and KSP14, O25 KSP8 and KSP13, O25 KSP21, O164 KSP28, and OUT KSP26 and KSP27) possessed bla_{TEM} while one strain (O1 KSP4) carried both $bla_{CTX\text{-}M}$ and bla_{TEM} (Table 2).

BOX-PCR profile

Genetic relationship among serotypes O1, O6, O25 and O164 were investigated for their genetic relationship by BOX-PCR. ExPEC serogroup O6 KSP20 and KSP24, and O164 KSP10, KSP19 and KSP28 demonstrated identical BOX-PCR profiles within each serogroup and serogroups O1 and O25 exhibited two profiles (Fig 1A). ExPEC O25 strains displayed 98% genetic

Table 2
Characteristics of 31 ExPEC strains isolated from humans.

Strain	Sex	Sero-type	Phylo-genetic group	ExPEC indicator gene						ExPEC-associated gene					Other *E. coli*-associated gene		Antimicrobial resistance[a]
				Gr.1		Gr.2	Gr.3	Gr.4	Gr.5								
				papA	*papC*	*sfaDE*	*afa*	*kpsMT*II	*iutA*	*fimH*	*traT*	*hlyA*	*cnf1*	*kpsMT*K1	*lpf*	*agn43*	
KSP1	F	O1	D	+	+	-	-	+	+	+	+	-	-	+	-	-	KF,S
KSP2[δ]	F	O1	B2	+	+	-	-	+	+	+	+	-	-	+	-	-	KF,S
KSP3	F	O1	D	+	+	-	-	+	+	+	+	-	-	+	-	-	C,CIP,IPM,KF
KSP4[Ω]	M	O1	D	-	-	-	+	-	+	+	+	-	-	-	-	+	CN,KF,S,SXT,TE
KSP5	F	O25	B2	-	-	-	-	+	+	+	+	-	-	-	-	-	C,CIP,KF,S,SXT,TE
KSP6[δ]	F	O1	D	+	+	-	-	+	+	+	+	-	-	-	-	-	FOS,KF
KSP7	M	O25	B1	-	-	-	-	+	+	+	+	-	-	-	-	-	CIP,CRO,FOS,KF,S,SXT,TE
KSP8[δ]	F	O25	D	-	-	-	-	+	+	+	+	-	-	-	-	-	CIP,FOS,KF,S,SXT,TE
KSP9	M	UN[b]	B2	-	-	-	-	+	+	+	-	-	-	-	-	-	TE
KSP10	F	O164	A	-	-	-	-	+	+	+	-	-	-	-	-	+	CRO
KSP11	F	UN	B2	-	-	+	-	+	-	+	+	+	+	+	-	-	KF,S
KSP12	F	UN	B2	-	-	-	-	+	+	+	-	-	-	-	-	-	TE
KSP13[δ]	F	O25	B2	-	-	-	-	+	+	+	+	-	-	-	-	-	CIP,KF,S,SXT,TE
KSP14[δ]	F	O1	D	-	-	-	+	+	+	+	+	-	-	-	-	+	C,CN,KF,S,SXT,TE
KSP15	F	UN	B2	-	-	-	-	+	+	+	-	-	-	-	-	-	C,CIP,KF
KSP16	M	O1	D	+	+	-	-	+	+	-	+	-	-	+	-	-	FOS
KSP17	F	UN	B2	-	-	+	-	+	-	+	+	+	+	+	-	-	KF,S
KSP18	F	UN	D	-	+	-	-	+	+	+	-	-	-	-	-	-	KF,S,SXT,TE
KSP19	F	O164	A	-	-	-	-	+	+	+	-	-	-	-	-	+	SA[c]
KSP20	F	O6	B2	+	+	+	-	+	-	+	+	-	-	-	-	-	KF,S,SXT,TE
KSP21[δ]	F	O25	B2	-	-	-	+	+	+	-	+	-	-	-	-	-	KF,SXT
KSP22	M	UN	B2	-	-	-	-	+	+	+	-	-	-	-	-	-	TE
KSP23	F	UN	B2	-	-	-	-	+	+	+	-	-	-	-	-	-	SA
KSP24	F	O6	B2	+	+	+	-	+	-	+	+	-	-	-	-	-	S,SXT,TE

Table 2 (Continued)

Strain	Sex	Sero-type	Phylo-genetic group	ExPEC indicator gene						ExPEC-associated gene					Other *E. coli*-associated gene		Antimicrobial resistance^{a}
				Gr.1		Gr.2	Gr.3	Gr.4	Gr.5								
				papA	*papC*	*sfaDE*	*afa*	*kpsMT*II	*iutA*	*fimH*	*traT*	*hlyA*	*cnf1*	*kpsMT*K1	*lpf*	*agn43*	
KSP25	F	UN	A	-	-	-	-	+	+	+	-	-	-	-	-	+	SXT,TE
KSP26$^{\delta}$	M	UN	B2	-	-	-	-	+	+	+	-	-	-	+	-	-	C,CIP,KF,S,SXT,TE
KSP27$^{\delta}$	M	UN	D	+	+	-	-	-	+	+	+	-	-	-	-	-	KF,S
KSP28$^{\delta}$	F	O164	A	-	-	-	-	+	+	+	+	-	-	-	-	+	S,SXT,TE
KSP29	M	UN	B2	-	-	-	-	+	+	+	-	-	-	-	-	-	S
KSP30	F	UN	B2	-	-	-	-	+	+	+	-	-	-	-	-	-	SA
KSP31	F	UN	A	-	-	-	-	+	+	+	-	-	-	-	-	+	SXT,TE

[a] C, chloramphenical; CIP, ciprofloxacin; CN, gentamicin; CRO, ceftriaxone; FOS, fosfomycin; KF, cephalothin; IPM, imipenem; S, streptomycin; SXT, cotrimoxazole; TE, tetracycline; SA, susceptible to all agents. [b] UN, untypeable. $^{\delta}$ carry *bla*$_{\text{TEM}}$. $^{\Omega}$ carry *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{TEM}}$.

similarity to one another and ExPEC O1 strains demonstrated 78% genetic similarity (Fig 1B).

DISCUSSION

Commensal *E. coli* is a natural inhabitant of human gut and is thought to be beneficial to its hosts (Duriez *et al*, 2001). However, some *E. coli* strains carry virulent traits, which are able to cause morbidity to the human host. Commensal *E. coli* can acquire virulent traits through a number of mechanisms, *viz*. acquisition of chromosomal or extra-chromosomal virulence operons (Ochman *et al*, 2000), random functional point mutation (Sokurenko *et al*, 1998), or genomic indels enhancing pathogenicity (Maurelli *et al*, 1998). ExPEC is outstanding among pathogenic *E. coli* strains in that, while residing in human gut, it is non-pathogenic, it causes diseases when dislodged from its natural habitat (Russo and Johnson, 2000).

Detection of ExPEC in healthy human fecal samples, can, at least in part, help forecast clinical symptoms, especially in women. Healthy women with ExPEC carrying adhesin (*afa*, *pap* and *sfa*) together with toxin (*cnf1* and *hlyA*,) genes are considered to have a higher risk of contracting UTI (Themphachana *et al*, 2015). In elderly females ExPEC may cause the highest severity due to host immunity impairment leading to high mortality rate (Nicolle *et al*, 2005).

Newborns delivered from mothers with ExPEC carrying K1 gene are at a greater risk of neonatal meningitis and sepsis (Mellata, 2013). Newborns can acquire K1-containing

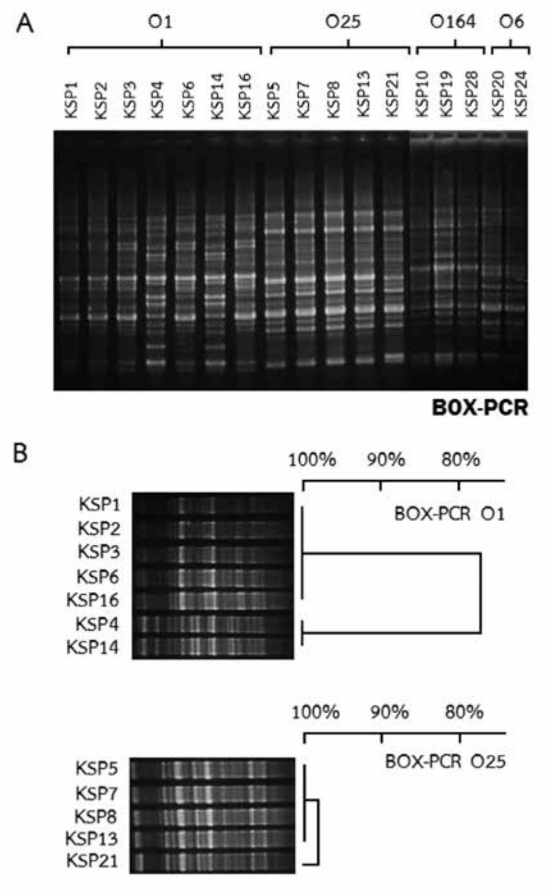

Fig 1-BOX-PCR profile of ExPEC strains obtained from healthy human fecal samples (A) and dendrogram of ExPEC O1 and O25 strains (B). Panel A. ExPEC *boxA* was PCR amplified and amplicons were separated by 1.5% agarose gel-electrophoresis and stained with ethidium bromide. Panel B. Dendrogram was constructed using unweighted-pair group method of arithmetic average (UPGMA). Scale bar indicates percent identity.

ExPEC through a number of ways: ExPEC infection of umbilical cord antenatally, passage through birth canal, or through upper respiratory/intestinal tracts (Smith *et al*, 2007). In the present study, 23% of healthy humans carried ExPEC possessing K1 gene, 71% of whom were females.

Distribution of ExPEC phylogenetic groups was similar to other studies (Duriez *et al*, 2001; Micenková *et al*, 2016). For example, Duriez *et al* (2001) reported 40% of commensal *E. coli* strains in three different geographical areas, namely, Croatia, France and Mali, belonged to group A. On the other hand, the largest portion of commensal *E. coli* carrying certain ExPEC virulence genes, belonged to group B2 (Duriez *et al*, 2001). In addition, Micenková *et al* (2016) reported among human ExPEC samples isolated from patients at the University Hospital in Brno, Czech Republic 52.6% belong to group B2, followed by groups A and D (both at 18.4%), and then group B1 (10.6%). Factors responsible for differences in ExPEC strains from healthy human colons include geographic/climate conditions, diet, antibiotics prescribed, and host genetic background (Duriez *et al*, 2001).

In conclusion, the study findings on virulent and multidrug-resistant ExPEC strains obtained from a healthy population is of importance not only to the heath of newborn babies and their mothers but to the population as a whole because Thailand will soon become a member of the aged nations, when demands on health care and costs become a major social and economic burden. Immunocompromised patients also constitute a group prone to such opportunistic infections. The data contribute to the current status of ExPEC epidemiology in southern Thailand should help provide a more complete picture of this group of commensal but potentially pathogenic *E. coli* in the Thai population.

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