CHARACTERIZATION OF EXTRAINTESTINAL PATHOGENIC *ESCHERICHIA COLI* FROM MEAT IN SOUTHERN THAILAND

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Abstract. Extraintestinal pathogenic *Escherichia coli* (ExPEC) is an *E. coli* group, which causes diseases in systems outside human intestinal tract. ExPEC isolates were recovered from fresh chicken (25%) and pork (10%) meats, but not beef and shrimp, from markets in southern Thailand. Among the 14 ExPEC strains isolated, all carried *iutA* and *fimH*, coding for aerobactin and type 1 fimbriae, respectively. Two ExPEC strains from chicken meat possessed *kpsMTK1* coding for K1 capsular antigen, responsible for neonatal meningitis. Antimicrobial susceptibility assay revealed that all ExPEC were resistant to streptomycin and carried bla_{TEM}, but susceptible to imipenem. Phylogenetic group analysis showed that 4, 4, and 6 ExPEC strains belonged to group A, B1 and D, respectively. ExPEC strains were classified into four serotypes, namely, O8 (2 strains), O15 (2 strains), O25 (1 strain), and O127a (1 strain), with the remaining untypeable. DNA profiling analysis by BOX-PCR revealed clonality of strains with the same serotype. The existence of ExPEC in meat products should cause concern regarding food safety and public health not only in southern Thailand but also throughout the country.

Keywords: *E. coli*, extraintestinal pathogen, virulence gene, BOX-PCR, meat, Thailand

INTRODUCTION

While a group of diarrheagenic *Escherichia coli* (DEC) pathotypes causes severe gastrointestinal illness, there is another group of *E. coli* in the intestine that is capable of causing pathology at several systems, *viz*, urinary tract, central nervous system and circulatory system once it released outside (Russo and Johnson, 2003). The latter group is termed extraintestinal pathogenic *E. coli* (ExPEC) (Russo and Johnson, 2000). ExPEC carries a large number of virulence genes, such as *fimH* (encoding Type 1 fimbriae), *pap* (encoding P fimbriae), and *sfaDE* (encoding S

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fimbriae), (all responsible for bacterial adherence), which are associated many aspects of its course of infection and pathogenesis (Köhler and Dobrindt, 2011; Themphachana *et al*, 2015). Pathogenesis also involves crucial effector proteins such as protectins and toxins (Köhler and Dobrindt, 2011).

In the past, ExPEC was shown to sporadically infected humans (George and Manges, 2010), but recently is reported to be associated with community-wide outbreaks (George and Manges, 2010), inferring that ExPEC is able to be transmitted in the community through common vehicles (Bergeron *et al*, 2012). One important ExPEC vehicles is raw meat, especially chicken, which is suspected to be a primary reservoir of ExPEC (Johnson *et al*, 2006; Cortés *et al*, 2010). Moreover, ExPEC strains from meat have demonstrated resistance to a wide variety of antimicrobials (Johnson *et al*, 2005, 2007).

Due to its potential impact on public health and the lack of information regarding its prevalence and characteristics in Thailand, this study investigated the presence of ExPEC in meat marketed throughout Hat Yai City, southern Thailand during June 2015 to May 2016. The data obtained should provide information on possible outbreaks of ExPEC infection in in this area of the country.

MATERIALS AND METHODS

Bacterial collection

A total of 134 raw meat samples comprising of beef (n = 34), pork (n = 30), chicken (n = 40), and shrimp (n = 30), were collected from fresh markets throughout Hat Yai City, Songkhla Province, southern Thailand during June 2015 to May 2016. All samples were processed within 2 hours after collection as previously described with slight modifications (Phetkhajorn *et al*, 2014). In brief, 10 g of meat were mixed with 90 ml of tryptic soy broth (TSB) (Beckton Dickinson, Sparks, MD), homogenized for 1 minute in a stomacher CIR-400 (Seward, West Sussex, UK) and supernatant was incubated statically at 37ºC for 6 hours. Then, 1 ml aliquot was pelleted and suspended in 100 µl of phosphate-buffered saline pH 7.4 (PBS). One loopful of suspension was streaked on eosin methylene blue (EMB) agar (Beckton Dickinson) and incubated at 37ºC for 18 hours. Five green metallic sheen colonies per sample were randomly selected for ExPEC identification.

ExPEC identification

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). The presence of these genes in the bacterial isolates was determined by PCR amplification as described previously (Sukkua *et al*, 2015). In short, an individual colony was grown in 3 ml of TSB at 37ºC for 6 hours with shaking and 1 ml aliquot of bacterial culture was boiled in 1 ml of PBS for 10 minutes, then sedimented and 10-fold dilution of supernatant in sterile deionized water was used as PCR template. PCR 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, 3.0 mM $MgCl_2$, 0.5 U Go*Taq* DNA polymerase (Promega, Medison, WI). Thermocycling was carried out in a thermal cycler (T100™, Bio-Rad, Hercules, CA) for 35 cycles of 94ºC for 1 minute; appropriate annealing temperature for each (Table 1) 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 and

ethidium bromide-stained bands were recorded using WSE5200 Printgraph 2M gel-imaging system (ATTO, Tokyo, Japan). *E. coli* identification was confirmed by detection of presence of *uidA* (annealing temperature at 59ºC).

ESBL gene determination

ESBL genes, bla_{SHV}, bla_{CTX-M}, and bla_{TEM}, were investigated by PCR (Monstein *et al*, 2007). Uniplex PCR was conducted as described above for 35 cycles of denaturation at 94ºC for 1 minute, annealing at 55ºC (bla_{CTX-M}, bla_{TEM}), or 50ºC (bla_{SHV}) for 1 minute, and extension at 72ºC for 1 minute. Amplicons were analyzed as mentioned above.

DNA profiling

DNA profiling of ExPEC strains was carried out using BOX-PCR (Versalovic *et al*, 1994). In brief, PCR was performed in a 25-µl reaction mixtures comprising of 0.2 µM of each specific primer (Table 1), 0.2 mM dNTPs, 1X Go*Taq* DNA polymerase buffer, 3.0 mM $MgCl_2$, 1.25 U Go*Taq* DNA polymerase (Promega) and 10 ng of DNA template (prepared by glass fiber matrix spin column; Geneaid, New Taipei City, Taiwan). Thermocycling conditions, conducted in a thermal cycler (T100™, Bio-Rad, Hercules, CA) 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 in 1.5 % agarose gel at 90 V for 2 hours.

Phylogenetic group determination

Phylogenetic group determination was performed using PCR amplifications of *chuA*, TspE4.C2 fragment and *yjaA* (Clermont *et al*, 2000). Uniplex PCR was carried out as described above using primers listed in Table 1 and the following thermocycling conditions: 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.

Serotype determination

O antigen serotyping was carried out by an agglutination assay using *E. coli*-specific antisera (set1) (Denka Seiken, Tokyo, Japan) as previously described with slight modifications (Sirikaew *et al*, 2014). In brief, a single colony of each bacterial isolate was cultured in 3 ml of TSB at 37ºC for 6 hours with shaking. Bacterial cells were harvested and re-suspended in physiological saline and heated to 121ºC for 15 minutes, centrifuged at 900*g* for 20 minutes and re-suspended in 0.5 ml of physiological saline. Agglutination was monitored on a glass slide by mixing cell suspension with a specific antiserum.

Antimicrobial susceptibility test

Antimicrobial susceptibility of ExPEC strains was performed by a disk diffusion method (CLSI, 2014) using amikacin (30 µg), ceftriaxone (30 µg), cephalothin (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), cotrimoxazole (25 µg), fosfomycin (200 µg), gentamicin (10 µg), imipenem (10 µg), streptomycin (10 µg), and tetracycline (30 µg) (Oxoid, Hampshire, UK).

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 ExPEC positive samples among meat types. Significant is set at $p < 0.05$.

RESULTS

Bacterial collection, ExPEC identification and phylogenetic group

Of 134 meat samples, 13 (10%) samples [10 (25% of chicken) and 3 (10% of

Table 1
Oligonucleotide primers used in the study.

Gene	Virulence factor	Primer name	Sequence (5′ to 3′)	Amplicon size (bp)	Reference
papA	P fimbriae subunit	papA f papA r	ATGGCAGTGGTGTCTTTTGGTG CGTCCCACCATACGTGCTCTTC	720	Johnson and Stell, 2000
papC	Outer membrane usher protein	papC f papC r	GTGGCAGTATGAGTAATGACCGTTA ATATCCTTTCTGCAGGGATGCAATA	200	Johnson and Stell, 2000
sfaDE	S fimbriae	sfaDE-F sfaDE-R	CTCCGGAGAACTGGGTGCATCTTAC CGGAGGAGTAATTACAAACCTGGCA	408	Le Bouguénec *et al*, 1992
afa	Afa adhesin	afa1 afa2	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	750	Le Bouguénec *et al*, 1992
kpsMTII	Capsular antigen	kpsII f kpsII r	GCGCATTTGCTGATACTGTTG CATCCAGACGATAAGCATGAGCA	272	Johnson and Stell, 2000
iutA	Aerobactin	AerJ f AerJ r	GGCTGGACATCATGGGAACTGG CGTCGGGAACGGGTAGAATCG	300	Johnson and Brown, 1998
fimH	Type 1 fimbrial tip	fimH-F fimH-R	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	508	Johnson and Stell, 2000
traT	Conjugal transfer surface exclusion protein	traT f traT r	GGTGTGGTGCGATGAGCACAG CACGGTTCAGCCATCCCTGAG	290	Johnson and Stell, 2000
hlyA	α-hemolysin	hly1 hly2	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	1,177	Yamamoto *et al*, 1995
cnf1	Cytotoxic necrotizing factor-1	cnf1-F cnf1-R	GGCGACAAATGCAGTATTGCTTGG GACGTTGGTTGCGGTAATTTTGGG	552	Yamamoto *et al*, 1995
kpsMTK1	K1 capsular antigen	K1-f kpsII r	TAGCAAACGTTCTATTGGTGC CATCCAGACGATAAGCATGAGCA	153	Johnson and Stell, 2000
lpf	Long polar fimbriae	lpfA1-F lpfA1-R	GGTCGTTTTTGCCTTAACCGC AGGTTGAAATCGACCTGCGC	≈500	Torres *et al*, 2004
agn43	Antigen 43	1-Kpn 2-Bam	GAACCTGTCGGTACCGATGCCCTCCC CGGGATCCGTTGCCACTGTACCGGGCTTGACGACC	≈900	Danese *et al*, 2000

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	GAGTAATGTCGGGGCATTCA	152	Clermont *et al*, 2000
		TspE4.C2-2	CGCGCCAACAAAGTATTACG		
bla_{SHV}	β-lactamase	bla-SHV.SE	ATGCGTTATATTCGCCTGTG	747	Paterson *et al*, 2003
		bla-SHV.AS	TGCTTTGTTATTCGGGCCAA		
bla_{CTX-M}	β-lactamase	CTX-M-U1	ATGTGCAGYACCAGTAARGTKATGGC	593	Boyd *et al*, 2004
		CTX-M-U2	TGGGTRAARTARGTSACCAGAAYCAGCGG		
bla_{TEM}	β-lactamase	TEM-164.SE	TCGCCGCATACACTATTCTCAGAATGA	445	Monstein *et al*, 2007
		TEM-164.AS	ACGCTCACCGGCTCCAGATTTAT		
uidA	β-glucoronidase	uidA-F	ATCACCGTGGTGACGCATGTCGC	486	Heninger *et al*, 1999
		uidA-R	CACCACGATGCCATGTTCATCTGC		
boxA	-	BOXA1R	CTACGGCAAGGCGACGCTGACG	Variable	Versalovic *et al*, 1994

pork], yielded 14 ExPEC/670 (2%) bacteria isolates [11/200 (6%) from chicken and 3/150 (2%) from pork]. No ExPEC isolate was found in beef and shrimp samples. Chicken was shown to be the most potent ExPEC reservoir compared to beef and shrimp ($p < 0.05$). Based on PCR amplifications of *chuA*, TspE4.C2 fragment and *yjaA*, the 14 ExPEC strains belonged to 3 phylogenetic groups, namely, A (4 strains), B1 (4 strains), and D (6 strains) (Table 2).

ExPEC serotypes

The 14 ExPEC strains were classified into 4 serotypes: strains PSU550 and PSU551 from different pork samples as O15; PSU562 and PSU563 from different chicken samples O8; PSU553 from chicken as O25; PSU561 from pork O127a; and the remaining 8 were untypeable (Table 2).

ExPEC- and *E. coli*-associated genes

In addition to the ExPEC indicator genes, the associated gene *fimH*, *kpsMT*K1 and *traT* were detected in 2 (14%), and 6 (43%) strains, respectively but not *E. coli*-associated genes, *lpf* and *agn43* (Table 2). For the 73 non-ExPEC *E. coli* strains carrying 1 of 5 ExPEC indicator genes, *fimH* was the most frequently found (69 strains), followed by *traT* (59 strains), and *kpsMT*K1 (6 strains) (data not shown).

Antimicrobial susceptibility and presence of ESBL genes

All 14 but 1 ExPEC strains were multidrug-resistant strains, namely, resistant to at least 3 different classes of antibiotics. Antibiogram showed that all ExPEC strains were resistant to streptomycin, followed by tetracycline (12 strains), cephalothin (11 strains) cotrimoxazole (9 strains), and ciprofloxacin (8 strains) (Table 2). All 14 strains were susceptible to imipenem. Among ESBL genes examined, all 14 ExPEC strains possessed bla_{TEM} but not bla_{SHV} and bla_{CTX-M} (data not shown).

Table 2
Characteristics of 14 ExPEC strains isolated from meat of fresh produce markets, Hat Yai City, Songkhla Province, southern Thailand during June 2015 to May 2016.

Strain	Meat type	Phylogenetic group	Serotype	ExPEC indicator gene						ExPEC-associated gene					Other *E. coli*-associated gene		Antimicrobial resistance[b]
				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*	
PSU550	Chicken	B1	O15	-	+	-	-	-	+	+	-	-	-	-	-	-	KF, S, SXT, TE
PSU551	Chicken	B1	O15	-	+	-	-	-	+	+	-	-	-	-	-	-	KF, S, SXT, TE
PSU552	Chicken	D	UN[a]	-	-	-	-	+	+	+	-	-	-	-	-	-	CIP, KF, S, SXT, TE
PSU553	Chicken	D	O25	-	-	-	-	+	+	+	+	-	-	+	-	-	CIP, KF, S, TE
PSU554	Chicken	D	UN	-	-	-	-	+	+	+	-	-	-	-	-	-	CIP, CRO, FOS, KF, S, SXT, TE
PSU555	Chicken	D	UN	-	-	-	-	+	+	+	-	-	-	-	-	-	CIP, CRO, KF, S, SXT, TE
PSU556	Chicken	B1	UN	-	+	-	-	+	+	+	+	-	-	-	-	-	KF, S,TE
PSU557	Chicken	A	UN	-	+	-	-	+	+	+	-	-	-	-	-	-	CIP, S
PSU558	Chicken	D	UN	-	-	-	-	+	+	+	-	-	-	-	-	-	C, CIP, KF, S, SXT, TE
PSU559	Chicken	A	UN	-	-	-	-	+	+	+	+	-	-	+	-	-	C, CIP, S, TE
PSU560	Chicken	D	UN	-	-	-	-	+	+	+	-	-	-	-	-	-	AK, C, CIP, CN, FOS, KF, S, SXT, TE
PSU561	Pork	B1	O127	-	+	-	-	-	+	+	+	-	-	-	-	-	C, CRO, KF, S, SXT, TE
PSU562	Pork	A	O8	+	+	-	-	-	+	+	+	-	-	-	-	-	CRO, S, TE
PSU563	Pork	A	O8	-	-	+	-	-	+	+	+	-	-	-	-	-	KF, S, SXT

[a]UN, untypeable; [b]AK, amikacin; C, chloramphenicol; CIP, ciprofloxacin; CN, gentamicin; CRO, ceftriaxone; FOS, fosfomycin; KF, cephalothin; S, streptomycin; SXT, cotrimoxazole; TE, tetracycline.

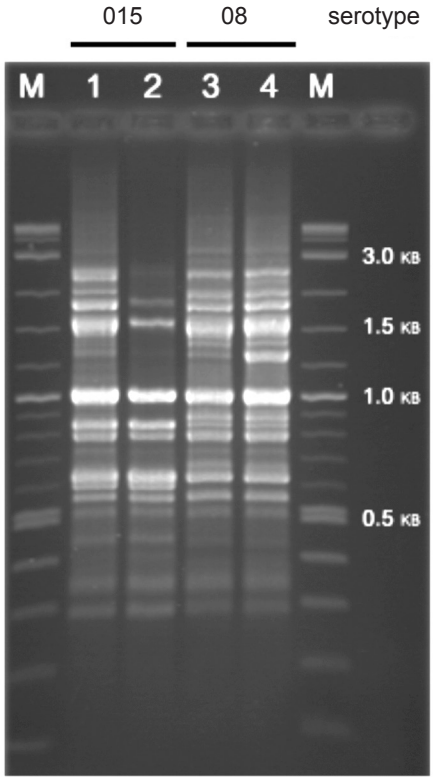

Fig 1–DNA profiles of ExPEC O8 and O15 strains by BOX-PCR. ExPEC *boxA* was amplified using BOXA1R primer (Table 1). Amplicons were analyzed by 1.5% agarose gel-electrophoresis at 90 V for 2 hours. Lane M, DNA size markers; lane 1, ExPEC O15 PSU550 strain; lane 2, ExPEC O15 PSU551 strain; lane 3, ExPEC O8 PSU562 strain; lane 4, ExPEC O8 PSU563 strain.

DNA profiling

As a number of ExPEC strains showed the same O-serotype but were isolated from different sources, *eg*, O15 serotype PSU550 and PSU551 (from chicken) and O8 serotype PSU562 and PSU563 (from pork), clonality of these strains was investigated by comparing their DNA profiles by BOX-PCR. ExPEC strains with the same serotype had identical DNA band size profiles (although intensities of some of the DNA were different), but dissimilar to those of strains with another serotype (Fig 1), suggesting that ExPEC strains of the same serotype may come from a common bacterial source.

DISCUSSION

ExPEC is now recognized as a distinct group of *E. coli* that plays a key role in a wide variety of diseases (Smith *et al*, 2007). Uropathogenic *E. coli* is a member of ExPEC group and has long been considered an important pathogen in Thailand (Polwichai *et al*, 2009; Themphachana *et al*, 2015). However, the ExPEC group especially from meat source has not been investigated previously in Thailand. Studies conducted in St Paul, Minneapolis, USA during 2000 demonstrated 21% of *E. coli* strains, both nalidixic acid-resistant and -susceptible, from chicken were ExPEC (Johnson *et al*, 2003). Continual surveys conducted in the same area during 2001-2003 indicated similar incidence (Johnson *et al*, 2005). A study from Finland demonstrated a prevalence of 23% of ExPEC in poultry meat (Lyhs *et al*, 2012). Our study revealed a similar (25%) prevalence of ExPEC contamination in chicken meat. This suggests that much more attention should be paid worldwide to the processes involved with poultry.

Bergeron *et al* (2012) demonstrated genetic similarity between *E. coli* from animals, mainly chicken, and ExPEC causing human urinary tract infection, suggesting an establishment of transmission of ExPEC from meat to humans. Additionally, our study also points to pork as another source of ExPEC, at least in the region of Thailand surveyed.

Newborn humans are very susceptible to bacterial infections including *E. coli*. Among the ExPEC virulence factors

that play a key role in neonatal meningitis is capsular polysaccharide K1 (Glode *et al*, 1977). In the United States, >50% of neonatal meningitis cases caused by *E. coli* are due to ExPEC, approximately 80% of which carry K1 (Pong and Bradley, 1999). K1 polysialic acid (PSA) capsule may mimic PSA chains that are attached to neonatal neural cell adhesion molecules (NCAM) (Cieslewicz and Vimr, 1997) and thereby interfere with normal neonatal brain maturation by competing with neural PSA on NCAM (Smith *et al*, 2007). Sequelae include hydrocephalus, seizures, mental retardation, cerebral palsy, and hearing loss (Harvey *et al*, 1999; Pong and Bradley, 1999). In this study, two ExPEC strains from chicken possessed K1 gene, suggesting the possible risk of severe infection in newborns.

Most ExPEC strains from humans have been shown to be members of phylogenetic group B2 or D while strains from animals belong to phylogenetic group A and B1 (Maynard *et al*, 2004), which corresponded to the current results indicating that 57% of ExPEC strains belonged to group A and B1. However, these strains are considered comparable as regards pathological capabilities, possessing genes of similar virulent properties (Table 2). Long polar fimbria (encoded by *lpf*) is responsible for bacterial adherence and increases pathogenicity while Antigen43 (encoded by *agn43*) is involved in autoagglutination and biofilm formation, processes which promote bacterial virulence (Schembri *et al*, 2004; Sukkua *et al*, 2016). The lack of these virulence genes in ExPEC in this study is not surprising because based on DEC group, *lpf* and *agn43*, are seldomly found (Sukkua *et al*, 2016).

Previous studies of ExPEC and uropathogenic *E. coli* (UPEC) characteristics of from southern Thailand showed UPEC from Phuket Island and Nakhon Si Thammarat Provinces tend to carry *sfaDE*, *afa*, *cnf1*, and *hlyA* (Themphachana *et al*, 2014, 2015), but ExPEC from meats in this present study did not carry such genes, except for PSU563 that carried *sfaDE*. Different geographical areas may affect the occurrence of dissimilar UPEC genotypes from patients and ExPEC from meat. Information regarding UPEC from Songkhla Province is needed to verify this notion. Alternatively, only ExPEC possessing high numbers of virulence genes is able to cause pathology. Therefore, this might be one of the reasons why a higher number of virulence genes can be found in UPEC compared to ExPEC.

In the current study, 73 *E. coli* isolates were not classified as ExPEC (non-ExPEC group) although they carried 1 of 5 ExPEC indicator gene. Having only a single ExPEC virulence gene is considered to be lower potential to cause the pathologies associated with this group of bacteria. However, the non-ExPEC group demonstrated similar antimicrobial resistant pattern to that of ExPEC group, with highest resistance to streptomycin, followed by tetracycline, then cephalothin (data not shown).

In conclusion, due to the detection of high prevalence of ExPEC strains in fresh market survey, chicken and pork meat in the area of southern Thailand, their potential to cause severe illnesses, and their capability to resist to a wide variety of antimicrobials should not be overlooked. Frequent surveillances for ExPEC should be carried out in all area of Thailand for developing public health measures for control and prevention.

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REFERENCES

Bergeron CR, Prussing C, Boerlin P, *et al*. Chicken as reservoir for extraintestinal pathogenic *Escherichia coli* in humans, Canada. *Emerg Infect Dis* 2012; 18: 415-21.

Boyd DA, Tyler S, Christianson S, *et al*. Complete nucleotide sequence of a 92-kilobase plasmid habouring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. *Antimicrob Agents Chemother* 2004; 48: 3758-64.

Cieslewicz M, Vimr E. Reduced polysialic acid capsule expression in *Escherichia coli* K1 mutants with chromosomal defects in *kpsF*. *Mol Microbiol* 1997; 26: 237-49.

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

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

Cortés P, Blanc V, Mora A, *et al*. Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. *Appl Environ Microbiol* 2010; 76: 2799-805.

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

George DB, Manges AR. A systematic review of outbreak and non-outbreak studies of extraintestinal pathogenic *Escherichia coli* causing community-acquired infections. *Epidemiol Infect* 2010; 138: 1679-90.

Glode MP, Sutton A, Robbins JB, *et al*. Neonatal meningitis due to *Escherichia coli* K1. *J Infect Dis* 1977; 136: S93-7.

Harvey D, Holt DE, Bedford H. Bacterial meningitis in the newborn: a prospective study of mortality and morbidity. *Semin Perinatol* 1999; 23: 218-25.

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

Johnson JR, Brown JJ. Colonization with and acquisition of uropathogenic *Escherichia coli* strains as revealed by polymerase chain reaction-based detection. *J Infect Dis* 1998; 177: 1120-4.

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

Johnson JR, Kuskowski MA, Menard M, Gajewski A, Xercavins M, Garau J. Similarity between human and chicken *Escherichia coli* isolates in relation to ciprofloxacin resistance status. *J Infect Dis* 2006; 194: 71-8.

Johnson JR, Kuskowski MA, Smith K, O'Bryan TT, Tatini S. Antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli* in retail foods. *J Infect Dis* 2005; 191: 1040-9.

Johnson JR, Murray AC, Gajewski A, *et al*. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. *Antimicrob Agents Chemother* 2003; 47: 2161-8.

Johnson JR, Sannes MR, Croy C, *et al*. Antimicrobial drug-resistant *Escherichia coli* from humans and poultry products, Minnesota and Wisconsin, 2002-2004. *Emerg Infect Dis* 2007; 13: 838-46.

Köhler CD, Dobrindt U. What defines extraintestinal pathogenic *Escherichia coli*?. *Int J Med Microbiol* 2011; 301: 642-7.

Le Bouguénec C, Archambaud M, Labigne A. Rapid and specific detection of the *pap*, *afa*, and *sfa* adhesin-encoding operons in

uropathogenic *Escherichia coli* strains by polymerase chain reaction. *J Clin Microbiol* 1992; 30: 1189-93.

Lyhs U, Ikonen I, Pohjanvirta T, Raninen K, Perko-Mäkelä P, Pelkonen S. Extraintestinal pathogenic *Escherichia coli* in poultry meat products on the Finnish retail market. *Acta Vet Scand* 2012; 54: 64.

Maynard C, Bekal S, Sanschagrin F, *et al*. Heterogeneity among virulence and antimicrobial resistance gene profiles of extraintestinal *Escherichia coli* isolates of animal and human origin. *J Clin Microbiol* 2004; 42: 5444-52.

Monstein HJ, Östholm-Balkhed A, Nilsson MV, Nilsson M, Dornbusch K, Nilsson LE. Multiplex PCR amplification assay for the detection of *bla*SHV, *bla*TEM and *bla*CTX-M genes in Enterobacteriaceae. *APMIS* 2007; 115: 1400-8.

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

Phetkhajorn S, Sirikaew S, Rattanachuay P, Sukhumungoon P. Most probable number-polymerase chain reaction-based quantification of enterotoxigenic *Escherichia coli* from raw meats in southern Thailand. *Southeast Asian J Trop Med Public Health* 2014; 45: 1385-91.

Polwichai P, Dejsirilert S, Panpetch S, Sawanpanyalert P, Aswapokee N, Mootsikapun P. Antimicrobial resistance of *Escherichia coli* isolated from urine in Thailand from 2000-2005. *J Med Assoc Thai* 2009; 92 (suppl 4): S59-67.

Pong A, Bradley JS. Bacterial meningitis and the newborn infant. *Infect Dis Clin North Am* 1999; 13: 711-33.

Russo TA, Johnson JR. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli* ExPEC. *J Infect Dis* 2000; 181: 1753-4.

Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasing important endemic problem. *Microbes Infect* 2003; 5: 449-56.

Schembri MA, Dalsgaard D, Klemm P. Casule shields the function of short bacterial adhesion. *J Bacteriol* 2004; 186: 1249-57.

Smith JL, Fratamico PM, Gunther NW. Extraintestinal pathogenic *Escherichia coli*. *Foodborne Pathog Dis* 2007; 4: 134-63.

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

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

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

Themphachana M, Kanobthammakul S, Nakaguchi Y, Singkhamanan K, Yadrak P, Sukhumungoon P. Virulence characteristics and antimicrobial susceptibility of uropathogens from patients on Phuket Island, Thailand. *Southeast Asian J Trop Med Public Health* 2014; 45: 1090-8.

Themphachana M, Kongpheng S, Rattanachuay P, Khianngam S, Singkhamanan K, Sukhumungoon P. Molecular characterization of virulence and antimicrobial susceptibility profiles of uropathogenic *Escherichia coli* from patients in a tertiary hospital, southern Thailand. *Southeast Asian J Trop Med Public Health* 2015; 46: 1021-30.

Torres AG, Kanack KJ, Tutt C, Popov V, Kaper JB. Characterization of the second long po-

lar (LP) fimbriae of *Escherichia coli* O157:H7 and distribution of LP fimbriae in other pathogenic *E. coli* strains. *FEMS Microbiol Letters* 2004; 238: 333-44.

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

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