*Original Article*

# Seroprevalence and molecular epidemiology of EAST1 gene-carrying *Escherichia coli* from diarrheal patients and raw meats

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**Abstract**
Introduction: Several *Escherichia coli* pathotypes have been reported in Thailand; however, information on enteroaggregative heat-stable enterotoxin 1 (EAST1)-carrying *E. coli* (EAST1-EC) is insufficient. Previous reports show that consumption of raw meats causes diarrheagenic *E. coli* infections. In this study, we investigated the seroprevalence and genetic relationship of EAST1-EC from clinical and raw meat samples.
Methodology: Diarrheal patients and raw meat samples were investigated for the presence of EAST1-EC by performing polymerase chain reaction (PCR) to detect *astA*. Serotyping, antimicrobial susceptibility tests, and PCR-based phylogenetic group assay were performed. Molecular epidemiology of *E. coli* strains from clinical and raw meat samples was determined using repetitive element-PCR typing, BOX-PCR, and ERIC2-PCR.
Results: Results showed that 11.2% (17/152) of clinical samples and 53.3% (16/30) of raw meat samples had EAST1-EC. In all, 24 and 36 EAST1-EC strains were successfully isolated from 17 clinical and 16 raw meat samples, respectively. These strains had *astA* but did not possess the indicative genes of other *E. coli* pathotypes and were therefore classified as EAST1-EC. Most of these strains were multidrug resistant and were classified into nine serogroups. Molecular genotyping showed identical DNA fingerprint among EAST1-EC serotype O15 strains from clinical and raw chicken samples, suggesting that they were derived from the same bacterial clone.
Conclusions: Our results indicated a high prevalence of multidrug-resistant EAST1-EC strains in clinical and environmental samples in Thailand belonging to nine serogroups. Moreover, the study highlighted the close association between infections caused by EAST1-EC serotype O15 and raw meat consumption.

**Key words:** *astA*; EAST1; diarrhea; raw meat; *Escherichia coli.*

## Introduction

*Escherichia coli* cause various diarrheal diseases worldwide [1]. An individual *E. coli* pathotype possesses virulence factor that cause diseases with different degrees of severity. One of the virulence genes implicated in the outbreaks of gastrointestinal illnesses is *astA* that encodes enteroaggregative heat-stable enterotoxin 1 (EAST1) [2-4]. *E. coli* expressing EAST1 is referred to as EAST1-EC. EAST1, a 38-amino acid peptide, was first reported in a prototype enteroaggregative *E. coli* (EAEC) strain 17-2 isolated from a Chilean child with diarrhea [5,6]. Recently, *astA* and its variants have been isolated from other *E. coli* pathotypes, including enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) [7,8]. Although ETEC produce EAST1, this toxin is genetically and immunologically distinct from heat-stable toxins (ST) produced by ETEC.

Outbreaks of EAST1-EC have been reported in both Western and Eastern hemispheres. A case-control study in Barcelona, Spain, reported that EAST1-EC was identified in 22 of 115 (19%) patients compared with 4 of 79 (5%) non-diarrheal controls [3]. Another study from Gifu prefecture, Japan, reported massive outbreaks of gastrointestinal illnesses caused by *E. coli* O untypeable (OUT): H10 in which 2,697 children in elementary and junior high schools developed severe diarrhea. Analysis of strains from these children showed an aggregative pattern of adherence to HEp-2 cells and presence of a 60-MDa plasmid and *astA* [2]. Moreover, these isolates lacked the indicative virulence genes of four standard diarrheagenic *E. coli* pathotypes. In 1999, a study reported an outbreak of *E. coli* O166 carrying *astA* [4]. These studies suggest the pathogenic potential of EAST1-EC.

However, inadequate information is available regarding the prevalence and characteristics of EAST1-

EC in clinical and environmental samples in Thailand. Moreover, no study has assessed the association between human EAST1-EC infections and raw meat consumption. In the present study, we showed for the first time that EAST1-EC serotype O15 isolated from diarrheal patients was associated with EAST1-EC serotype O15 from raw chicken. Virulence profiles, including antimicrobial susceptibility patterns of EAST1-EC from clinical and environmental samples, were also determined.

## Methodology

### Isolation and identification of E. coli strains

Clinical samples obtained from rectal swab samples collected from Hat-Yai and Pattani hospitals were screened for the presence of *astA*. Bacterial isolation was performed between August 2013 and June 2014. An individual colony from each patient was selected and stored at -80°C for further investigation. The study protocols were approved by the ethical committee of the Faculty of Medicine, Prince of Songkla University, Thailand (EC code 56-225-19-2-3).

Raw meat samples were investigated for the presence of EAST1-EC. Three common types of raw meats, *i.e.*, pork, beef, and chicken, were collected from fresh markets across Hat-Yai city, Songkhla, Thailand, from June 2014 to February 2015. Briefly, 25 g of the meat sample was homogenized in 225 mL tryptic soy broth (TSB) (Beckton Dickinson, Sparks, USA) for 1 minute using a Stomacher 400 (Seward, West Sussex, UK). The liquid portion was statically incubated at 37°C for 6 hours. Subsequently, the bacterial culture was diluted tenfold, and 1 loopful of bacteria was streaked on eosin methylene blue (EMB) agar (Beckton Dickinson, Sparks, USA) to obtain *E. coli* candidates. Next, 7–10 colonies/samples with green-metallic sheen were selected for confirming the *E. coli* strain and for further analysis.

### Detection of EAST1-EC by polymerase chain reaction (PCR)

PCR template was prepared using a boiling method described previously [9]. Briefly, a single colony was inoculated in 3 mL TSB and was incubated at 37°C for 6 hours with aeration at 150 rpm. Subsequently, 1 mL bacterial culture was harvested and washed with 0.1 M phosphate saline buffer (pH 7.4) before boiling for 10 minutes, after which it was immediately immersed in ice for 5 minutes. The boiled bacterial suspension was centrifuged at $11,000 \times g$ for 10 minutes. The boiled supernatant was diluted tenfold using sterile deionized water and was used as the PCR template.

The gene encoding EAST1 (*astA*) was amplified using primers EAST11a and EAST11b, as described by Yamamoto and Echeverria [10]. *E. coli* were identified by performing PCR targeting *uidA* [11]. PCR for *astA* was performed in a 25 μL reaction consisting of 0.4 μM of each primer pair, 0.1 mM of dNTPs, 1X Go*Taq* DNA polymerase buffer, 0.5 unit of Go*Taq* Flexi DNA polymerase (Promega, Madison, USA), and 2 μL of DNA template. PCR conditions were as follows: initial denaturation at 95°C for 3 minutes; 35 cycles of denaturation at 94°C for 50 seconds, annealing at 50°C for 50 seconds, and extension at 72°C for 10 seconds; and final extension at 72°C for 5 minutes. The amplified products were electrophoresed on 1.5% agarose gel and visualized by ethidium bromide staining in a WSE-5200 Printpraph 2M gel imaging system (ATTO Corporation, Tokyo, Japan).

### Detection of diarrheagenic E. coli by PCR

To determine whether the isolated EAST1-EC belonged to one of the six *E. coli* pathotypes, the presence of indicative genes of each *E. coli* pathotype (*est/elt* for ETEC [12], *aggR* for EAEC [13], *ipaH* for EIEC [14], *bfpA* [15] and *eae* [16] for EPEC, *stx* [17] and *eae* for EHEC, and *daaE* [18] for diffusely adherent *E. coli*) was examined using PCR. PCRs were performed using specific primers, and the amplicons were electrophoresed on agarose gel as described above.

### Serotype determination

O-antigen serotyping was performed using agglutination assays with the available 8 polyvalent and 43 monolavent *E. coli*-specific antisera (Denka Seiken, Tokyo, Japan), as previously described [8]. Briefly, a single colony of each bacterial isolate was grown in 5 mL TSB (Beckton Dickinson, Sparks, USA) at 37°C for 6 hours with aeration. Next, the bacterial cells were harvested, resuspended in physiological saline (Loba, Mumbai, India) (pH 7.4), and heated to 121°C for 15 minutes. Antigenic suspension was obtained by centrifuging the cell suspension at $900 \times g$ for 20 minutes and by resuspending the bacterial cell debris in 500 μL physiological saline (pH 7.4). Agglutination pattern was observed on a glass slide by mixing the antigenic suspension with specific antisera.

### Phylogenetic group analysis

The phylogenetic origin of the EAST1-EC strains was determined by the method of Clermont *et al.* [19] based on a uniplex PCR carried out with primers for the genes *chuA*, *yjaA*, and TspE4.C2 fragment.

Amplification was performed using uniplex PCR with specific oligonucleotide primers for each gene. PCRs were performed in a T100 Thermal Cycler (Bio-Rad, Hercules, USA) using the following conditions: initial denaturation at 95°C for 3 minutes; 35 cycles of denaturation at 94°C for 50 seconds, annealing at 54°C for 50 seconds, and extension at 72°C for 30 seconds; and final extension at 72°C for 5 minutes. The amplified products were electrophoresed on 1% agarose gel, as described above.

*Antimicrobial susceptibility test*

Antimicrobial susceptibility of EAST1-EC was determined using the disk diffusion method [20] with the following 10 antimicrobial agents: cephalothin (30 μg), ciprofloxacin (5 μg), amikacin (30 μg), streptomycin (10 μg), gentamicin (10 μg), chloramphenicol (30 μg), tetracycline (30 μg), imipenem (10 μg), cotrimoxazole (25 μg), and ceftriaxone (30 μg). All the antimicrobial agents were obtained from Oxoid (Hampshire, UK).

*Hemolysis assay*

EAST1-EC were cultured on blood agar (Merck, Darmstadt, Germany) to monitor their erythrocyte destruction ability. Briefly, one colony of each strain was grown in 5 mL TSB, inoculated on blood agar containing human O-antigen, and incubated at 37°C for 24 hours to monitor the type of hemolysis around the bacterial colony.

*DNA fingerprinting*

DNA fingerprinting of EAST1-EC serotype O15 was performed using enterobacterial repetitive intergenic consensus (ERIC) 2-PCR and BOX-PCR using ERIC2 and BOX-A1R primers, respectively [21]. PCR was performed in a 25-μL reaction mixture containing 0.2 μM of each primer, 0.2 mM dNTPs, 1× GoTaq DNA polymerase buffer, 3.0 mM MgCl$_2$, 1.25 U GoTaq DNA polymerase, and 50 ng of DNA template (prepared using glass fiber matrix spin column; Geneaid, Taiwan). PCR was performed in a T100 Thermal Cycler (Bio-Rad, Hercules, USA) using

**Table 1.** Characteristics of 24 EAST1-EC isolates from 152 diarrheal patients of Hat-Yai and Pattani Hospitals during August 2013 to June 2014.

| Sample no. | Strain | Hospital | Date of isolation | Sex/age (years) | O-serotype | Hemolysis | Phylogenetic group | Antibiogram |
|---|---|---|---|---|---|---|---|---|
| 1 | PSU240 | HY | 22 Aug 2013 | M/2 | ONT | γ | A | SA |
| 2 | PSU241 | HY | 27 Aug 2013 | F/9 | O86a | γ | B1 | KF, TE |
|  | PSU242 | HY | 27 Aug 2013 | F/9 | O86a | γ | B1 | TE |
| 3 | PSU243 | HY | 29 Aug 2013 | M/97 | ONT | γ | B1 | SXT |
| 4 | PSU244 | HY | 29 Aug 2013 | F/1 | ONT | γ | D | CRO, KF, S, SXT, TE |
|  | PSU245 | HY | 29 Aug 2013 | F/1 | ONT | γ | D | CRO, KF, S, SXT, TE |
| 5 | PSU246 | HY | 31 Aug 2013 | M/38 | ONT | γ | D | C, CIP, CN, KF, S, SXT, TE |
|  | PSU247 | HY | 31 Aug 2013 | M/38 | ONT | γ | D | C, CIP, CN, KF, S, SXT, TE |
|  | PSU248 | HY | 31 Aug 2013 | M/38 | ONT | γ | D | C, CIP, CN, KF, S, SXT, TE |
| 6 | PSU249 | HY | 31 Aug 2013 | M/19 | O15 | γ | D | SA |
| 7 | PSU258 | HY | 25 Sep 2013 | M/70 | O8 | γ | B1 | C, CIP, S, SXT |
|  | PSU259 | HY | 25 Sep 2013 | M/70 | O8 | γ | B1 | C, CIP, S, SXT |
| 8 | PSU265 | HY | 13 Jun 2014 | F/89 | O15 | γ | A | C, CIP, CN, S, SXT, TE |
|  | PSU266 | HY | 13 Jun 2014 | F/89 | O15 | γ | B1 | C, CIP, CN, S, SXT, TE |
| 9 | PSU279 | HY | 31 Aug 2013 | F/20 | ONT | γ | D | CRO, KF, S, SXT |
| 10 | PSU282 | HY | 22 Sep 2013 | F/3 | ONT | γ | D | CRO, KF, S, SXT, TE |
| 11 | PSU284 | PT | 12 Oct 2013 | ND | ONT | γ | B1 | CIP, CN, CRO, KF, S, SXT, TE |
| 12 | PSU287 | HY | 10 Jun 2014 | M/4 | ONT | γ | D | CRO, KF, S, SXT, TE |
|  | PSU288 | HY | 10 Jun 2014 | M/4 | ONT | γ | D | C, CIP, CRO, IPM, KF, S, SXT, TE |
| 13 | PSU289 | HY | 10 Jun 2014 | F/5 | O15 | γ | B1 | S, SXT, TE |
| 14 | PSU290 | HY | 11 Jun 2014 | F/4 | O18 | γ | D | C, CRO, KF, TE |
| 15 | PSU291 | HY | 11 Jun 2014 | F/71 | ONT | γ | B1 | CRO, KF, S, SXT, TE |
| 16 | PSU292 | HY | 13 Jun 2014 | M/50 | ONT | γ | A | C, S, TE |
| 17 | PSU293 | HY | 13 Jun 2014 | F/89 | O15 | γ | B1 | C, CIP, CN, S, SXT, TE |

HY: Hat-Yai Hospital; PT: Pattani Hospital; C: chloramphenicol; CIP: ciprofloxacin; CN: gentamicin; CRO: ceftriaxone; KF: cephalothin; IPM: imipenem; S: streptomycin; SXT: co-trimoxazole; TE: tetracycline; SA: susceptible to all antimicrobial agents; ONT: O-non-typeable; ND: no data

the following conditions: initial denaturation at 95°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 3 seconds and 92°C for 30 seconds, annealing at 50°C for 1 minute, and extension at 65°C for 8 minutes. The amplified products were electrophoresed on 1.5% agarose gel at 100 V for 2 hours. The images were captured as described previously. A dendrogram was constructed using unweighted pair-group method of arithmetic average (Vilber Lourmat, Torey, France).

### Statistical analysis

Data were computerized using SPSS for Windows version 11.0 (IBM, Armonk, USA). A one-way ANOVA was employed to analyze the presence of EAST1-EC among raw meat samples. Significance was set at p < 0.05.

## Results
### Detection of EAST1-EC by PCR

In total, 379 isolates from 152 rectal swab samples (335 isolates from 108 samples obtained from Hat-Yai Hospital and 44 isolates from 44 samples obtained from Pattani Hospital) were screened for *astA*. Of the 152 samples, 11.2% (17) had EAST1-EC (Table 1). In addition, EAST1-EC was detected in 53.3% (16 of 30) of raw meat samples (Table 2). Of the raw meat samples analyzed, EAST1-EC was detected in 66.7% (10 of 15) of samples from chicken, 57.1% (4 of 7) of samples

**Table 2.** Characteristics of 36 EAST1-EC isolates from 30 raw meat samples during June 2014 to February 2015.

| Sample no. | Strain | Origin | Date of isolation | O-serotype | Hemolysis | Phylogenetic group | Antibiogram |
|---|---|---|---|---|---|---|---|
| 1 | PSU250 | Chicken | 16 Jul 2014 | ONT | $\gamma$ | B2 | CIP, S, KF, CN |
| | PSU251 | Chicken | 16 Jul 2014 | ONT | $\gamma$ | B1 | TE, S, C |
| 2 | PSU252 | Chicken | 16 Jul 2014 | ONT | $\gamma$ | B1 | TE, CIP, S, KF, SXT |
| | PSU253 | Chicken | 16 Jul 2014 | O15 | $\gamma$ | B1 | TE, S |
| | PSU254 | Chicken | 16 Jul 2014 | ONT | $\gamma$ | B1 | TE, S, C, KF, CN |
| 3 | PSU295 | Chicken | 30 Jun 2014 | O153 | $\gamma$ | B2 | CIP, KF |
| | PSU296 | Chicken | 30 Jun 2014 | O153 | $\gamma$ | B2 | CIP, KF |
| | PSU297 | Chicken | 30 Jun 2014 | O153 | $\gamma$ | B1 | CIP, S |
| | PSU298 | Chicken | 30 Jun 2014 | O15 | $\gamma$ | B1 | TE, CIP, S, C, SXT |
| 4 | PSU299 | Chicken | 30 Jun 2014 | ONT | $\gamma$ | B1 | TE, S, KF |
| | PSU300 | Chicken | 30 Jun 2014 | ONT | $\gamma$ | B1 | TE, S |
| 5 | PSU302 | Chicken | 16 Jul 2014 | ONT | $\gamma$ | B1 | TE, S |
| | PSU303 | Chicken | 16 Jul 2014 | ONT | $\gamma$ | B1 | KF, SXT |
| 6 | PSU304 | Chicken | 16 Jul 2014 | ONT | $\gamma$ | B1 | TE, C, KF, SXT |
| 7 | PSU305 | Chicken | 9 Jul 2014 | ONT | $\gamma$ | B1 | TE, CIP, S, KF |
| | PSU306 | Chicken | 9 Jul 2014 | ONT | $\gamma$ | B1 | TE, CIP, S, KF |
| 8 | PSU307 | Chicken | 9 Jul 2014 | ONT | $\gamma$ | B1 | TE, CIP, S |
| | PSU308 | Chicken | 9 Jul 2014 | ONT | $\gamma$ | B1 | TE, CIP, S |
| 9 | PSU309 | Chicken | 9 Jul 2014 | ONT | $\gamma$ | B1 | TE, CIP, S, KF, CN |
| | PSU310 | Chicken | 9 Jul 2014 | ONT | $\gamma$ | B1 | CIP, S, KF, CN |
| | PSU311 | Chicken | 9 Jul 2014 | ONT | $\gamma$ | B1 | CIP, S, KF, CN |
| | PSU312 | Chicken | 9 Jul 2014 | ONT | $\gamma$ | B1 | TE, IPM, S, SXT |
| | PSU313 | Chicken | 9 Jul 2014 | ONT | $\gamma$ | B1 | TE, S, SXT |
| | PSU314 | Chicken | 9 Jul 2014 | O152 | $\gamma$ | B1 | CIP, S, KF, CN |
| | PSU315 | Chicken | 9 Jul 2014 | O152 | $\gamma$ | B1 | CIP, S, KF, CN |
| 10 | PSU316 | Chicken | 9 Jul 2014 | O142 | $\gamma$ | B1 | TE, CIP, S, KF, CN |
| | PSU317 | Chicken | 9 Jul 2014 | ONT | $\gamma$ | B1 | CIP, KF |
| 11 | PSU318 | Beef | 18 Jan 2015 | ONT | $\gamma$ | B1 | IPM |
| | PSU319 | Beef | 18 Jan 2015 | ONT | $\gamma$ | B1 | SA |
| 12 | PSU320 | Beef | 18 Jan 2015 | O29 | $\gamma$ | B1 | S, KF |
| 13 | PSU321 | Pork | 28 Jan 2015 | ONT | $\gamma$ | B1 | S, KF |
| | PSU322 | Pork | 28 Jan 2015 | ONT | $\gamma$ | B1 | S, KF |
| | PSU323 | Pork | 28 Jan 2015 | O146 | $\gamma$ | B1 | C, SXT |
| 14 | PSU324 | Pork | 28 Jan 2015 | ONT | $\gamma$ | B1 | TE, S, C, SXT |
| 15 | PSU325 | Pork | 4 Feb 2015 | ONT | $\gamma$ | B1 | TE, S, KF, CN |
| 16 | PSU326 | Pork | 4 Feb 2015 | ONT | $\gamma$ | B1 | TE, S, KF |

C: chloramphenicol; CIP: ciprofloxacin; CN: gentamicin; KF: cephalothin; IPM: imipenem; S: streptomycin; SXT: co-trimoxazole; TE: tetracycline; SA: susceptible to all antimicrobial agents; ONT: O-non-typeable

from pork, and 25.0% (2 of 8) of samples from beef. The presence of EAST1-EC in these meat types was found to be comparable (p > 0.05). All EAST1-EC strains analyzed in this study did not carry the indicative genes of other diarrheagenic *E. coli* pathotypes and displayed gamma hemolysis.

*Serotype determination*

EAST1-EC strains isolated in this study were classified into 9 serogroups. EAST1-EC strains isolated from clinical samples were classified into 4 serogroups, namely O8 (2 strains), O15 (5 strains), O18 (1 strain), and O86a (2 strains). A total of 15 EAST1-EC strains could not react with any of the available antisera and were therefore classified in the non-typeable (ONT) group (Table 1). EAST1-EC strains isolated from raw meat samples were classified into 6 serogroups, namely O15 (2 strains), O29 (1 strain), O142 (1 strain), O146 (1 strain), O152 (2 strains), and O153 (3 strains). In addition, 26 strains were classified in the ONT group (Table 2).

*Phylogenetic group analysis*

The phylogenetic group of EAST1-EC from clinical samples was more diverged than that of EAST1-EC from raw meat samples. Of the strains isolated from clinical samples, 3 (12.5%) belonged to phylogenetic group A, 10 (41.67%) belonged to group B1, and 11 (45.83%) belonged to phylogenetic group D. None of

**Figure 1.** Antimicrobial susceptibility pattern of EAST1-EC strains from patients and raw meats during August 2013 and February 2015.
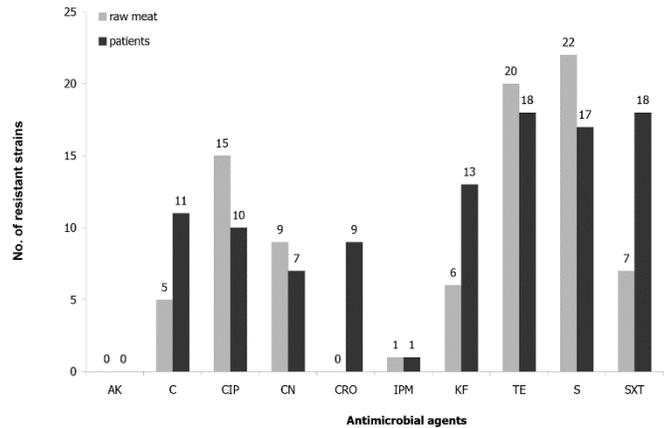


AK: amikacin; C: chloramphenicol; CIP: ciprofloxacin; CN: gentamicin; CRO: ceftriaxone; IPM: imipenem; KF: cephalothin; TE: tetracycline; S: streptomycin; SXT: co-trimoxazole

the isolated strains belonged to group B2. Of the strains isolated from raw meat samples, 33 (90%) belonged to group B1, and 3 (10%) belonged to group B2 (Tables 1 and 2). Based on data of virulent extra-intestinal strains, which belong mainly to groups B2 and D [19], phylogenetic results indicated that most EAST1-EC in the present study were in a virulent group.

*Antimicrobial susceptibility test*

**Figure 2.** ERIC2-PCR and BOX-PCR based-dendrogram of EAST1-EC O15 among clinical and raw meat samples.
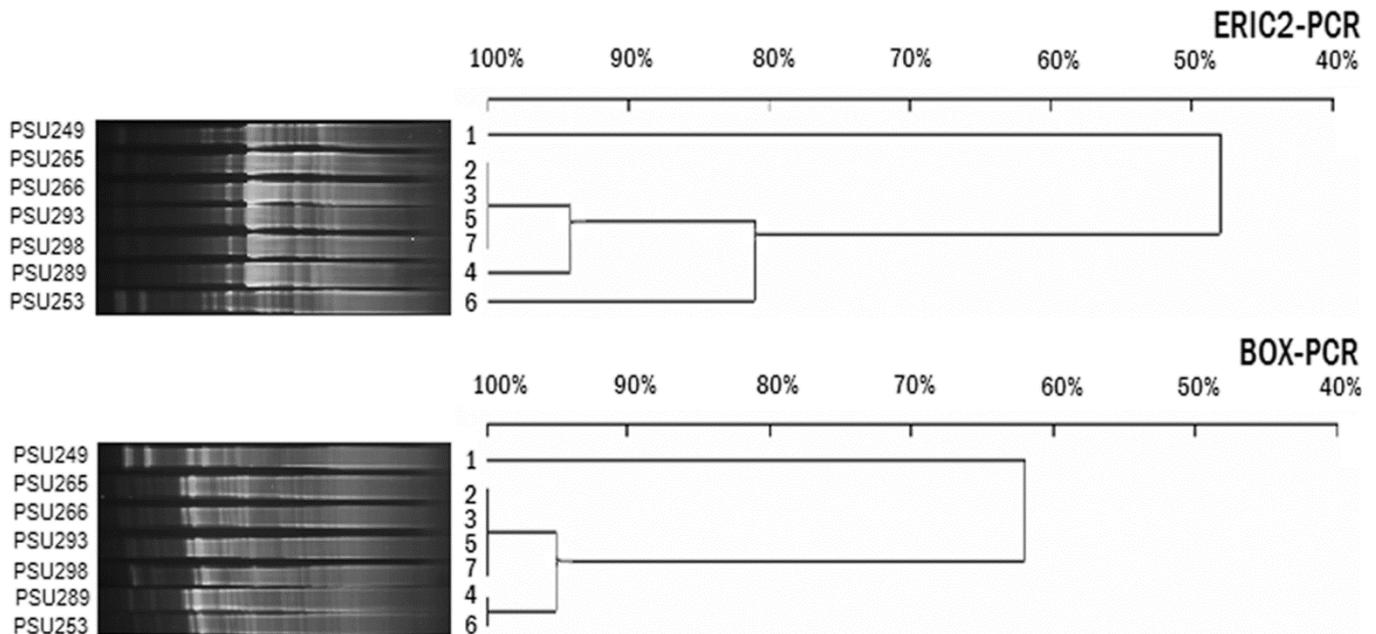


Clinical samples: PSU249, PSU265, PSU266, PSU289, and PSU293; raw meat samples: PSU253 and PSU298.

EAST1-EC resistance to antibiotics of strains from clinical sources was 75% (18/24) against tetracycline and cotrimoxazole, and 70.8% (17/24) against streptomycin (Figure 1). For EAST1-EC strains isolated from raw meat samples, resistance to antibiotics was 61.1% (22/36) against streptomycin, and 55.6% (20/36) against tetracycline (Figure 1). These findings suggest that tetracycline, streptomycin, and cotrimoxazole are not antibiotics to treat EAST1-EC infection in this area. In addition, all the strains isolated from raw meat samples were susceptible to ceftriaxone and amikacin.

*DNA fingerprinting*

In this study, EAST1-EC serotype O15 strains were isolated from both the sample sources (clinical samples: five strains, namely PSU249, PSU265, PSU266, PSU289, and PSU293; raw meat samples: two strains, PSU253 and PSU298). To determine whether these strains shared a genetic relationship, two specific molecular typing PCRs, ERIC2-PCR and BOX-PCR, were performed. Both BOX-PCR and ERIC2-PCR showed that the DNA fingerprint of EAST1-EC serotype O15 strains PSU265, PSU266, and PSU293 isolated from diarrheal patients was identical to that of EAST1-EC serotype O15 strain PSU298 isolated from raw chicken sample (Figure 2). In addition, BOX-PCR showed that the DNA fingerprint pattern of EAST1-EC strain PSU289 from a 5 year-old child was identical to that of EAST1-EC serotype O15 strain PSU253 isolated from the raw chicken sample (Figure 2).

## Discussion

The gene encoding EAST1 is widely distributed among diarrheagenic *E. coli* [22,23]. These *E. coli* strains have been implicated in many outbreaks. Nishikawa *et al.* [4] described a gastroenteritis outbreak caused by *E. coli* serotype O166 in Osaka prefecture, Japan in 1996. In this outbreak, 54 of 91 persons became ill after eating lunch served in their office. *E. coli* O166 strains isolated during this outbreak did not have any identifiable pathogenic properties and could not be assigned to any diarrheagenic pathotype, suggesting the pathogenic potential of EAST1-EC against humans. In the present study, sporadic infections by the nine serogroups of EAST1-EC were reported in individuals of different ages (Table 1). With respect to seroprevalence, our results were consistent with those obtained by Fujihara *et al.* [24], who showed that EAST1-EC serotypes O8, O15, O18, and O86a isolated from humans were the most abundant. However, the prevalence of EAST1-EC was higher in the present study (12% compared to 4.8% in the study by Fujihara *et al.* [24]).

Raw meats are potential vehicles for diarrheagenic *E. coli* [17,25,26]. In Thailand, the prevalence of EAST1-EC in raw meats has been rarely reported. In addition, there is a scarcity of data on EAST1-EC seroprevalence. The present study showed the seroprevalence of EAST1-EC in raw meats marketed in Thailand, and the level of EAST1-EC contamination reported in this study (53.3%) was consistent with that previously reported [27-29]. Choi *et al.* [27] screened *astA* in 476 *E. coli* isolates from weaned pigs with diarrhea and/or neurological symptoms across Korea between 1996 and 2000 and found that 149 (31.3%) isolates had this gene. A study in Iran also showed a comparable prevalence of EAST1-EC in raw chickens (33.3%) [28]. These data indicate the importance of raw meats, which are able to transfer EAST1-EC strains to human. Raw meats should be handled carefully to decrease the exposition of the population to the pathogen. This is crucial from the perspective of public health.

In bacterial typing, interspersed repetitive-element PCR (rep-PCR) is extensively used because repetitive elements are highly conserved in bacterial genomes and because the PCR is easy to perform and generates reproducible fingerprints. Of the BOX subunits boxA (57 bp), boxB (43 bp), and boxC (50 bp), only boxA is highly conserved among different bacterial species [30]. In the present study, double confirmation by BOX-PCR and ERIC2-PCR, which are reliable molecular source tracking tools, showed an identical fingerprint pattern among EAST1-EC strains isolated from clinical and raw meat samples, suggesting that they originated from the same bacterial clone (Figure 2). Moreover, these strains were isolated during the same time period, confirming their role in human infections (Tables 1 and 2). Based on our results, we propose that consumption of EAST1-EC-contaminated raw meats may result in human infection. Oral or intravenous rehydration in severe cases should be rapidly administered at the onset of diarrhea to prevent morbidity and mortality, especially in children.

To the best of our knowledge, this is the first study to report infections caused by EAST1-EC serotype O15 due to the consumption of raw chicken meat in Thailand. Our results indicate that proper hygienic care should be taken to manage and store raw meat because of the increased risk of contamination by EAST1-EC.

## Conclusions

Our results show that EAST1-EC plays an important role in human infections in Thailand. Reports on EAST1-EC infections in Thailand are rare because most studies focus on other important pathotypes of diarrheagenic *E. coli*. Raw meat samples are highly contaminated with EAST1-EC strains. Although 40 EAST1-EC strains from all samples could not be typed, seroprevalence analysis of 20 EAST1-EC strains from clinical and raw meat samples showed that these strains belonged to nine serogroups, with serotype O15 being present in both sample types. Analysis of genetic relatedness of EAST1-EC serotype O15 from both sample types by rep-PCR showed that these strains were derived from the same clone, suggesting raw meat-associated human infection.

## Author's contributions

KS was a principal investigator and performed data entry and analysis. SM participated in part of the clinical samples investigation. PS designed and performed the experiments, and extensively revised the manuscript.

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