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

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isolates digested with cnf set1B saa pic efa1, tia lpfA E. coli gyrB E. coli (3). The (F: 5 on the basis iha isolates could not be classified into the 6 recA E. coli O112ac:H19. Although the kitchen worker ate aggA O112ac:H19 stx papC pCVD432 cnf1 E. coli aatA stx cdt afaD aggB irp2 (F: 5 afaB-afaC < purA isolates (F: 5 strain 042 cnf2 shf sfaD-sfaE subA (F: 5 lpfA hly aap agg3A (? (F: 5 and katP but non-O112ac:H19. paa O112ac:H19 isolates E. coli ? (F: 5 O112ac:H19 isolate, including K12 strain HB101 toxB fumc allelic profile or ST among all 30 sequence types (STs). MLST identified an identical database (3) for generating allelic profiles and assigning 7.1 software and submitted to an international public sequences were analyzed using DNASTAR Lasergene website performed according to the method described in 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 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 stx1 (F: 5’-CAG TTAATGTGGTGCGGGAAGG-3’, R: 5’-CAGCAGAC AATGTAACCAGCCTG-3’), stx2 (F: 5’-ATCTCTATT CCCCGGAGTTTACG-3’, R: 5’-GGCTCATGCTATACACAGGAGGC-3’) (4), lt (F: 5’-GGGGACAGATT ATACCGTCAG-3’, R: 5’-CGGTCTCTATATTTCCGT TT-3’) (5), st (F: 5’-ATTTTTTCGTTATTGCR R: 5’-CACCCCCGTACARGCAGGATT-3’) (5), aggR (F: 5’-GTATACACAAAAAGAAGGAAGGC-3’, R: 5’-ACAAGATCGTACATCAGC-3’) (6), eae (F: 5’-TCAATTGCACTTCCGGTTATCAGT-3’, R: 5’-GTAAAGTCCGTTACCCCAACCTG-3’) (7), ipaH (F: 5’-CTCGGAGACGTTTTATATGTCGG-3’, R: 5’-GGAAGACTGAATATGCGTTATT-3’) (8), daaD (F: 5’-TGAAACGGAGGTATAAGGAGATG-3’, R: 5’-GTCCGCCCATACATCACAATAA-3’-3’) (9). The isolates were tested for 37 other virulence and adherence genes (asaA, pap, iha, saa, toxB, efa1, lpfA0157/0158, lpfA0157/0158, pet, set1A, set1B, set2, cdt, pilS, aap, attA, irp2, ssh, papC, sfaD-sfaE, afaB-afaC, aeaA, hly, cnf, aggB, afaD, uia, pic, aggA, agg3A, hadA, pCVD432, cnf1, cnf2, subA, and katP). However, except lpfA0157/0158, 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 $5 \times 10^4$ 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% CO$_2$. Enteropathogenic _E. coli_ (EPEC) 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$1,000 (oil immersion) (Nikon 80i; Nikon, Tokyo, Japan). Infections were repeated 3 times in duplicates. An invasion assay was performed as described above, except that the extracellular bacteria were removed by incubating the plate with gentamicin (100 μg/ml/well) for 1 h at 37°C in 5% CO$_2$. _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.

**REFERENCES**


