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Molecular Characterization of Enterotoxigenic *Escherichia coli* in Foodborne Outbreak

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Diarrheagenic *Escherichia coli* (*E. coli*) is a main cause of diarrhea worldwide. This study reports the investigation on the occurrence of enterotoxigenic *E. coli* (ETEC) serotype O27:H7-associated foodborne gastrointestinal disease that occurred at two schools, one middle school and one high school, in Seoul, Korea in June 2015. The immediate government investigation in 1,216 students and 19 food handlers in these two schools revealed that 116 students, 32 students in the middle school and 84 students in the high school, and 2 food handlers, one from middle school and the other from high school, developed gastrointestinal illness symptoms including diarrhea. Following lab investigation identified 29 ETEC serotype O27:H7 strains, 27 from 116 students and 2 from 19 food handlers. Pattern of pulsed-field gel electrophoresis analysis of ETEC isolates suggested that ETEC serotype O27:H7 caused the diarrheal outbreak in June 2015 in Seoul, Korea was a specific clone. In addition, these ETEC serotype O27:H7 isolates were highly resistance to the several antibiotics. The results from the present study provide the evidence that ETEC serotype O27:H7 can be an important cause of domestic foodborne outbreak in Korea.

Key Words: Enterotoxigenic *E. coli*; Pulsed-field gel electrophoresis, Foodborne outbreak

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INTRODUCTION

Enterotoxigenic *Escherichia coli* (ETEC), one of the six recognized diarrheagenic *E. coli* (enterohemorrhagic; EHEC, enterotoxigenic; ETEC, enteropathogenic; EPEC, enteroaggregative; EAEC, enteroinvasive; EIEC, and diffuse-adherent *E. coli*), is the most common cause of diarrhea in children of the developing countries and in travelers to these areas (1, 2). Annually, ETEC is estimated to cause 200 million diarrheal episodes and approximately 380,000 deaths (3, 4). In addition, ETEC is the most common cause of acute travelers' diarrhea globally (5).

Among many enterotoxins of ETEC, heat-labile toxin (LT) encoded by the *eltA* and *eltB* genes and heat-stable toxin (ST) encoded by two different genes *estA* and *st1*, which produce STh (originally isolate), are the main enterotoxins associated with ETEC-associated diarrhea. Phenotypic detection of one or both toxins or the genes encoding these toxins in isolates of *E. coli* is used to diagnose the ETEC

association in foodborne diarrhea (6, 7).

The purpose of the present study is to investigate the epidemiological characteristics and determine the antibiotic susceptibilities of ETEC isolates from the outbreak of foodborne diarrhea in two schools, one middle and one high schools, in Seoul, Korea in June 2015.

MATERIALS AND METHODS

Subjects and specimen collection

On June 22th, 2015, Public Health Centers in two different districts in Seoul, Korea were notified of complaints of gastrointestinal illness among the students from two different schools, one middle school and one high school. The immediate investigation revealed that one school catering facility in Seoul provided meals for the students in these two schools on June 19th, 2015. Fecal samples were taken from the patients who had gastrointestinal disease symptoms and all the food handlers. In addition, 7 swabs from knife, cutting board, dish cloth, and etc. were collected from the kitchen, and 57 food samples served to the patients including kimchi, golden kiwifruit, barbecue bossam, and etc. were collected to explore the possible cause of foodborne pathogens.

Microbiological analysis of specimens

Stool specimens were collected during the investigation to monitor the pathogens, which are routinely isolated from the patients with foodborne diarrhea, including 10 bacterial pathogens (pathogenic *E. coli*, *Salmonella* spp., *Shigella* spp., *Vibrio parahaemolyticus*, *Campylobacter* spp., *Staphylococcus aureus*, *Clostridium perfringens*, *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Bacillus cereus*) and 5 viruses (rotavirus, norovirus, adenovirus, sapovirus, and astrovirus) as described previously (8, 9).

Identification of virulence genes and serotyping of *E. coli* isolates

To identify ETEC, stool specimens were plated onto MacConkey agar plate, and individual colonies or sweeps of confluent growth were tested by polymerase chain reaction (PCR) for the heat-labile (LT) and heat-stable (ST) enterotoxin genes using a PowerChek™ Diarrheal *E. coli* 4-plex Detection Kit (KogeneBiotech, Seoul, Korea). In addition, the LT- and/or ST-positive ETEC isolates were tested for O (somatic) and H (flagella) antigens using the standard agglutination methods as described previously (10).

Pulsed-field gel electrophoresis (PFGE) analysis

PFGE was performed in ETEC isolates using *Xba*I as a restriction endonuclease (Roche Applied Science, Mannheim, Germany) according to the PulseNet standardized protocol (11). The electrophoretic parameters were as follows; initial switch time, 2.2 s; final switch time, 54.2 s; sum time, 18 h; angle, 120°; gradient, 6.0 V/cm; temperature, 14℃; ramping factor, linear. Following the electrophoresis, gels were stained for 15~20 min in 250 ml of deionized water containing 25 μl of ethidium bromide (10 mg/ml) and destained by three washes of 20~30 min each using 500 ml of deionized water. PFGE banding pattern was analyzed using BioNumerics software (version 4.6; Applied Math, Sint-Martens-Latem, Belgium). Analysis of banding patterns was performed using the Dice coefficient with a 1.0% tolerance for the band migration distance. Clustering of the patterns was performed using the un-weighted pair-group method with arithmetic averages.

Antimicrobial susceptibility test

Antimicrobial susceptibility of ETEC isolates was determined with the VITEK 2 automated system using ASTN169 Card (bioMéieux, Durham, France) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The following antibiotics were tested: ampicillin (AM), amoxicillin/clavulanic acid (AMC), ampicillin/sulbactam (SAM), cephalothin (CF), cefotaxime (CTX), cefotetan (CTT), cefoxitin (FOX), cefazolin (CFZ), ceftriaxone (CRO), imipenem (IPM), chloramphenicol (CHL), gentamicin (GEN), amikacin (AMK), nalidixic acid (NAL), ciprofloxacin (CIP), tetracycline (TET), trimethoprim/sulfamethoxazole (TMP/SMX). *E. coli* strain ATCC 25922 (American Type Culture Collection, Manassas, VA, USA) was used as a reference strain for quality control.

RESULTS

Standard questionnaires administered by health agencies revealed that 116 of 1,216 students and 2 of 19 food handlers in two schools developed clinical symptoms between 19th and 22th, June. All identified patients experienced diarrhea, and the median age of the patient was 18 years (range from 14 to 89 years); 81 patients were male, and 37 patients were female.

Among the 10 bacterial and 5 viral pathogens tested, which are commonly involved in the foodborne diarrhea, 29 ETEC strains were isolated which were serotyped as serotype O27:H7. Two food handlers having gastrointestinal illness symptoms were ETEC positive, one from middle school and the other from high school.

PCR analysis for the LT and ST toxin genes identified that the 29 ETEC isolates represented 2 different enterotoxin profiles: ETEC-ST strains (16/29, 55.2%) or ETEC-LT/ST strains (13/29, 44.8%) (Table 1).

Several ETEC serotype O27:H7 isolates were resistant to many antimicrobial drugs. The highest resistance rate was found in nalidixic acid (100%; n=29), followed by ampicillin and trimethoprim/sulfamethoxazole (24.1%; n=7), gentamicin (20.7%; n=6), ampicillin/sulbactam (17.2%; n=4), amoxicillin/clavulanic acid (17.2%; n=4), cefalotin (17.2%; n=4), cefoxitin (6.9%; n=2), and tetracycline (3.4%; n=1). Five resistance profiles were noted among the isolates: AM-AMC-CF-GM-NA-SAM-SXT, n=5; AM-NA-SXT-TE, n=2; GM-NA-SXT, n=1; FOX-NA, n=2 and NA, n=20 (Table 2).

PFGE (*Xba*I) types of all isolates are presented in Fig. 1. Dendrogram analysis of the PFGE profiles showed that 29 ETEC isolates belonged to the different PFGE profiles including ETCX01.066, ETCX01.127, ETCX01.128, ETCX01.129, and ETCX01.130. The most predominated PFGE profile was ETCX01.066, in which 20 out of 29 isolates belonged. Five isolates belong to profile ETCX01.129, and 2 isolates were profile ETCX01.127. Each one isolate belonged to profile ETCX01.128 or ETCX01.130. Based on the PFGE analysis in 29 ETEC serotype O27:H7 isolates from the fecal samples of students and food handlers, the similarity of 29 ETEC serotype O27:H7 isolates were 65.46~80.57%.

DISCUSSION

ETEC is one of the most common etiologic agents associated with severe childhood diarrhea in Korea (12, 13). There have been several outbreaks of ETEC infection throughout the world (14~16). The study from the largest ETEC outbreak in the United States reported the ETEC serotype O6:H16 (LT/ST)-associated gastrointestinal disease, in which 3,300 people were affected by a disease due to the food prepared by the delicatessen that catered several events over the span of a few days (17). The further outbreaks were reported from a sushi restaurant in Nevada (18) and a buffet-style lunch in Illinois (16). However, this study is the first report of an acute gastroenteritis outbreak associated with ETEC serotype O27:H7 in Korea in middle and high school students, as far as we know.

Table 1. Profiles of enterotoxin of 29 ETEC isolates

Strain number	PFGE pattern	Virulence gene	Note*
SE-2015-01	ETCX01.127	ST, LT	SS**
SE-2015-02	ETCX01.127	ST, LT	SD
SE-2015-03	ETCX01.066	ST	SS
SE-2015-04	ETCX01.066	ST	SS
SE-2015-05	ETCX01.066	ST	SS
SE-2015-06	ETCX01.066	ST	SS
SE-2015-07	ETCX01.066	ST	SS
SE-2015-08	ETCX01.066	ST	SS
SE-2015-09	ETCX01.066	ST, LT	SD
SE-2015-10	ETCX01.066	ST	SD
SE-2015-11	ETCX01.066	ST, LT	SD
SE-2015-12	ETCX01.066	ST, LT	SD
SE-2015-13	ETCX01.066	ST	SD
SE-2015-14	ETCX01.066	ST	SD
SE-2015-15	ETCX01.066	ST, LT	SD
SE-2015-16	ETCX01.066	ST, LT	SD
SE-2015-17	ETCX01.066	ST	SD
SE-2015-18	ETCX01.066	ST, LT	SD
SE-2015-19	ETCX01.066	ST	SD
SE-2015-20	ETCX01.066	ST	SD
SE-2015-21	ETCX01.066	ST, LT	SD
SE-2015-22	ETCX01.066	ST	SD
SE-2015-23	ETCX01.129	ST	SD
SE-2015-24	ETCX01.129	ST, LT	SD
SE-2015-25	ETCX01.129	ST	SD
SE-2015-26	ETCX01.129	ST, LT	SD
SE-2015-27	ETCX01.129	ST	SD
SE-2015-28	ETCX01.128	ST, LT	SS
SE-2015-29	ETCX01.130	ST, LT	SD

*SS indicates the middle school investigated, and SD indicates the high school investigated.
**ETEC was isolated from the food hanler, a cooking employ

Foods can be contaminated by infected food handlers (18), by asymptomatic carriers (19), or when vegetable crops are irrigated with untreated water (20). In this study, inspection of the school catering facility that provided the implicated meal revealed no obvious source of contamination. A recent study, which investigated the sources of ETEC infection, reported that implicated food is not easy to be found as a source of infection, because ETEC is difficult to be isolated from the contaminated food (21).

Table 2. Antibiotic resistance phenotypes of ETEC serotype O27:H7 isolates

Resistant phenotype	No. of isolate
AM-AMC-CF-GM-NA-SAM-SXT	5
AM-NA-SXT-TE	1
GM-NA-SXT	1
FOX-NA	2
NA	20

Abbreviations: AM, Ampicillin; AMC, Amoxicillin/Clavulanic Acid; CF, Cefalotin; FOX, Cefoxitin; GM, Gentamicin; IPM, Imipenem; NA, Nalidixic Acid; SAM, Ampicillin/Sulbactam; SXT, Trimethoprim/Sulfamethoxazole; TE, Tetracycline

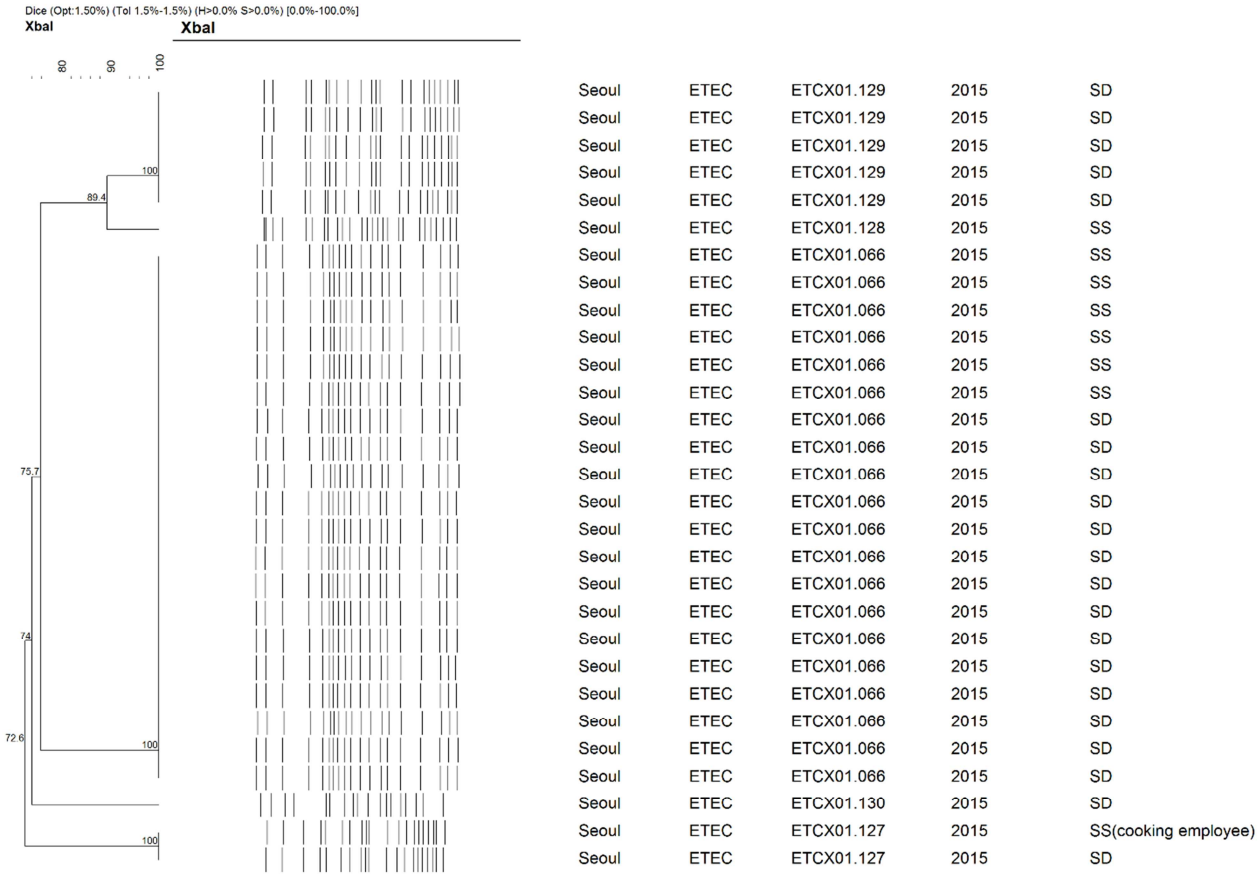

Figure 1. Dendrogram of *Xba*I PFGE profiles of ETEC serotype O27:H7 isolates. The dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean method. Degrees of similarity (% values) are shown. SS indicates the middle school investigated, and SD indicates the high school investigated.

Moreover, in most of the cases, food assumed to be the vehicle had been discarded by the patients during the period between ingestion and disease development (18). For example, in the outbreak of enterotoxigenic *Escherichia coli* associated with sushi restaurants, any food samples for enteric pathogens were not able to be tested, because all foods had been discarded by the start of the formal investigation (18). Although, there is no direct evidence, we speculated that ETEC serotype O27:H7 strain

responsible for current outbreak spread via a contaminated Suri rice cake because it was the only food commonly found in served table at two schools. Taken together, all of these findings highlight the need to collect food specimens from outbreaks as soon as possible.

Based on the results obtained from the analysis of toxin profile in current ETEC isolates, ETEC expressing ST alone was the most common (16/29, 55.2%), followed by the strains producing both ST and LT (13/29, 44.8%) in ETEC serotype O27:H7. This finding is consistent with the report from Japan, in which ST-producing *E. coli* O27:H7 is commonly found (22).

The resistances to common antibiotics, such as ampicillin, tetracycline and trimethoprim-sulfamethoxazole in ETEC have been reported worldwide (23~28). In our study, we also found that ETEC strain isolated from the foodborne diarrhea outbreak in Korea showed similar pattern of high antibiotic resistance, mainly to ampicillin (24.1%) and trimethoprim-sulfamethoxazole (SXT) (24.1%) with previous reports conducted in Nicaragua during March 2005 - September 2006 (29). However, unlike previous studies, we rarely found tetracycline resistance strain in our study.

Subtyping of outbreak associated isolates using PFGE revealed five similar but distinct *Xba*I PFGE patterns. Current ETEC serotype O27:H7 isolates were grouped in five distinct groups based on the PFGE analysis of *Xba*I restriction digest patterns. Among 29 ETEC serotype O27:H7 isolates, 20 showed the major PFGE pattern, ETCX01.066, that was often found in Seoul, Korea during 2012~2013. Interestingly, one isolate from the cooking employee in one middle school showed ETCX01.127 PFGE pattern, which was also found from one student in the other high school. This find suggest that one infection source which is commonly served in two schools may attribute to the diarrhea outbreak in two different schools. We also found ETCX01.129 and ETCX01.130 PFGE patterns in current ETEC serotype O27:H7 isolates, which are not reported in PulseNetKorea yet.

The objective of this study was to describe the epidemiology of ETEC outbreak in Korea. Although, there is no direct evidence, we speculated Suri rice cake, which was commonly served in two different schools affected by foodborne diarrhea, as the food vehicle responsible for ETEC transmission during this outbreak. Poor food-handling practices and infected food handlers are thought to be ongoing transmission vehicles of the pathogen. Although there are several limitations in this investigation, this report describes the first investigation of a foodborne outbreak of ETEC serotype O27:H7 infections in Seoul, Korea. This outbreak highlights the need of attention that ETEC should be considered as a cause of outbreak in non-endemic countries when common gastrointestinal pathogens are not found. Constant surveillance is also important for public health practitioners and clinicians to successfully monitor the emergence of ETEC as a domestic cause of gastroenteritis.

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