



An Acute Gastroenteritis Outbreak Caused by Enteropathogenic Escherichia Coli (EPEC) in China

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Keywords: Enteropathogenic *Escherichia coli*; Foodborne disease; Outbreak; WGS.

Abstract

Background: Foodborne diseases or foodborne diarrhea are important reasons affecting the global population. Diarrheagenic *E. coli* is the most important pathogens, which causes diarrhea in humans. On May 27, 2022, Anji County Hospital of Chinese Medicine treated several patients with acute onset of gastroenteritis. It was found that this was an outbreak caused by two different EPECs.

Methods: According to the case definition, the local public health workers started the investigation. Stool samples collected from probable case patients and delicatessen owners were forwarded for *Salmonella* spp., *Shigella* spp., *V. parahaemolyticus*, and Diarrheagenic *E. coli* testing. Environment and food samples were tested for the same pathogens. All detections were carried out according to the "National foodborne disease surveillance manual for 2022" (China National Center for Food Safety Risk Assessment, 2022). The isolated strains were then subjected to PFGE analysis, antibiotic susceptibility tests, and Whole Genome Sequencing analysis.

Results: Six families had purchased and eaten the cold pork purchased from the delicatessen produced by the owner of the shop. 37 people had ate the cold pork, 20 of them were ill. All of them met the definition of possible cases. The incidence rate was 54.05% (20/37). 37 suspected cases, 20 probable cases and 6 laboratory-confirmed cases were determined in this outbreak. A total of 15 stool samples and 5 food samples were collected. Nine isolates (five cases, one food producer and three food samples) were confirmed as Enteropathogenic *E. coli*-positive. The results of PFGE were obviously clustered into two banding patterns, with an overall similarity of 58.92%. Four cases presented 100% similarity with the food producer. Another case and 3 cold pork formed a band pattern with a similarity of 100%. Two serotypes were observed among the nine isolates: Out: H4, O16:H4, and two STs (ST226, ST10) were found.

Conclusions: This study presented here a foodborne outbreak event caused by eating cold pork contaminated by EPEC. The detected pathogenic bacteria were not homologous after analysis.



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Introduction

Foodborne diarrhea is important reason affecting the global population [1-2]. Diarrheagenic *Escherichia coli* (Diarrheagenic *E. coli*) is one of the most common bacterial agents of foodborne diarrheal disease [3]. *E. coli* is a Gram-negative, facultative anaerobic and rod-shaped bacterium, which is a common symbiotic bacteria in mammalian large intestine [4]. However, some *E. coli* strains can cause serious infection [4].

Diarrheagenic *E. coli*, which causes humans diarrhea, can be divided into seven different pathotypes according to its specific virulence characteristics, distinct epidemiological and clinical features: Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Diffuse-Adhering *E. coli* (DAEC), Cytotolethal distending toxin-producing *E. coli*, Enteropathogenic *E. coli* (EPEC), and Enterohaemorrhagic *E. coli* (EHEC) [5].

Enteropathogenic *E. coli* (EPEC) is the uppermost pathogen infecting children in the worldwide, and can cause fatal diarrhea in infants in developing countries [6]. Previous studies demonstrated that in China, EPEC is one of the most common diarrheagenic *E. coli* [7-8] and were found in many food sources [9-10]. In this study, we investigated an outbreak of gastroenteritis caused by EPEC infection in China.

Materials and Methods

The outbreak

On May 27, 2022, Anji County Hospital of Chinese Medicine treated several patients with acute onset of gastroenteritis. The patients reported having eaten cold pork purchased from the same delicatessen before the onset of the illness. This suspected outbreak of foodborne diseases was subsequently reported to the market supervision department and the health administrative authority. An investigation was conducted by Anji CDC.

Case definition

A suspected case was defined as a person with one of the symptoms including abdominal pain, diarrhea or vomiting (diarrhea meant three or more episodes within 24 hours, or vomiting meant more than once within 24 hours) after having eaten cold pork purchased from the delicatessen since May 25, 2022. A probable case could be determined if two or more symptoms described above presented. A probable case could be defined as a laboratory-confirmed case, if Enteropathogenic *Escherichia coli* (EPEC) was detected in the vomit or diarrhea of the patient.

Epidemiologic investigation

According to the case definition, the local public health workers interviewed the delicatessen owners, physician-in-charge, and checked the outpatient and hospitalization records to determine the cases. At the same time, with the help of the foodborne disease monitoring and reporting system, the case search was carried out among people who had eaten the cold pork purchased from the delicatessen. Probable cases were further confirmed based on questionnaire investigation. Probable cases were analyzed by clinical symptoms and descriptive epidemiology. On May, 27, a detailed sanitary investigation of the delicatessen was carried out, and environment and food samples were obtained for laboratory testing.

Laboratory investigation

Stool samples collected from probable case patients and del-

icatessen owners were forwarded for *Salmonella* spp., *Shigella* spp., *V. parahaemolyticus*, and Diarrheagenic *E. coli* testing. Environment and food samples were tested for the same pathogens. All detections were carried out according to the "National foodborne disease surveillance manual for 2022" (China National Center for Food Safety Risk Assessment, 2022).

For Diarrheagenic *E. coli* testing, environment and food samples were enriched in Enterobacteriaceae Enrichment Broth (Hopebio, Qingdao, China) at 42°C up to 18h, followed by plating on Levine- eosin-methylene blue agar (Hopebio, Qingdao, China), and MacConkey agar (Hopebio, Qingdao, China), for incubation at 37°C up to 24 h. The Enrichment procedure could be omitted when stool samples were processed. Presumptive colonies were identified by using Diarrheagenic *Escherichia coli* detection kit (real-time PCR method) (ABT, Beijing, China).

Pulsed field gel electrophoresis

PFGE (Pulsed field gel electrophoresis) typing for *E. coli* isolates was carried out according to the recommended protocol [11]. *Xba* I was selected as the restriction enzyme, and the digestion fragments were subjected to PFGE in a 1% Seakem Gold Agarose gel (Lonza Company, Swiss) in 0.5% Tris-boric acid-ethylenediaminetetraacetic acid (EDTA) buffer using a CHEF Mapper XA system (Bio-Rad Laboratories, Richmond, CA, USA). The electrophoresis time was 19 h. The *Xba* I digested DNA from *Salmonella* strain H9812 was used as a standard. BioNumerics software v. 7.6 (Applied Maths, Kortrijk, Belgium) was used for Gel images analysis.

Antibiotic susceptibility tests

The antimicrobial resistance patterns of the 9 isolates were determined by the broth microdilution method, following the standards of the Clinical and Laboratory Standards Institute [12]. The following 16 antibiotics were tested using the Sensititre™ CHNENF (Thermo scientific, American. B1481A): Chloramphenicol (CHL); Colistin (CT); Ertapenem (ETP); Meropenem (MEM); Cefotaxime (CTX); Ceftazidime(CAZ); Ceftazidime/avibactam (CZA); Tetracycline (TET); Tigecycline (TIG); Ciprofloxacin (CIP); Nalidixic (NAL); Azithromycin (AZM); Amikacin (AMI); Streptomycin (STR); Ampicillin (AMP); and Ampicillin/sulbactam (AMS). The breakpoints for resistance, intermediate and susceptible, were referred to CLSI [12].

Whole genome sequencing analysis

The total DNA from each strain was extracted from overnight cultures using the bacterial genomic DNA extraction kit (TIANGEN biotech co., Beijing, China), according to the manufacturer's instructions. The DNA concentration was determined using the Qubit 4 (Thermo Fisher Scientific, Waltham, MA, USA) and Qualified DNA was stored in -80°C until use.

The WGS libraries were constructed with Metagenomic DNA library Kit (Cat No: MD001T-P1, Hangzhou Matridx Biotechnology Co.,Ltd) and then sequenced on the NextSeq 500/550 High Output Reagent Cartridge v2 300 cycles (Illumina, San Diego, CA 92122, USA). After sequencing, the low-quality reads were removed, and then all the original sequences belonging to *E. coli* were screened through BWA comparison; The genome was assembled with SPAdes v3.15.3 [13].

The serotype of each strain was determined using the genes deposited in the Center for Genomic Epidemiology (www.genomicepidemiology.org, accessed on 20 September 2022) for *E. coli* as part of their web-based serotyping tool (Serotype-

Finder 2.0, <https://cge.food.dtu.dk/services/SerotypeFinder/>), with a similarity of 85% and minimum length of 60%; the ST of each strain was analyzed using the MLST 2.0 *Escherichia coli*#1 approach provided by the Center for Genomic Epidemiology (<https://cge.food.dtu.dk/services/MLST/>, accessed on 20 September 2022). The virulence factor of each strain were determined using the genes deposited in the Center for Genomic Epidemiology for *E. coli* as part of their web-based VirulenceFinder 2.0 tool (<https://cge.food.dtu.dk/services/VirulenceFinder/>, accessed on 20 September 2022), with the similarity of 90% and minimum length of 60%.

Statistical analysis

Descriptive analysis was performed with SPSS 23.0 (SPSS, Inc.). The chi-square test or Fisher's test was used to compare qualitative variables. The case-control odds ratio (OR) and 95% confidence intervals (95% CIs) were calculated to measure the association between exposure and outcome. Statistical significance was defined as $p < 0.05$.

Ethics

This study was approved by the Ethics committee of Huzhou Center for Disease Control and Prevention. Informed consent for the stool samples was obtained from the patients or their guardians.

Results

Epidemiological investigation

The delicatessen is located in the Chengnan Community of Lingfeng Street, Anji County. It sells bulk cooked meat and cold dishes all the year round. The customers are basically residents living in the community. The bulk cooked meat and cold dishes in the shop are produced by the owner and his wife in the shop. The processing place is about 15 square meters. Only one sink, a processing table of about 2 square meters, and two freezers are set up. There are no windows in the processing place, and the indoor ambient temperature is high. There is no disinfection facility on site.

Six families had purchased and eaten the cold pork purchased from the delicatessen produced by the owner of the shop. 37 people had ate the cold pork, 20 of them were ill. All of them met the definition of possible cases. According to the statistics of possible cases, the incidence rate was 54.05% (20/37). 37 suspected cases, 20 probable cases and 6 laboratory-confirmed cases were determined in this outbreak. The 20 probable cases with the main symptoms of diarrhea and vomiting were from six families. All the families had no history of eating together. 20 cases all the cold pork purchased from the shop.

Table 1: Symptoms of 20 cases.

Symptom	Number of cases	Percentage%
vomiting	18	90
abdominal	5	10
diarrhea	18	90

The first case, a male patient, aged 40, began to have nausea (six times) and diarrhea (five times) at 20:00 on May 26, without fever. The final case occurred at 00:00 on May 27. The incidence peak presented at 20:30 on May 26, and the time between the first case and the last case was about 4h. According to the epidemiological curve, this outbreak was a homologous exposure event. The suspicious meal was the dinner on May 26 (at about

18 o'clock). The suspicious food is the cold pork purchased on May 26. The 20 cases were mainly concentrated in 2 to 4 hours after dinner, and the peak appeared within 2.5 to 3 hours, with the longest incubation period of 6 hours, the shortest incubation period of 2 hours, and the average incubation period of 3 hours (Figure 1). The specific distribution of onset time is shown in Figure 1. Among the 20 possible cases, the youngest patient was 4 years old and the oldest was 44 years old. There were 14 males and 6 females. In all probable cases, the main clinical symptoms were abdominal pain, diarrhea and vomiting. Fever and other symptoms were rare (Table 1). All the 20 patients received emergency treatment in local hospitals. After giving symptomatic support treatment, including rehydration and anti-diarrheal medication, they were all cured without any severe cases or deaths.

Laboratory results

A total of 15 stool samples were collected (including 13 cases, 1 for the owner of the shop and 1 for the owner's wife). Five food samples were collected (two cold pork left by sick households, one cold pork being sold in the shop, one other cold mixed vegetables, and one chili oil in the shop). Two kitchen environment samples were collected.

All the samples were all negative for *Salmonella* spp., *Shigella* spp., and *V. parahaemolyticus*. Nine isolates (five cases, one food producer and three cold pork samples) confirmed as Enteropathogenic *E. coli*-positive. Enteropathogenic *E. coli* isolates were further typed with PFGE (Figure 2). The results of PFGE were obviously clustered into two banding patterns, with an overall similarity of 58.92%. Four cases presented 100% similarity with the food producer. Another case and 3 cold pork formed a band pattern with a similarity of 100%. Strains from the three cases and the food producer had multiple drug resistance to NAL-AZM-AMP-AMS. Other strains had resistance to TET-NAL (Figure 2). All the isolates were resistant to NAL.

Regarding the 9 EPEC isolates, 2 serotypes were observed: Out: H4, O16:H4,, and 2 STs were found, including ST226, ST10. Serotype was highly correlated with ST (Table 2). The distribution of virulence genes was divided into two types. There were 12 virulence genes shared by 9 isolates. The strain from food source had 10 more other virulence genes, while the strain from food producer had 2 more other virulence genes.

Table 2 Serotype, ST, and virulence factor of the EPEC isolates in this study.

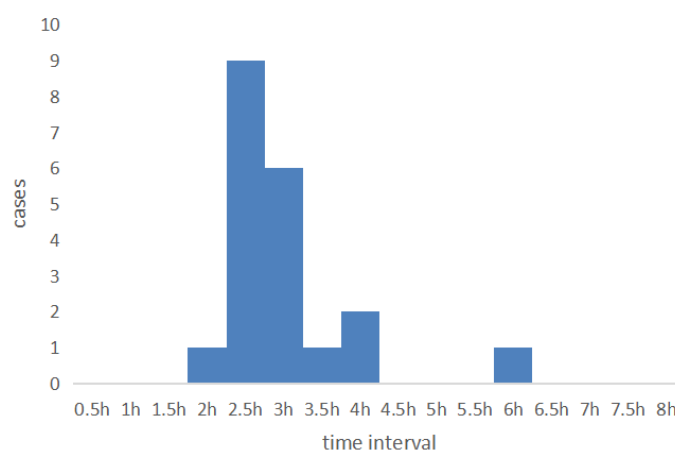


Figure 1: The onset time distribution of 20 probable cases.

using the data may contact the corresponding author

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