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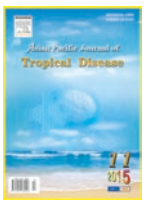

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Potential routes of transmission of an emerging hospital strain: Enterotoxigenic *Escherichia coli* O169:HUT from Southern Thailand

Kannika Sukkua[1], Pattamarat Rattanachuay[2], Pharanai Sukhumungoon[1,3*]

[1]*Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkhla, Thailand*

[2]*Department of Pre-Clinic, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus, Pattani, Thailand*

[3]*Food Safety and Health Research Unit, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkhla, Thailand*

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ABSTRACT

Objective: To assess the potential routes of transmission of clinical enterotoxigenic *Escherichia coli* (ETEC) from a patient in Southern Thailand.

Methods: The fate of ETEC PSU192 was monitored in four potential ETEC vehicles (ice, acidic food, pasteurization temperature and natural water) in different time intervals. Bacterial survival was determined by surface plate count on MacConkey agar.

Results: We clearly showed that ice, acidic food and natural water were able to behave as the potential vehicles transmitting the clinical ETEC PSU192 to humans. While ETEC PSU192 was deactivated under pasteurization temperature, suggesting that pasteurized milk could not act as a vehicle for ETEC transmission.

Conclusions: This is one of rare studies assessing the route of transmission of clinical ETEC through food and natural water in Thailand. Data clearly emphasized the potential routes of transmission of ETEC in this area. These findings will be of benefit for more appropriate prevention of massive losses by ETEC infection in Southern Thailand.

1. Introduction

An updated report of global child mortality shows that diarrhea undertakes 751 000 deaths (9.9%) out of 7.6 million children (aged 1–59 months) in 2010[1]. Diarrhea caused by enterotoxigenic *Escherichia coli* (ETEC) is as a public health concern worldwide since hundreds of thousands of fatal cases are reported annually[2]. Illnesses are triggered mainly by two enterotoxins, a 18–19 amino acids heat-stable enterotoxin and the 84 kDa oligomeric heat-labile enterotoxin protein, leading to the production of diarrheal stools caused by an imbalance of the bowel absorptive capacity and resulting in a severe form of diarrhea contributing to the mortality especially in children[3]. Outbreaks and infections caused by ETEC have been reported worldwide, but they are seldom reported in Thailand[4]. Recently, the emerging infection caused by ETEC serotype O169:H Untypeable (HUT) is reported from Hat-Yai Hospital, Southern Thailand[5]. No data about the natural behavior of this clinical ETEC in water and foods are known. This study aimed to investigate the possible potential routes of transmission of clinical ETEC in Southern Thai area, which is important to the public health.

2. Materials and methods

2.1. Bacterial strains and culture preparation

ETEC O169:HUT strain PSU192, a multi-drug resistant strain isolated from a diarrheal patient, Hat-Yai Hospital in 2014, was determined for its fate in several circumstances to assess its importance to the public health. This isolate contains *est* gene encoding heat-stable enterotoxin and *astA* gene encoding

*Corresponding author: Pharanai Sukhumungoon, Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkhla, Thailand.
Tel: +66 74 288322
Fax: +66 74 446661
E-mail: pharanai82@gmail.com

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enteroaggregative heat-stable enterotoxin 1[5]. For bacterial culture preparation, an individual colony of ETEC PSU192 was inoculated into 3 mL of tryptic soy broth (Merck, USA) and incubated at 37 ˚C for 6 h with aeration. Bacteria were harvested by centrifugation and washed using phosphate buffer saline, pH 7.4. The concentration of bacteria was adjusted to 0.5 McFarland turbidity standards by densitometer (Biosan, Latvia). A working culture of bacteria was prepared to a concentration of 10^7 CFU/mL for using in all experiments.

2.2. Evaluation of ETEC routes of transmission

In order to obtain the data, we focused on some ETEC specific vehicles that have been reported by Centers for Disease Control and Prevention and broadly implicated with the people in this area[6]. Tolerations to chilled, acidic and pasteurized conditions were evaluated.

For the tolerance to chilled condition, a working culture was spiked into distilled water with final bacterial numbers of 10^5 CFU/mL and the water temperature was cooled down to become ice (0 ± 1) ˚C for 6 h. Bacterial survival was monitored every hour. Survival of ETEC PSU192 in acidic food was examined using green-papaya salad (pH 4.0) as a medium. A final concentration of ETEC PSU192 of 10^5 CFU/mL was assessed for their survival in green-papaya salad's sauce (pH 4.0) for 6 h. Tolerance to pasteurization temperature was assessed as recommended by Food and Drug Administration[7]. A final concentration of ETEC PSU192 of 10^5 CFU/mL was spiked into pasteurized milk (9.0% fat without sweetener) and their fates were monitored at 63 ˚C for 30 min. Survival of PSU192 in natural water was evaluated by adding a working culture into a sterile natural water with final concentration of 10^5 CFU/mL and incubated at 30 ˚C for one week. Bacterial survival was monitored every 24 h.

Survival bacteria in all experiments were assessed by surface plate count on MacConkey agar. The experiment was performed in triplicate.

2.3. Total organic carbon (TOC) and total nitrogen (TN) quantification

To support the role of organic matter as nutrient supplier in natural water, TOC and TN were determined by non-purgeable organic carbon method and total bound nitrogen, respectively [TOC analyzer, multi N/C 3100 machine (Analytik Jena AG, Germany)]. Water sample was filtered through sterile Whatman filter paper No. 3 before subjecting to the machine and the test was performed in triplicate. Tap water was used as a control.

2.4. Statistical analysis

The data were computerized using SPSS for Windows software, version 11.0. The statistical comparison in the number of ETEC in different time points in all experiments, was analyzed by ANOVA. All significant levels were set as $P < 0.05$.

3. Results

A total of 10^5 CFU/mL of ETEC PSU192 was applied to test their fate under specific conditions and it was found that it could well tolerate to chilled [ice, (0 ± 1) ˚C] and acidic condition (green-papaya salad, pH 4.0) for at least 6 h without the significant decrease of bacterial populations ($P < 0.05$) (Table 1). In addition, it could survive in natural water up to one week with slightly increase in bacterial numbers (Table 2). Further examination was performed using the non-purgeable organic carbon method and total bound nitrogen to reveal the TOC and TN, respectively and the results showed that the TOC and TN in natural water were much higher than tap water (control group) as 8 and 5 folds, respectively (Table 3). Thus, we suggested that organic matters, perhaps, in soil sediment in natural water sources indeed play a key role in bacterial propagation. Although this ETEC strain could survive in various stressed conditions, it could be destroyed by pasteurization temperature (Table 4).

Table 1
Survival of ETEC PSU192 under chilled, acidic and pasteurization conditions, and in natural water. LogCFU/mL. Mean ± SD.

Time (h)	No. of survival bacteria	
	Chilled condition	Acidic food
0	5.70 ± 0.07[a]	4.72 ± 0.05[a]
1	5.55 ± 0.09[a]	4.72 ± 0.01[a]
2	5.48 ± 0.12[a]	4.74 ± 0.01[a]
3	5.42 ± 0.09[a]	4.76 ± 0.01[a]
4	5.29 ± 0.08[a]	4.61 ± 0.02[a]
5	5.34 ± 0.33[a]	4.57 ± 0.02[a]
6	5.25 ± 0.41[a]	4.53 ± 0.05[a]

Different lowercase letters indicate significant difference in the same column ($P < 0.05$) analyzed by ANOVA. The experiment was performed in triplicate.

Table 2
Survival of ETEC PSU192 in natural water. LogCFU/mL. Mean ± SD.

Time (day)	Natural water
0	4.77 ± 0.01[a]
1	6.00 ± 0.75[a]
2	5.97 ± 0.56[a]
3	6.02 ± 0.74[a]
4	5.78 ± 0.71[a]
5	5.81 ± 0.90[a]
6	5.70 ± 0.85[a]
7	5.58 ± 0.96[a]

Different lowercase letters indicate significant difference ($P < 0.05$) analyzed by ANOVA. The experiment was performed in triplicate.

Table 3
Contents of TOC, TN in water. mg/L. Mean ± SD.

Water categories	TOC	TN
Natural water	17.13 ± 0.07	2.23 ± 0.07
Tap water	2.17 ± 0.03	0.49 ± 0.02

Table 4
Survival of ETEC PSU192 under pasteurization conditions. LogCFU/mL. Mean ± SD.

Time (min)	No. of survival bacteria
0	4.69 ± 0.07[a]
5	4.37 ± 0.08[a]
10	2.93 ± 0.06[a]
15	1.00 ± 0.24[b]
20	0.00 ± 0.00[c]
25	0.00 ± 0.00[c]
30	0.00 ± 0.00[c]

Different lowercase letters indicate significant difference ($P < 0.05$) analyzed by ANOVA. The experiment was performed in triplicate.

4. Discussion

ETEC is the most common diarrheagenic *Escherichia coli* pathotypes known to infect human, which lead to the vast economic loss and massive human death annually[2]. About 88% of diarrhea-associated deaths are attributed by one of the important causes, such as unsafe water[8]. In Thailand and some countries, water from natural sources is processed by alum and/or lime treatments followed by chlorination to prepare raw water supply[9]. Nonetheless, certain studies reported the existence of ETEC in surface raw water supplying for drinking[10,11]. Furthermore, in Bangladesh, 18–26 household water samples were shown to be positive for ETEC with at least one enterotoxin[12]. ETEC contamination in lakes and ponds was also shown in high numbers.

In the laboratory condition for milk pasteurization experiment, the use of 63 ˚C for 30 min instead of 72 ˚C for 15 seconds is more suitable. Based on the Grade A pasteurized milk ordinance, recommended by Food and Drug Administration, the temperature of 63 ˚C for 30 min is approved for pasteurization of raw milk in the condition of fat less than 10% and without sweeteners[7]. Therefore, we thought that the result in this study reflected the fact that ETEC O169:HUT strain PSU192 is naturally deactivated under pasteurization temperature and cannot harm people by this route.

At any rates, with these capabilities of ETEC strain to survive in various conditions, we suggest that ice, acidic food and raw water can be potential vehicles for clinical ETEC transmission. Release of information regarding the potential route of transmission of ETEC is essential for the people in Southern Thai area and is important for the public health issue, helping prevent ETEC infection in the future.

Conflict of interest statement

We declare that we have no conflict of interest.

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