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Foodborne Outbreak Associated with *Escherichia coli*
O112ac:H19 in China

Yujuan Jin¹, Hui Yang¹, Yanwen Xiong², Ailan Zhao², Liping Gan¹, Qu Liu¹, Lin Lin¹,
Yingjian Chen^{1*}, Jingmei Li¹, Bin Li¹, Jiazhi Wu¹, and Wenjuan Lin¹

¹Longgang Center for Disease Control and Prevention in Shenzhen, Shenzhen; and

²State Key Laboratory for Infectious Disease Prevention and Control,
National Institute for Communicable Disease Control and Prevention,
Chinese Center for Disease Control and Prevention, Beijing, China

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The Longgang Center for Disease Control and Prevention in Shenzhen city received a report of a food poisoning outbreak among employees of the Xiang-Mu-Yuan furniture-manufacturing factory on March 16, 2013. Thirty-six of 170 employees who ate dinner at the cafeteria of the factory on March 14 were admitted to a local hospital owing to diarrhea and abdominal pain. The key signs and symptoms included diarrhea (36/36, 100%), rectal tenesmus (36/36, 100%), and abdominal pain (23/36, 63.9%). A few patients also complained of dizziness (8/36, 22.2%), headache (4/36, 11.1%), vomiting (2/36, 5.5%), and low-grade fever (1/36, 2.8%). Stools were soft but without blood. All 36 patients (32 males and 4 females; age range, 22–57 years) recovered after fluid and electrolyte replacement and were discharged 4–21 h after admission. Onset of symptoms reported by the diagnosed patients was from 7:00 on March 15 to 19:40 on March 16. Workers who ate lunch, but not dinner, on March 14 at the cafeteria did not show any symptoms. Thus, we inferred the cause for the food poisoning to be the dinner served at 18:00 on March 14, 2013. The median incubation period was 23 h (range, 13–37 h). Unfortunately, no food samples were available for analysis.

Fecal samples from 34 patients and 3 kitchen workers

as well as 7 environmental samples from the kitchen that included tap water, foods, and surface swabs of innate objects were examined. Standard microbiological techniques showed that all samples were negative for *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Vibrio parahaemolyticus*, *Listeria monocytogenes*, and *Campylobacter jejuni*. For *Norovirus* detection, total RNA was extracted from all samples using the Roche High Pure Viral RNA Kit (Roche, Mannheim, Germany) and subjected to real-time RT-PCR analysis (Shanghai ZJ Bio-Tech Co., Shanghai, China); however, all were negative. To isolate diarrheagenic *Escherichia coli*, all samples were plated on ECC (CHROMagar; CHROMagar Microbiology, Paris, France) and MacConkey (Oxoid, Hampshire, England) agars after overnight enrichment in EC broth (Land Bridge, Beijing, China). More than 5 typical colonies were picked from each plate and inoculated on triple sugar iron agar slants (Hangwei, Hangzhou, China). Suspected colonies were further identified using VITEK 2 compact (bioMérieux, Marcy l'Etoile, France). *E. coli* was isolated from 29 fecal samples, namely from 28 patients and 1 symptomless kitchen worker as well as 1 chopping board swab.

Serotyping of all 30 isolates was performed by slide agglutination using conventional *E. coli* antisera (Denka Seiken Co., Tokyo, Japan), and all were confirmed to be of serotype O112ac:H19. The antimicrobial susceptibility profiles were determined using the disk diffusion method as described by the Clinical and Laboratory Standards Institute (1). All isolates were susceptible to amikacin, amoxycillin/clavulanic acid, ampicillin/sul-

*Corresponding author: Mailing address: Department of Microbiology, Shenzhen Longgang Center for Disease Control and Prevention, No. 39 Hexie Road, Longgang Center Town, Shenzhen, Guangdong Province, 518172 P.R. China. Tel: +86-755-28945111, E-mail: sz645651@126.com

Fig. 1. PFGE profiles of all *E. coli* isolates digested with *Xba*I, using Bionumerics V4.0 (Applied Maths BVBA, Sint-Martens-Latem, Belgium) with percent similarity calculated using the Dice coefficient (tolerance 1.0%). All except isolate 13, which was obtained from a chopping board swab, were isolated from human feces. Isolate 17 was acquired from a healthy kitchen worker. Isolates 34 and 19 isolated from healthy persons were identified as *E. coli* but non-O112ac:H19.

bactam, cefepime, cefotaxime, ceftazidime, ceftizoxime, cefoxitin, chloramphenicol, ciprofloxacin, gentamicin, imipenem, kanamycin, meropenem, nalidixic acid, sulphamethoxazole/trimethoprim, tetracycline, trimethoprim, levofloxacin, and cephalothin; intermediately resistant to streptomycin; and resistant to ampicillin.

The genetic relatedness of the 30 *E. coli* O112ac:H19 isolates was evaluated by pulsed-field gel electrophoresis (PFGE) using *Xba*I according to standard methods (2). Results showed that all 30 *E. coli* O112ac:H19 isolates had identical PFGE banding patterns (Fig. 1).

Multilocus sequence typing (MLST) of all isolates was performed according to the method described in the website <<http://mlst.ucc.ie/mlst/dbs/Ecoli>> (3). The following 7 housekeeping genes were used for MLST: *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*. The sequences were analyzed using DNASTAR Lasergene 7.1 software and submitted to an international public database (3) for generating allelic profiles and assigning sequence types (STs). MLST identified an identical allelic profile or ST among all 30 *E. coli* O112ac:H19 isolates (ST 1972; 64-196-5-83-8-8-6). Thus, the epidemiological investigation and laboratory tests indicated that this food poisoning outbreak may be due to

Fig. 2. (Color online) HEp-2 cells assay. (A) *E. coli* O112ac:H19, (B) enteropathogenic *E. coli* strain E2348/69 (localized adherence), (C) enteroaggregative *E. coli* strain 042 (aggregative adherence), and (D) *E. coli* K12 strain HB101 (non-adherence).

E. coli O112ac:H19. Although the kitchen worker ate the same foods as the patients and had the isolate cultured from his stool sample, he experienced no clinical symptoms.

The kitchen was dirty and untidy. A single cloth was used to clean different surfaces. Cooked foods were often served with the same utensils that had been used to handle raw foods. This outbreak was therefore probably related to unhygienic food-handling techniques.

None of the 8 virulence genes were present in the *E. coli* O112ac:H19 isolate, including *stx*₁ (F: 5'-CAG TTAATGTGGTGGCGAAGG-3', R: 5'-CACCAGAC AATGTAACCGCTG-3'), *stx*₂ (F: 5'-ATCCTATT CCCGGGAGTTTACG-3', R: 5'-GCGTCATCGTAT ACACAGGAGC-3') (4), *lt* (F: 5'-GGCGACAGATT ATACCGTGC-3', R: 5'-CGGTCTCTATATCCCTG TT-3') (5), *st* (F: 5'-ATTTTMTTTTCTGTATTTC TT-3', R: 5'-CACCCGGTACARGCAGGATT-3') (5), *aggR* (F: 5'-GTATACACAAAAGAAGGAAGC-3', R: 5'-ACAGAATCGTCAGCATCAGC-3') (6), *eae* (F: 5'-TCAATGCAGTTCCGTTATCAGTT-3', R: 5'-GT AAAGTCCGTTACCCCAACCTG-3') (7), *ipaH* (F: 5'-CTCGGCACGTTTTAATAGTCTGG-3', R: 5'-GT GGAGAGCTGAAGTTTCTCTGC-3') (8), *daaD* (F: 5'-TGAACGGGAGTATAAGGAAGATG-3', R: 5'-GTCCGCCATCACATCAAAA-3') (9). The isolates were tested for 37 other virulence and adherence genes (*astA*, *paa*, *iha*, *saa*, *toxB*, *efal*, *lpfA*_{O157/O1-154}, *lpfA*_{O157/O1-141}, *lpfA*_{O113}, *pet*, *set1A*, *set1B*, *set2*, *cdt*, *pilS*, *aap*, *aatA*, *irp2*, *shf*, *papC*, *sfaD-sfaE*, *afaB-afaC*, *aerA*, *hly*, *cnf*, *aggB*, *afaD*, *tia*, *pic*, *aggA*, *agg3A*, *hadA*, *pCVD432*, *cnf1*, *cnf2*, *subA*, and *katP*). However, except *lpfA*_{O113}, all were absent. Therefore, the *E. coli* isolates could not be classified into the 6 known pathovars of diarrheagenic *E. coli* on the basis of the presence of virulence genes. The presence of other virulence and adherence genes in these *E. coli* isolates

remains unknown.

We tested the pathogenesis of *E. coli* O112ac:H19 using HEp-2 cells (10–12), which were seeded in 24-well tissue culture plates at a density of 5×10^4 cells per well 24 h before infection. The medium was replaced before infection with RPMI-1640 medium containing 1% mannose. A mixture with a multiplicity of infection (number of bacteria per number of mammalian cells) of 100:1 was added to the HEp-2 monolayers in 1 ml of culture medium. Bacteria were allowed to adhere for 3 h at 37°C in 5% CO₂. Enteroaggregative *E. coli* (EAEC) strain 042 was used for aggregative adherence, and enteropathogenic *E. coli* (EPEC) strain E2348/69 was used for localized adherence. *E. coli* strain HB101 was included as a non-adhering control. After incubation for 3 h, each well was rinsed 5 times with phosphate-buffered saline, the cells were fixed with pre-cooled methanol (–20°C) for 15 min, stained with 1% Giemsa stain (Solarbio, Beijing, China) for 30 min, and examined under a light transmission microscope at a magnification of $\times 1,000$ (oil immersion) (Nikon 80i; Nikon, Tokyo, Japan). Infections were repeated 3 times in duplicates. An invasion assay was performed as described above, except that the extracellular bacteria were removed by incubating the plate with gentamicin (100 µg/ml/well) for 1 h at 37°C in 5% CO₂. *Shigella flexneri* 2a strain 301 was used as a positive control for the invasion assay. Unlike EPEC strain E2348/69, which exhibited localized adherence, EAEC strain 042 displayed aggregative adherence. *S. flexneri* 2a strain 301 invaded the HEp-2 cells, and none of the *E. coli* O112ac:H19 isolates adhered to or invaded the HEp-2 cells. However, the isolates may have been toxic to the HEp-2 cells because compared with *E. coli* strain HB101, 68% of the HEp-2 cells displayed morphological changes (based on light transmission microscope observations), such as rounding, swelling, nuclear division, aging, and loss of viability (Fig. 2).

Diarrheagenic *E. coli* can be classified into 6 pathovars on the basis of virulence, type of clinical infection, and pathological features: EPEC, EAEC, enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enterohemorrhagic *E. coli*, and diffusely adherent *E. coli* (13,14). Other pathovars have been previously described but are not well studied. Cell-detaching *E. coli*, which secretes cytotoxic necrotizing factor 1 and a hemolysin, may be associated with diarrhea in children (13). Adherent invasive *E. coli* adheres to carcinoembryonic antigen-related cell adhesion molecule 6 in the ileum and has been implicated

in ileal Crohn's disease (15). However, further investigations are required to elucidate the pathogenesis of the isolate that caused the present foodborne outbreak.

Conflict of interest None to declare.

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