CHARACTERIZATION OF DIARRHEAGENIC *ESCHERICHIA COLI* ISOLATED FROM FOOD IN KHON KAEN, THAILAND

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Abstract. Four categories of 186 ready-to-eat food samples in Khon Kaen municipality, Thailand, were collected and investigated for fecal contamination by enumeration of *Escherichia coli* using the most probable number (MPN) method. Then, the *E. coli* isolates were presumptively identified as diarrheagenic *E. coli* by agglutinating with polyvalent O-antisera and monovalent O-antisera commonly found in diarrheagenic strains and were subsequently investigated for the presence of the recognized virulence genes for enteroaggregative (EAEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), and shiga toxin-producing *E. coli* (STEC or EHEC) by multiplex PCR assays. All *E. coli* isolates were examined for antimicrobial susceptibilities by the agar disc diffusion method, and the results were compared with those obtained from clinical samples. The percentage of each type of food with *E. coli*, including no heat food, low heat food, high heat food, and fruit juices and beverages, was higher than accepted standards at 60.4, 46.5, 38.6 and 20%, respectively. Of 140 *E. coli* isolates obtained from food samples, 11 isolates (7.9%) agglutinated with 6 monovalent O-antisera, including one isolate each of O6, O8, O114 and O159, two isolates of O1, and five isolates of O157. None of the 11 isolates harbored the virulence genes for EPEC, ETEC, EAEC, EIEC and STEC. Although O157 *E. coli* isolates were found, the most frequent, *E. coli* O157:H7, was not found in this study. The *astA* gene, however, was found in 1 *E. coli* isolate that showed weakly positive agglutination against the polyvalent antisera. Approximately 50% of the 140 *E. coli* isolates were resistance to at least one antimicrobial agent. The resistant strains showed high resistance to tetracycline (43%), co-trimoxazole (36%), ampicillin (26%) and chloramphenicol (23%), respectively. The resistance of *E. coli* was high for nearly all antimicrobial agents, particularly ampicillin (76%), tetracycline (70%), co-trimoxazole (69%) and nalidixic acid (44%). The results show that nearly half of the ready-to-eat food samples evaluated in Khon Kaen Municipality had levels of *E. coli* higher than acceptable standards. Of the diarrheagenic *E. coli* classified by serogroup, almost none of the isolates had virulence genes. These results indicate the disadvantage of relying on serogrouping alone for the recognition of diarrheagenic *E. coli*. *E. coli* isolated from food may not be an enteropathogenic strain. We also found that *E. coli* antimicrobial resistant strains are widespread in both food and humans.

INTRODUCTION

Escherichia coli have been identified as an indicator microorganism for food safety (Adams and Moss, 2000). Pathogenic *E. coli* have been recognized as an increasingly important human diarrheagenic pathogen in all parts of the world, especially in young children in developing countries (Porat *et al*, 1998). Five major categories of diarrheagenic *E. coli* have been defined on the basis of their pathogenic mechanisms: enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC) or shiga toxin-producing *E. coli* (STEC) and enteroaggregative *E. coli* (EAEC) (Porat *et al*, 1998). Traditionally, diarrheagenic *E. coli* belong to a number of distinct serogroups and were once defined solely on the basis of their sero-

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types (Gomes *et al*, 1989). Recently, molecular biology and the knowledge of specific genes encoding for characteristic virulence factors such as *invE* for EIEC, *LTh* and *STh* for ETEC, *eaeA* for EPEC, and *stx* for STEC, have been used to categorize these *E. coli* (Porat *et al*, 1998; Rosa *et al*, 1998). Increasing antimicrobial resistance of *E. coli* in both humans and animals over the world has been reported (Schroeder *et al*, 2002).

Extensive studies on food-borne pathogens, such as *Salmonella*, have been conducted in Thailand, but not much has been reported on the characterization of diarrheagenic *E. coli* (especially O157) and the antimicrobial susceptibilities of *E. coli* strains isolated from food. Our study therefore investigated the serogroups and the virulence genes of diarrheagenic *E. coli* isolated from foods, and their antimicrobial susceptibilities.

MATERIALS AND METHODS

Sample collection

A total of 186 food samples were randomly collected from food vendors and food shops in Khon Kaen Municipality. The samples were grouped into four categories, 57 samples of no heat foods, 71 samples of low heat foods, 48 samples of high heat foods, and 10 samples of juices and beverages. All the samples were collected aseptically, placed in sterile containers, kept at 4ºC, and then transferred to the laboratory.

MPN test for *E. coli*

The MPN test for *E. coli* was determined as described previously (Ohashi *et al*, 1978; Adams and Moss, 2000). Fifty grams of food samples were suspended in 450 ml of phosphate-buffered saline, blended for 2 minutes, and then diluted 10-fold to $1:10^4$. One ml of each diluted sample was incubated in triplicate tubes containing 10 ml of EC broth (Oxoid, Unipath Ltd, Basingstroke, Hamshire, England) at 44.5ºC for 24 hours. The MPN for *E. coli* was calculated from the number of the tubes which showed bacterial growth and gas production. All positive tubes were sub-cultured on Eosin methylene blue (EMB) agar (Oxoid) and incubated at 35ºC for 18-24 hours. One to three typical colonies were picked up and identified as *E. coli* by biochemical testing (Edwards and Ewing, 1986; Mahon and Manuselis, 2000). The *E. coli* isolates were kept in nutrient agar for further assay.

Isolation of *E. coli* O157

Isolation of *E. coli* O157 was performed according to the Dentorou method with modification (Dontorou *et al*, 2003). Twenty grams of each food sample was added to 200 ml sterile modified EC broth (Oxoid), supplemented with 2% novobiocin (Sigma, Germany) and incubated at 42ºC for 6 hours. After incubation, the culture was divided into two parts: the first part was streaked on Sorbitol MacConkey agar (SMAC) (Oxoid) and incubated at 42ºC for 24 hours. Two white colonies were picked up and confirmed before serotyping. A 1 ml aliquot of the EC broth was added to 20 µl of magnetic beads coated with specific antibody against *E. coli* O157 (Dynol, Norway). Immunomagnetic seperation (IMS) was performed according to the manufacturer's instructions. The final 50 µl of suspension obtained after IMS was plated on to SMAC and CHROM agar O157 at 42ºC for 24 hours. Two sorbitol non-fermenting colonies (white colonies) on SMAC and two mauve colonies on CHROM agar were picked up and identified as *E. coli* by biochemical testing. The *E. coli* isolates were kept in nutrient agar for further assay.

Confirmation of *E. coli* O157 by agglutination

The suspicious colonies were purified on plate count agar (Oxoid) by overnight incubation at 37ºC and were tested for agglutination with an *E. coli* O157 latex test kit (Oxoid).

O-serogroups

E. coli isolates were evaluated for the O-serogroup by slide agglutination. A heat suspension of bacterial cells (100ºC for 1 hour) was mixed with eight polyvalent antisera to screen for EPEC, EIEC, and ETEC according to the manufacturer's instruction (Denka Seiken, Tokyo, Japan). The isolates that agglutinated with polyvalent antisera were subsequently tested with monovalent O antisera (Denka Seikin, Tokyo, Japan) which reacted against common O-serogroups of EPEC, ETEC, EIEC, and STEC. The following 43 monovalent O-antisera were

detected: O1, O6, O8, O15, O18, O20, O25, O26, O27, O28ac, O29, O44, O55, O63, O78, O86a, O111, O112ac, O114, O115, O119, O124, O125, O126, O127a, O128, O136, O142, O143, O144, O146, O148, O152, O151, O153, O157, O158, O159, O164, O166, O167, O168, and O169. The results were confirmed by the tube agglutination test as described by Ewing (Edwards and Ewing, 1986).

Multiplex PCR assays

The presumptive diarrheagenic *E. coli* isolates were examined for the presence of heat-labile enterotoxin (*LTh*) and heat-stable enterotoxin (*STh*) encoding genes for ETEC, for the invasiveness (*invE*) encoding gene for EIEC, for the *stx*1/*stx*2 genes for EHEC, for the *eaeA*, *bfpA* and EAF genes for EPEC, and for the *aggR* and *astA* genes for EAEC by using 3 sets of multiplex PCR assays. The first set was used to identify ETEC, EIEC, and STEC (Ratchtrachenchai *et al*, 2004). The second and the third sets were used to identify EPEC and EAEC, respectively. The oligonucleotide primers used in this study are shown in Table 1. The PCR assays were performed as described below:

Template DNA was prepared by suspending overnight-grown colonies from an LB agar plate in 100 μl of sterile distilled water to give a final concentration of 10^5 to 10^6 organisms per ml. Bacterial cell suspension was then boiled for 20 minutes and spun down to obtain template DNA in the supernatant. The PCR assays were carried out in 0.2 ml PCR tubes with 25 μl of reaction mixture consisting of PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$), 0.25 mM of each dNTPs (New England Biolabs, Beverly, MA), 0.1μM of each primer (except 0.2 μM for ST primers), 1 unit of *Taq* DNA Polymerase (Gibco), and 1 μl of template DNA. The reaction mixtures were run in a thermal cycler (model 9700, Perkin-Elmer Corp, Norwalk, USA) with the following cycling profile: 94ºC for 5 minutes, 25 cycles of denaturation at 94ºC for 1 minutes, primer annealing at 48ºC for 1.5 minutes, primer extension at 72ºC for 2 minutes, and a final extension at 72ºC for 5 minutes. The annealing temperature was 50ºC for the EPEC and EAEC PCRs. Positive, negative, and background controls were included with each PCR. DNA templates of *E. coli* strains 1298 (*invE*+), EDL931 (*stx1/2*+), 682 (*LTh*), 825 (*STp*), 1296 (*STh*), 1228 (*eaeA*+, *bfpA*+, EAF), EAEC 6 (*aggR*, *astA*), and JM109 were used as positive and negative controls, respectively. *E. coli* positive controls were kindly provided by the National Institute of Infectious Diseases, Tokyo, Japan. Sterile distilled water (2 μl) was used in place of the DNA template in the PCR mixture as a reagent control. The amplified DNA products were resolved by agarose gel electrophoresis. DNA fragments were visualized under 320 nm UV light and photographed with a Polaroid camera. The sizes of the DNA fragments were calculated against the DNA size marker. The amplified products for each specific gene for the diarrheagenic *E. coli* strains are shown in Fig 1.

Drug susceptibility testing

Antimicrobial susceptibility testing of 140 *E. coli* isolates was performed using the disk agar diffusion method (National Committee for Clinical Laboratory Standards, 2002; Schroeder *et al*, 2002) with commercially manufactured disks (Oxoid). All *E. coli* isolates were examined for resistance to ampicillin (10 μg), chloramphenicol (30 μg), co-trimoxazole (25 μg), gentamicin (10 μg), nalidixic acid (30 μg), norfloxacin (10 μg), tetracycline (30 μg), colistin (10 μg), ofloxacin (5 μg), and cephalothin (30 μg). *E. coli* ATCC 25922, sensitive to all the drugs, was used as the control strain.

RESULTS

MPN for *E. coli*

The enumeration of *E. coli* in 186 food samples was determined by the MPN method. This was compared to the health standards for food safety set by the Department of Medical Sciences, Ministry of Public Health, Thailand. About 53% and 46% of the food samples were higher and lower than the health standard, respectively, as shown in Table 2.

Serogroups

A total of 140 *E. coli* isolates obtained from 186 food samples were presumptively identified as diarrheagenic *E. coli*, such as EPEC, ETEC, EIEC and EHEC, by agglutinating with O-polyvalent antisera and identified (Table 3). Of the

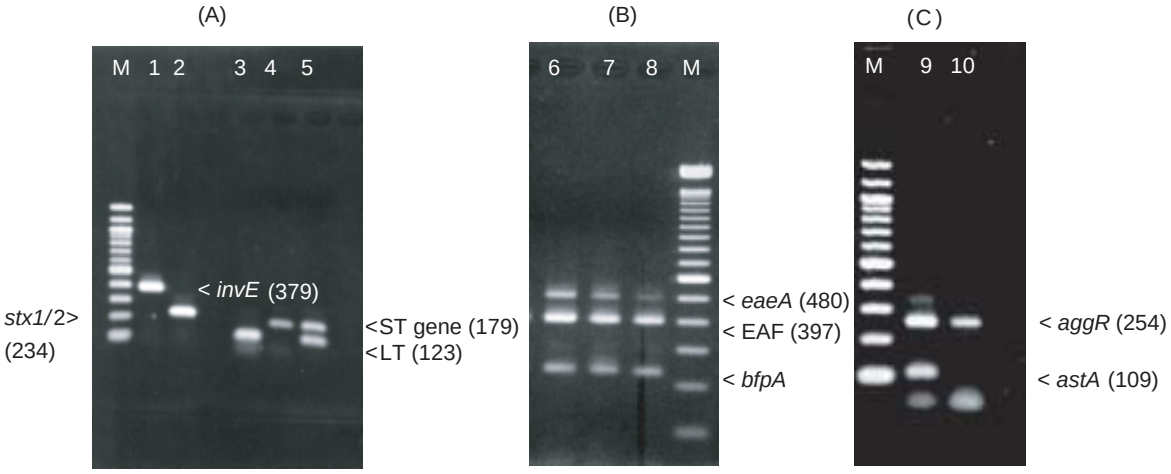

Fig 1–The products from PCR assays for EIEC, STEC and ETEC (A), EPEC (B), and EAEC (C). Lanes: 1, EIEC; 2, STEC; 3-5, ETEC; 6-8, EPEC; 9-10 EAEC; M, 100-bp DNA size marker. Virulence marker sizes (in bp) are indicated.

Table 1
Three multiplex PCR primer sets used to identify the recognized virulence markers for ETEC, EIEC, STEC, EPEC, and EAEC.

Patho-types	Virulence factors (genes)	Sequences (5′-3′)	PCR products (bp)	References
ETEC	*LTh*	(F) AGC AGG TTT CCC ACC GGA TCA CCA (R) CGT GCT CAG ATT CTG GGT CTC	132	(Ratchtrachenchai *et al*, 2004)
	STh/STp	(F) ATT TCT GTA TTG TCT TT (R) ATT ACA ACA CAG TTC ACA G	171	(Ratchtrachenchai *et al*, 2004)
EIEC	Regulator for cell invasion (*invE*)	(F) ATA TCT CTA TTT CCA ATC GCG T (R) GAT GGC GAG AAA TTA TAT CCC G	382	(Ratchtrachenchai *et al*, 2004)
	SLT1/2 (*stx*1/2)	(F) TTT ACG ATA GAC TTC TCG AC (R) CAC ATA TAA ATT ATT TCG CTC	228	(Ratchtrachenchai *et al*, 2004)
EPEC	Intimin (*eaeA*)	(F) GCT TAG TGC TGG TTT AGG AT (R) TCG CCG TTC AGA GAT CGC	488	(Ratchtrachenchai *et al*, 2004)
	EAF	(F) CAG GGT AAA AGA AAG ATG ATAA (R) TAT GGG GAC CAT GTA TTA TCA	397	(Ratchtrachenchai *et al*, 2004)
	BFP (*bfpA*)	(F) GAA GTA ATG AGC GCA ACG TC (R) ACA TGC CGC TTT ATC CAA CC	234	(Ratchtrachenchai *et al*, 2004)
EAEC	Transcriptional activator genes of AAF (*aggR*)	(F) GTA TAC ACA AAA GAA GGA AGC (R) ACA GAA TCG TCA GCA TCA GC	254	(Itoh *et al*, 1992; Rachtrachenchai *et al*, 1997)
	EAST (*astA*)	(F) GCC ATC AAC ACA GTA TAT CCG (R) CGC GAG TGA CGG CTT TGT AG	109	(Itoh *et al*, 1992; Rachtrachenchai *et al*, 1997)

LTh = heat-labile enterotoxin; *STh/STp*= heat-stable enterotoxin (human and pig alleles); SLT1/2= Shiga-like toxins 1 and 2; EAF= EPEC adherence factor; BFP= bundle-forming pili; AAF= aggregative adherence fimbriae; EAST1= heat-stable enterotoxin of EAEC.

Table 2
MPN for *E. coli* in 186 food samples collected in Khon Kaen, Thailand.

Types of food	No. of food samples (%)		Total
	MPN of *E. coli* below standard[a]	MPN of *E. coli* above standard[a]	
No heat food	29 (60.4)	19 (39.6)	48
Low heat food	33 (46.5)	38 (53.5)	71
High heat food	22 (38.6)	35 (61.4)	57
Fruit juice and beverage	2 (20)	8 (80)	10
Total	86 (46.2)	100 (53.8)	186

[a]The MPN for *E. coli* in 186 food samples were determined and scored as below or above health standards according to the criteria for food safety of the Medical Sciences Department, Ministry of Public Health, Thailand.

Table 3
The primary screening of diarrheagenic *E. coli* detected from 140 *E. coli* isolates found in various types of 186 food samples.

Type of foods	No. of food samples	EPEC	ETEC	EIEC	EHEC	Total (%)
No heat food	48	1	1	-	2	4
Low heat food	71	1	-	-	1	2
High heat food	57	1	2	-	2	5
Fruit juices and beverages	10	-	-	-	-	0
Total food samples	186	3	3	-	5	11
(%)		(1.6%)	(1.6%)	-	(2.7%)	(5.9%)

EPEC, ETEC, EIEC and EHEC were presumptively identified as diarrheagenic strains by agglutinating with O-polyvalent antisera.

Table 4
Diarrheagenic *E. coli* identified from 140 *E. coli* isolates found in four types of food samples.

Type of foods	No. of *E. coli* isolates	No.(%) of diarrheagenic *E. coli*
No heat food	31	4 (12.9%)
Low heat food	54	2 (3.7%)
High heat food	45	5 (11.1%)
Fruit juices and beverages	10	0 (0%)
Total	140	11 (7.9%)

E. coli strains were identified as diarrheagenic strains by agglutinating with O-polyvalent antisera against diarrheagenic strains.

140 *E. coli* isolates, 11 isolates (7.9%) were identified as diarrheagenic strains (or 5.9% of the food samples) by agglutinating with 43 O-monovalent antisera (Table 4). Then, they were tested with 6 O-antisera, including O1, O6, O8, O114, O157, and O159 (Table 5). Though *E. coli* O157 isolates were most frequently found, they were non-reactive with H7 antiserum.

Presence of virulence genes

The 11 *E. coli* isolates were examined for the presence of virulence genes ETEC (*LTh*, *STh*/*STp*), EIEC (*invE*), STEC (*stx*1/*stx*2), EPEC (*eaeA*, *bfpA*, EAF), and EAEC (*aggR*, *astA*) by multiplex PCR assays. None of the 11 *E. coli* isolates were positive for the recognized virulence genes. However, 1 of 17 potential diarrheagenic *E. coli* iso-

Table 5
O-serogroups of *E. coli* isolates in food samples.

Types of food	No. of isolates					
	O1	O6	O8	O114	O157	O159
Spicy quick-fried squid (Pud Pet Pramouk)	1					
Marinated raw fish (Mook Pra Sew)	1					
Vegetable sour curry (Gang Som)		1				
Quick fried tripe (Pud Krungnai)			1			
Fermented pork sausage (Nam Mue)				1		
Fermented pork sausage					1	
Sweet coconut pasties (Kanom Morgang)					1	
Fermented vegetables (Puck Dong)					1	
Quick-fried basil leaf with chicken (Krapao Kai)					1	
Shrimp chili paste mixed rice (Koa Klug Kapi)					1	
Unripe-papaya coleslaw (Som Tum)						1

Table 6
Virulence gene markers of diarrheagenic *E. coli* in each serotype isolated from food.

Serotype	No.	PCR detection of virulence gene markers								
		*eae*A	*bfp*A	EAF	*agg*R	*ast A*	*LTh*	*STh*	*Inv*E	*stx*
O1	2	-	-	-	-	-	-	-	-	-
O114	1	-	-	-	-	-	-	-	-	-
O6	1	-	-	-	-	-	-	-	-	-
O8	1	-	-	-	-	-	-	-	-	-
O159	1	-	-	-	-	-	-	-	-	-
O157	5	-	-	-	-	-	-	-	-	-
Untypable[a]	1	-	-	-	-	+	-	-	-	-

[a]17 isolates of untypable *E. coli* which showed very weak agglutination against diarrheagenic *E. coli* polyvalent antisera.

lates that was weakly positive for polyvalent O-antisera, possessed the *astA* gene (Table 6).

Antimicrobial susceptibility profile

All 140 *E. coli* food isolates were tested for resistance to 10 antimicrobial agents. Resistance was observed most commonly to tetracycline (43%), co-trimoxazole (36%), ampicillin (26%) and chloramphenicol (23%) (Fig 2). Sixty-nine (49%) of the *E. coli* isolates were sensitive to all the antimicrobial agents, and approximately 50% were resistant to at least one antimicrobial agent. Thirty-six percent of *E. coli* isolates were resistant to three or more antimicrobial agents (Table 7).

The resistance profiles for the various *E. coli* isolates from both food and humans were compared (Fig 2). The percentages of isolates from humans resistant to ampicillin, tetracycline and co-trimoxazole were 76, 70 and 69%, respectively. The majority of *E. coli* isolated from food were resistant to the same antimicrobial agents as the *E. coli* isolated from humans.

DISCUSSION

MPN for *E. coli* has been used extensively as an indicator of food quality. The results of MPN for *E. coli* found in this study indicate that more than 50% of ready to eat foods surveyed in Khon Kaen Municipality were below public health standards. This result suggests that these foods may

Table 7
Combinations of antimicrobial resistance of *E. coli* isolated from foods.

No. of combinations of antimicrobial resistance	No. of resistance isolates (%)
0	69 (49.3)
1	11 (7.9)
2	10 (7.1)
3	24 (17.1)
4	20 (14.1)
> 4	6 (4.3)

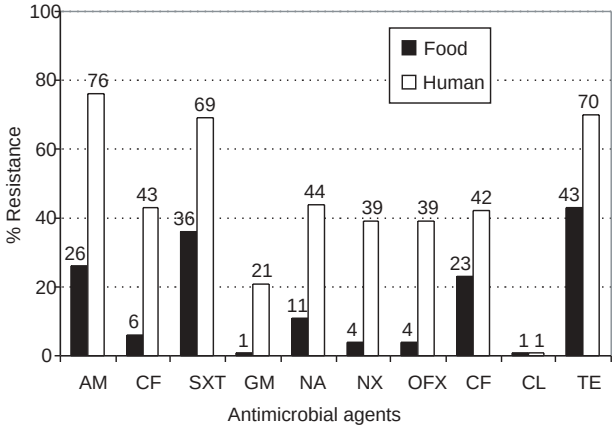

Fig 2–Antimicrobial resistance patterns of 140 *E. coli* isolated from food and humans.

Abbreviations: AM, ampicillin; CF, cephalothin; SXT, co-trimoxazole; GM, gentamicin; NA, nalidixic acid; NX, norfloxacin; OFX, ofloxacin; C, chloramphenicol; CL, colistin; TE, tetracycline. The number on each bar represents the percentage of resistance.

be a source for food-borne disease.

For pathogenic *E. coli*, several virulence gene markers have been identified. EPEC, a common cause of watery diarrhea in children especially in developing countries, generally posseses the *eaeA* gene, encoding for intimin, which mediates actin aggregation (Ohno *et al*, 1997; Baldwin, 1998; Murray, 2002). EIEC causing an illness like *Shigella* dysentery, is identified by the presence of the *invE* gene, which is involved in an invasive mechanism (Ohno *et al*, 1997). ETEC, the commonest cause of traveler's diarrhea, especially in tropical, developing countries, is identified by the presence of a heat-stable enterotoxin (*STh*) and/or a labile enterotoxin (*LTh*). *LTh* is closely related to the toxin produced by strains of *Vibrio cholerae* (Ayulo *et al*, 1994; Ohno *et al*, 1997; Rosa *et al*, 1998; Murray, 2002). EHEC or Shiga toxin-producing *E. coli* (STEC), causes a range of symptoms from mild, watery diarrhea, to severe diarrhea with bloody stools and hemolytic-uremic syndrome (HUS) in children. It is identified on the basis of having Shiga like toxins (SLT) and the attaching and effacing (AE) gene. *E. coli* O157 is the most common serogroup found in human STEC infection (Rios, 1999; Chinen, 2001). EAEC, which is associated with persistent childhood diarrhea, has been isolated from travelers and AIDS patients with diarrhea (Debroy *et al*, 1994). EAEC are characterized by distinctive aggregative adherence to Hep-L cells and Hela cells (Vial *et al*, 1988). The genes associated with EAEC are *aggR* and *astA* gene. The *aggR* gene encodes for bundle forming fimbriae and the *astA* gene encodes for EAEC heat-stable enterotoxin 1 (EAST1) (Kotler *et al*, 1995; Nishikawa *et al*, 2002; Osek, 2003). EAST1 was originally detected in EAEC strains, but has subsequently been found in ETEC, EHEC, and EPEC (Huang *et al*, 2004).

Even though several serotypes of pathogenic *E. coli* were found in food, virulence genes were not detected. Our results highlight a disagreement between the genotype and phenotype. This indicates that the serotyping method originally used for identifying pathogenic *E. coli*, such as EPEC, ETEC and EHEC, is not sufficient. The detection of pathogenic genes is necessary and more important than using the serotype method. Our results agree with other researchers who have reported that the possession of specific O-antigens did not necessarily correspond with the pathogenic characteristics (Barlow *et al*, 1999). In our study, 1 of 17 potential diarrheagenic *E. coli* isolates showed weakly positive agglutination against the polyvalent O-antisera for serogrouping, and possessed the *astA* gene. It is possible that the number of EAEC detected were less than the actual numbers,

because serotyping antisera was used, which did not cover EAEC and the diarrheagenic strains. Thus, diagnosing EAEC infection by serotyping can not identify the EAEC phenotype (Law and Chart, 1998). We found 5 strains of *E. coli* O157, which showed typical colonies on CHROM agar and SMAC. These strains agglutinated with antiserum for *E. coli* O157 with the latex test kit, but they did not react with H7 antiserum. In addition, *stx* genes were not found in these 5 strains. The biochemical characteristics of these *E. coli* O157 bacteria were different from those of *E. coli* O157:H7. They produced β-glucoronidase and were sorbitol-fermenters. Thus, these 5 isolates of *E. coli* O157 might not be virulent strains.

Although several serotypes of pathogenic *E. coli* were detected, the actual number of serotypes might be higher because the antisera used to identify the serotypes did not cover all the pathogenic types of *E. coli*, of which there are nearly 200 serotypes (Tamura *et al*, 1996; Barlow *et al*, 1999).

Of the 140 *E. coli* strains isolated from food samples, approximately half displayed resistance to one or more antimicrobial agents (tetracycline, co-trimoxazole, ampicillin, and chloramphenicol) indicating that some *E. coli* isolated from food were multi-drug resistant. This finding agrees with several previous studies of antimicrobial resistant diarrheagenic *E. coli* found in Thailand, suggesting improper control of these drugs fostered the emergence of antimicrobial resistant strains of *E. coli* (Paveenkittiporn *et al*, 1994). The transfer of resistant *E. coli* from food to humans may occur from several sources, notably animals and vegetables. The use of inappropriate drugs in animals and their release into the ecosystem may affect antimicrobial resistance patterns in human. Therefore all organizations investigating antimicrobial susceptibilities should cooperate with each other.

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