Outbreak Reports

# A Large Acute Gastroenteritis Outbreak Associated with Both *Campylobacter coli* and Human Sapovirus — Beijing Municipality, China, 2021

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**Summary**

**What is already known about this topic?**

*Campylobacter* is a significant foodborne pathogen that leads to global outbreaks of acute gastroenteritis (AGE) usually affecting less than 30 individuals. Human sapovirus (HuSaV) is an enteric virus responsible for sporadic cases and outbreaks of AGE worldwide. In a study conducted in Beijing, HuSaV detection ranked second after norovirus.

**What is added by this report?**

We present a discussion of the first large-scale outbreak of AGE caused by both *Campylobacter coli* (*C. coli*) and HuSaV. The outbreak involved a total of 996 patients and exhibited two distinct peaks over a period of 17 days. Through case-control studies, we identified exposure to raw water from a secondary water supply system as a significant risk factor. Among 83 patients, 49 samples tested positive for *C. coli*, 39 samples tested positive for HuSaV, and 27 samples tested positive for both pathogens using real-time polymerase chain reaction detection. Furthermore, whole-genome sequencing of 17 *C. coli* isolates obtained from 17 patients revealed that all isolates belonged to a highly clonal strain of *C. coli*.

**What are the implications for public health practice?**

Outbreaks of AGE resulting from multiple pathogen infections warrant increased attention. This report emphasizes the significance of ensuring the safety of drinking water, particularly in secondary supply systems.

Numerous acute gastroenteritis (AGE) outbreaks have been attributed to *Campylobacter* infection in recent years in China (*1*–*5*). The main species responsible for these outbreaks is *Campylobacter jejuni* (*3*–*4*) except one outbreak caused by *Campylobacter coli* (*C. coli*) in Shunyi reported in 2018 (*2*). Human sapovirus (HuSaV) is a pathogen that causes sporadic cases and outbreaks of acute gastroenteritis worldwide. An acute gastroenteritis outbreak involving 996 patients occurred in Beijing in July 2021. In this study, we present a comprehensive analysis of the epidemic and laboratory findings related to this outbreak.

## INVESTIGATION AND RESULTS

On July 15, 2021, a sentinel hospital in Beijing identified 13 patients with AGE in the same school. Epidemiological investigation revealed a total of 996 patients in this outbreak, including 958 students (221 male) and 38 staff members. The attack rate among students and staff members were 67.7% (958/1,413) and 21.7% (38/175), respectively. The most common clinical symptoms, ranked from highest to lowest, were abdominal pain (75.6%, 753/996), diarrhea (73.8%, 735/996), nausea (53.3%, 531/996), vomiting (23.0%, 229/996), and fever ≥37.3 ℃ (13.7%, 136/996).

A total of 828 valid survey questionnaires were accomplished (341 AGE cases and 487 controls). From the investigation, one direct drinking water (DDW) system supplied by a secondary water supply system (WSS-S) was identified in the school building. This DDW system offered two types of water: unboiled direct drinking water (UDDW) and boiled. The water source for WSS-S is a groundwater source well (WSW) located within the commercial campus and one neighbor hotel in the same campus also has its own secondary water supply system (WSS-H), which also utilizes the WSW as its water source. Both WSS-H and WSS-S have separate facilities including a water storage tank, water pump, and pipeline. The WSS-H conducted disinfection using chlorine dioxide and the water storage tank and pump were located inside of the hotel. The water quality from WSS-H was supervised

effectively. In contrast, the water storage tank and water pump of WSS-S are not located inside the school and lack effective management. Additionally, the sewage well is uncovered and lacks protective facilities during rainfall (Figure 1). Based on the results of the case-control studies, exposure to UDDW was found to increase the risk of illness [risk ratio=3.895, 95% confidence interval (*CI*): 2.90–5.22; Table 1].

The first identified patient on July 10, 2021 was designated as D0. Subsequent days were labeled as D1 to D17. Two peaks of patient onset were observed at D4–D5 and D11–D12. On D1–D2, after a rainstorm occurred, some students reported that the water in the school appeared turbid and had an unpleasant odor following the rainstorm. After the DDW was replaced with commercially bottled water, the number of patients decreased significantly. All patients were quarantined after the second peak, and the number of daily patients continued to decline until no new patients were reported after D17 (Figure 2). No cases were identified in the neighbor hotel during this period.

Stool or anal swabs were collected from a total of 83 patients and 5 canteen staff. In addition, 18 water samples, each 2L, 12 food samples, and 17 environmental smear specimens from the canteen were collected and stored in sterile containers. The laboratory investigation was conducted by local CDC.

Real-time polymerase chain reaction (PCR) was conducted using commercial kits to detect common enteric pathogens. Among the patient samples, the positive rates for *C. coli* and HuSaV GI, as determined by real-time PCR, were 59.04% (49/83) and 39.83% (39/83), respectively. The mixed positive rate for *C. coli* and HuSaV GI was 32.53% (27/83). Two out of 17 environmental smear samples, specifically those collected from school toilet pans, tested positive for *C. coli*. No other enteric pathogens were detected in any of the collected samples. The distribution of pathogen detection results in patient samples collected on various dates is provided in Table 2.

*Campylobacter* isolation and antibiotic resistance were performed using an isolation kit and agar dilution method as previously described (*2*). Seventeen *C. coli* isolates were obtained from 17 patients. The antibiotic resistance profile of all 17 strains showed co-resistance to nalidixic acid, ciprofloxacin, streptomycin, and tetracycline.

Aerobic plate counting and coliform detection were conducted on a total of 18 water samples. Five samples collected on D6 and 8 samples collected on D12–D14 from the WSS-S did not meet the criteria, with aerobic plate counts exceeding 100 CFU/mL and coliforms being detected. Water samples from WSS-H and WSW satisfied the criteria for both aerobic plate counting and coliform detection (Table 2).

The draft genomes of 17 *C. coli* isolates from patients were sequenced on the Illumina HiSeq 2500 platform at the Tianjin Genomics Institute. High-quality reads were assembled using the SPAdes genome assembler software (version v3.11.0) (https://github.com/ablab/spades). The average nucleotide identity (ANI) and in silico DNA-DNA hybridization (isDDH) values of these 17 genomes and one reference (CP047137), were analyzed using pyani v0.2 software (https://github.com/widdowquinn/pyani) employing the ANIm and GGDC modules (http://ggdc.dsmz.de/ggdc.ph) with Formula 2, respectively. Both ANI

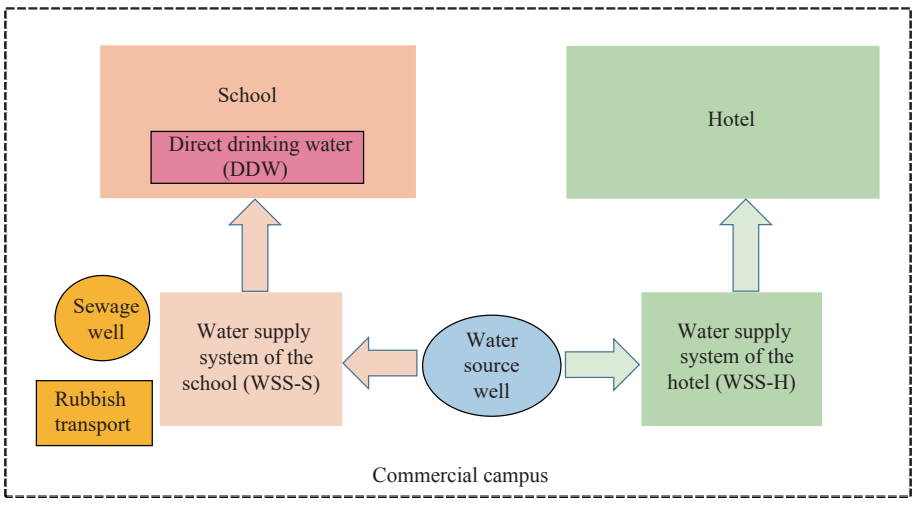



FIGURE 1. Schematic diagram of the commercial campus and water supply system.

TABLE 1. Case-control results for potentially suspicious risk factors.

| Exposure to uncooked direct drinking water | Case number | Control number | *RR* value | 95% *CI* |
|---|---|---|---|---|
| Yes | 210 | 142 | 3.895 | 2.906−5.220 |
| No | 131 | 345 | | |
| Total | 341 | 487 | − | − |

Note: "−" means not applicable.
Abbreviation: *RR*=risk ratio; *CI*=confidence interval.

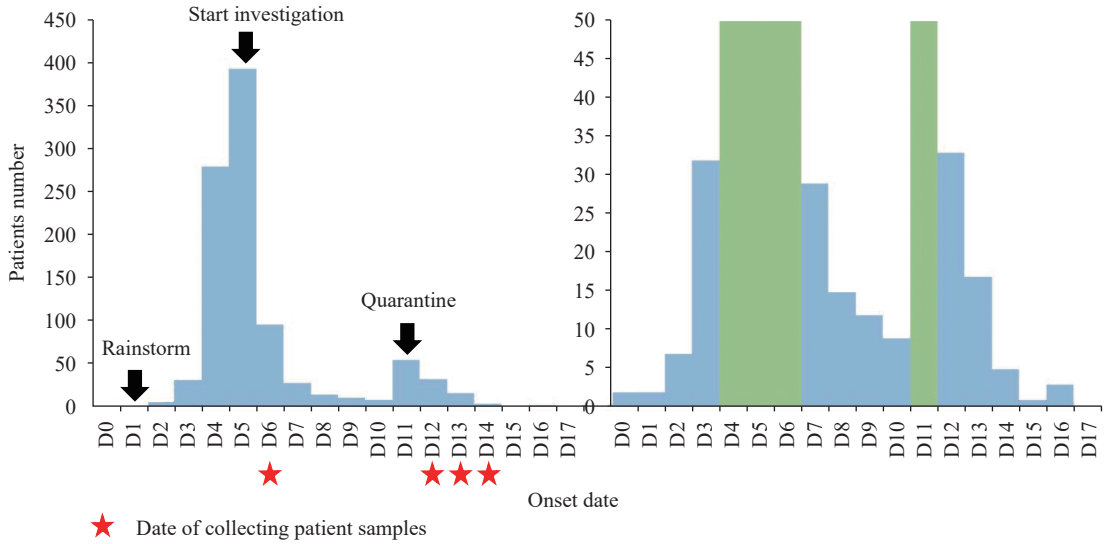



FIGURE 2. Time distribution of patient onset.
Note: The onset time for patients corresponding to the samples collected on day 6 (D6) was between day 3 and day 6 (D3D6), while the onset time for cases corresponding to the samples collected on day 12 to day 14 (D12D14) was between day 3 and day 12 (D3D12). The number of new patients which onset at D4, D5, D6 and D11 all exceed the maximum value of 50 on the Y-axis (green bar).

TABLE 2. Detection results in samples collected on different dates.

| Sample type | Sample collection date | Time interval for the first onset of clinical symptoms of patients | Sample name | Sample number | Real-time PCR positive rate for *C. coli* | *C. coli* culture positive rate | HuSaV GI positive number | *C. coli* and HuSaV GI positive number | Number of unqualified samples for aerobic plate counting | Number of unqualified samples for coliform detection |
|---|---|---|---|---|---|---|---|---|---|---|
| Patients | D6 | D3−D6 | Anal swab | 19 | 11 | 1 | 0 | 0 | − | − |
| | D12 | D3−D11 | Anal swab | 15 | 10 | 4 | 7 | 6 | − | − |
| | D13 | D4−D12 | Stool | 24 | 11 | 5 | 17 | 8 | − | − |
| | D14 | D4−D12 | Stool | 25 | 17 | 6 | 15 | 13 | − | − |
| Canteen workers | D6 | − | Anal swab | 5 | 0 | 0 | 0 | 0 | − | − |
| Retained food | D6 | − | Food | 12 | 0 | 0 | 0 | 0 | − | − |
| Environment smear | D6 | − | Smear | 17 | 2 | 0 | 0 | 0 | − | − |
| Water | D6 | − | WSW | 1 | − | − | − | − | 0 | 0 |
| | | − | WSS-H | 1 | − | − | − | − | 0 | 0 |
| | | − | WSS-S | 5 | − | − | − | − | 5 | 5 |
| | D12−D14 | − | WSS-H | 3 | − | − | − | − | 0 | 0 |
| | | − | WSS-S | 8 | − | − | − | − | 8 | 8 |

Note: "−" means not applicable.
Abbreviation: HuSaV=human sapovirus; WSW=water source well; WSS-H=secondary water supply system for hotel; WSS-S=secondary water supply system for school.

and isDDH values among the genomes exceeded 96% and 70%, respectively, which meet the taxonomic criteria for classification within the same species (Figure 3A–B and Table 3).

Multilocus sequence typing (MLST) for each isolate was determined using the Genome Comparator tool on the PubMLST website (http://pubmlst.org/campylobacter). All isolates were identified as sequence type (ST) 1068 and clonal complex (CC) 828. An *ad hoc* whole-genome MLST (wgMLST) analysis was performed using fast-GeP, with strain RM5611 from the PubMLST database as the reference. This analysis identified 1,697 gene loci in the reference strain and 1,595 core gene loci across all strains, assessing the sequence characteristics of each gene locus. A phylogenetic tree was constructed using SplitsTree 4 and the neighbor-net method (Figure 4) based on wgMLST analysis. Subsequently, core genome SNPs (cgSNPs) were identified by mapping sequencing reads to the draft genome of isolate RM5611 using Snippy version 4.3.5 (https://github.com/tseemann/snippy). A total of 6,203 cgSNPs were identified, using RM5611 as the reference genome. The cgSNP analysis (Figure 5A) shows high clonality among the 17 strains, with cgSNP differences between them presented in Figure 5B.

## DISCUSSION

Case-control studies showed that exposure to UDDW increased the risk of disease. All 17 *C. coli* isolated from the patients were highly clonal. Water samples from the WSW and treated WSS-H yielded satisfactory results for aerobic plate counting and coliform detection. Exposure to WSS-S was the primary risk factor for the outbreak. *C. coli* and HuSaV GI were finally identified as the major pathogens in this outbreak, and transmission primarily occurred via UDDW from the contaminated secondary water supply system. This is the first reported outbreak in China where a mixed infection of *C. coli* and HuSaV caused AGE.

Outbreaks caused by *C. coli* have been linked to contaminated surface water exposure (*6*), male sexual activity (*7*), and transmission through food (*2*). This outbreak occurred following a rainstorm, where the "rinsing water" from WSS-S appeared turbid and had an odor. Water samples collected from WSS-S on D6 and D12–D14 showed unqualified results for aerobic plate counting and coliform detection, which indicated the contamination. The nearby garbage station and sewage well were likely sources of contamination. Regrettably, during the initial stages of this outbreak, *Campylobacter* was not given attention. Even though 19 anal swab samples were collected on Day 6, *Campylobacter* detection was not performed until Day 11, potentially explaining why 11 out of the 19 samples tested positive for *Campylobacter* through real-time PCR, but only one sample was positive through bacterial isolation.

The *C. coli* strains isolated in this outbreak were ST1068. There have been no previous reports of ST1068 in China. However, sporadic reports of ST1068 have been observed in Japan, the USA, the UK, Italy, and Canada (*8–11*). In Japan, ST1068 is the second most dominant type of *C. coli* isolated in the clinic but no reports of outbreaks caused by ST1068 worldwide. ST1068 is mainly found in

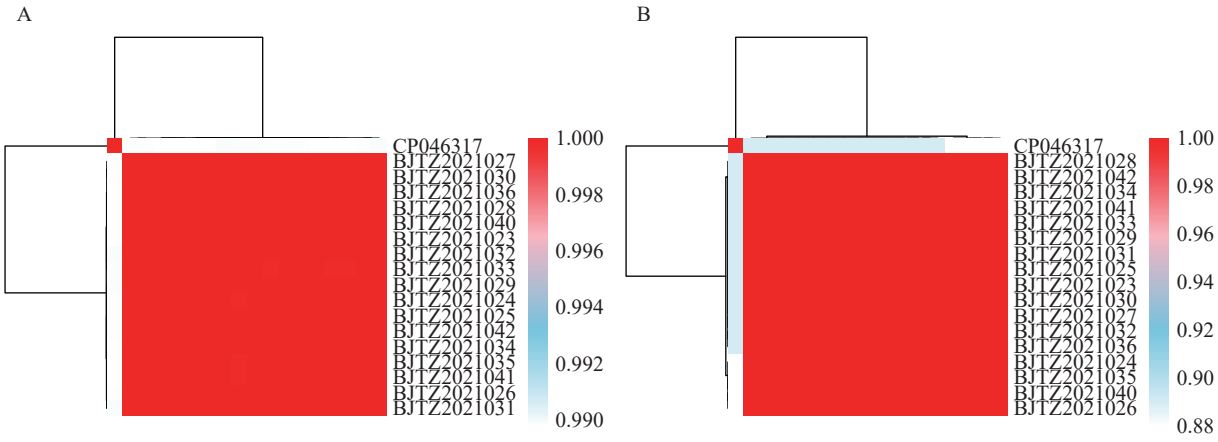



FIGURE 3. Heatmap of average nucleotide identity (A) and in silico DNA-DNA hybridization (B) values of 17 outbreak *C. coli* isolate genomes and reference genome CP047137.

TABLE 3. Average nucleotide identity and in silico DNA-DNA hybridization results for *C. coli* (%).

| | CP 046317 | BJTZ 2021032 | BJTZ 2021036 | BJTZ 2021040 | BJTZ 2021026 | BJTZ 2021027 | BJTZ 2021035 | BJTZ 2021030 | BJTZ 2021023 | BJTZ 2021025 | BJTZ 2021031 | BJTZ 2021029 | BJTZ 2021024 | BJTZ 2021033 | BJTZ 2021041 | BJTZ 2021034 | BJTZ 2021042 | BJTZ 2021028 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CP046317** | | 88.50 | 88.50 | 88.40 | 88.40 | 88.50 | 88.40 | 88.50 | 88.50 | 88.50 | 88.60 | 88.60 | 88.40 | 88.50 | 88.50 | 88.50 | 88.50 | 88.50 |
| **BJTZ2021032** | **98.99** | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **BJTZ2021036** | **98.98** | **100.00** | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **BJTZ2021040** | **98.98** | **00.00** | **00.00** | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **BJTZ2021026** | **98.99** | **99.99** | **99.99** | **99.99** | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **BJTZ2021027** | **98.99** | **100.00** | **100.00** | **100.00** | **99.99** | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **BJTZ2021035** | **98.98** | **99.99** | **99.99** | **99.99** | **100.00** | **99.99** | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **BJTZ2021030** | **98.99** | **100.00** | **100.00** | **100.00** | **99.99** | **100.00** | **99.99** | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **BJTZ2021023** | **98.98** | **100.00** | **100.00** | **100.00** | **99.99** | **100.00** | **99.99** | **100.00** | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **BJTZ2021025** | **98.99** | **99.99** | **99.99** | **99.99** | **100.00** | **99.99** | **100.00** | **99.99** | **99.99** | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **BJTZ2021031** | **99.00** | **99.99** | **99.99** | **99.99** | **100.00** | **99.99** | **100.00** | **99.99** | **99.99** | **100.00** | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **BJTZ2021029** | **98.99** | **99.99** | **99.99** | **99.99** | **100.00** | **99.99** | **100.00** | **99.99** | **99.99** | **100.00** | **100.00** | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **BJTZ2021024** | **98.99** | **99.99** | **99.99** | **99.99** | **100.00** | **99.99** | **100.00** | **99.99** | **99.99** | **100.00** | **100.00** | **100.00** | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **BJTZ2021033** | **98.99** | **100.00** | **100.00** | **100.00** | **99.99** | **100.00** | **99.99** | **100.00** | **100.00** | **99.99** | **99.99** | **99.99** | **99.99** | | 100.00 | 100.00 | 100.00 | 100.00 |
| **BJTZ2021041** | **98.98** | **99.99** | **99.99** | **99.99** | **100.00** | **99.99** | **100.00** | **99.99** | **99.99** | **100.00** | **100.00** | **100.00** | **100.00** | **99.99** | | 100.00 | 100.00 | 100.00 |
| **BJTZ2021034** | **98.98** | **99.99** | **99.99** | **99.99** | **100.00** | **99.99** | **100.00** | **99.99** | **99.99** | **100.00** | **100.00** | **100.00** | **100.00** | **99.99** | **100.00** | | 100.00 | 100.00 |
| **BJTZ2021042** | **98.99** | **99.99** | **99.99** | **99.99** | **100.00** | **99.99** | **100.00** | **99.99** | **99.99** | **100.00** | **100.00** | **100.00** | **100.00** | **99.99** | **100.00** | **100.00** | | 100.00 |
| **BJTZ2021028** | **98.98** | **100.00** | **100.00** | **100.00** | **99.99** | **100.00** | **99.99** | **100.00** | **100.00** | **99.99** | **99.99** | **99.99** | **99.99** | **100.00** | **99.99** | **99.99** | **99.99** | |

Note: Numbers in bold represent average nucleotide identity values, and numbers in plain text represent the DNA-DNA hybridization values.

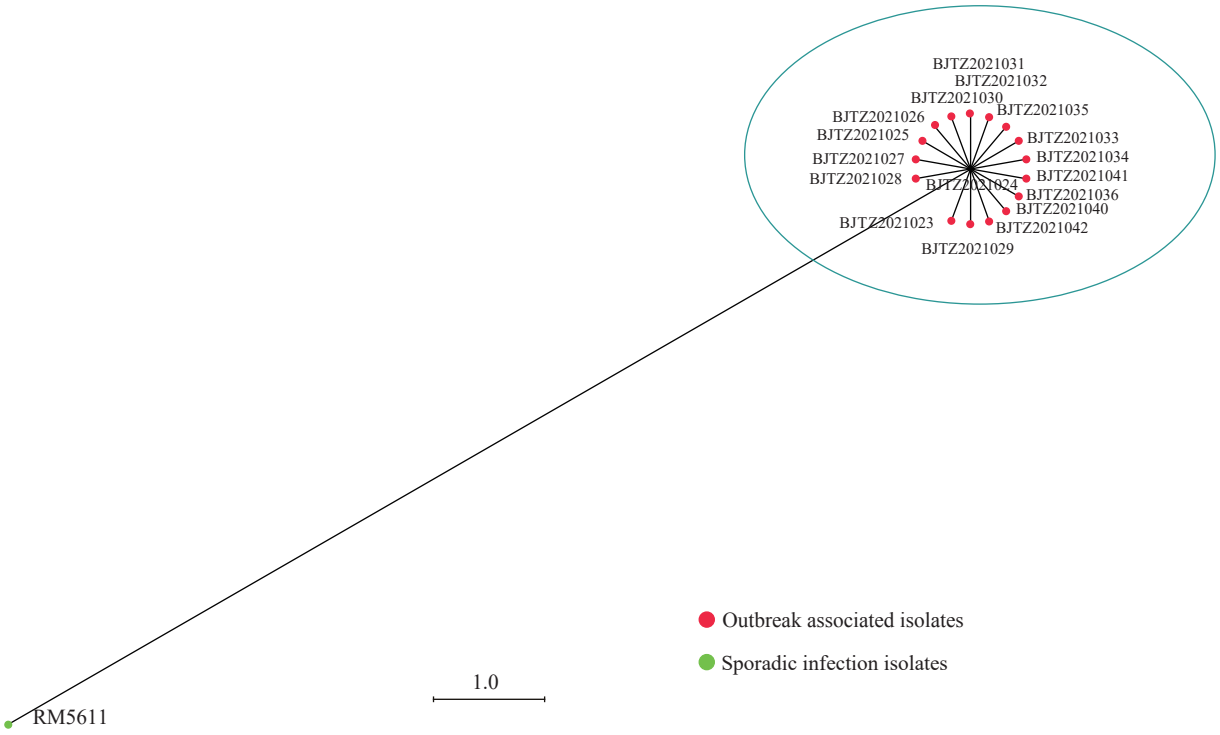
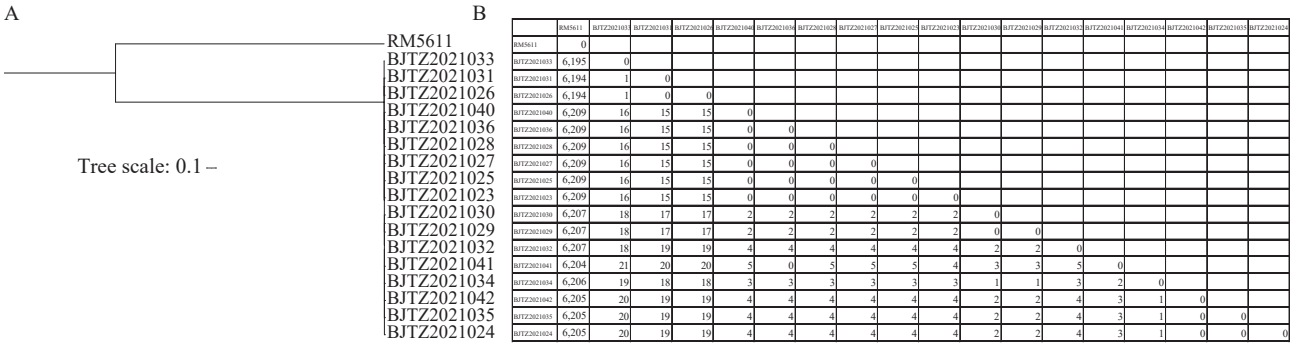



FIGURE 4. Neighbor-net phylogeny for alleles of wgMLST loci of 18 *C. coli* isolates.
Note: All 18 isolates are classified as ST-1068. The red points represent the 17 isolates associated with the outbreak, while the green points represent sporadic isolates from the pubMLST database (reference). The outbreak cluster is indicated by blue circles.




| | RM5611 | BJTZ2021033 | BJTZ2021031 | BJTZ2021026 | BJTZ2021040 | BJTZ2021036 | BJTZ2021028 | BJTZ2021027 | BJTZ2021025 | BJTZ2021023 | BJTZ2021030 | BJTZ2021029 | BJTZ2021032 | BJTZ2021041 | BJTZ2021034 | BJTZ2021042 | BJTZ2021035 | BJTZ2021024 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RM5611 | 0 | | | | | | | | | | | | | | | | | |
| BJTZ2021033 | 6,195 | 0 | | | | | | | | | | | | | | | | |
| BJTZ2021031 | 6,194 | 1 | 0 | | | | | | | | | | | | | | | |
| BJTZ2021026 | 6,194 | 1 | 0 | 0 | | | | | | | | | | | | | | |
| BJTZ2021040 | 6,209 | 16 | 15 | 15 | 0 | | | | | | | | | | | | | |
| BJTZ2021036 | 6,209 | 16 | 15 | 15 | 0 | 0 | | | | | | | | | | | | |
| BJTZ2021028 | 6,209 | 16 | 15 | 15 | 0 | 0 | 0 | | | | | | | | | | | |
| BJTZ2021027 | 6,209 | 16 | 15 | 15 | 0 | 0 | 0 | 0 | | | | | | | | | | |
| BJTZ2021025 | 6,209 | 16 | 15 | 15 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | |
| BJTZ2021023 | 6,209 | 16 | 15 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | |
| BJTZ2021030 | 6,207 | 18 | 17 | 17 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | | | | | | | |
| BJTZ2021029 | 6,207 | 18 | 17 | 17 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | | | | | | |
| BJTZ2021032 | 6,207 | 18 | 19 | 19 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 2 | 0 | | | | | |
| BJTZ2021041 | 6,204 | 21 | 20 | 20 | 5 | 0 | 5 | 5 | 5 | 4 | 3 | 3 | 5 | 0 | | | | |
| BJTZ2021034 | 6,206 | 19 | 18 | 18 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 1 | 3 | 2 | 0 | | | |
| BJTZ2021042 | 6,205 | 20 | 19 | 19 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 2 | 4 | 3 | 1 | 0 | | |
| BJTZ2021035 | 6,205 | 20 | 19 | 19 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 2 | 4 | 3 | 1 | 0 | 0 | |
| BJTZ2021024 | 6,205 | 20 | 19 | 19 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 2 | 4 | 3 | 1 | 0 | 0 | 0 |

FIGURE 5. Phylogenetic tree and difference matrix of 18 isolates based on SNPs. (A) A phylogenetic tree was constructed using SNP data from 18 *C. coli* isolates. (B) In addition, a difference matrix was generated based on the SNP data for the 18 *C. coli* isolates.
Note: SNPs were identified by mapping sequencing reads to the draft genome of isolate RM5611 using Snippy version 4.3.5. A total of 6,203 cgSNPs were included, with strain RM5611 serving as the reference genome.

livestock, particularly cows and pigs, and it is closely associated with diarrhea in patients. Future research should pay attention to ST1068.

Previous research has shown that HuSaV outbreaks typically last from 10 hours to 6 days with an attack rate of 16%–52% (*12*). These characteristics align with the second peak observed in this outbreak, as well as the high positive rate of HuSaV GI in samples collected on D12–D14. Notably, no positive results were found in 19 samples collected at D6, while 39 positive results were obtained from 64 samples collected at D12–D14. This suggests that HuSaV GI infection is likely to occur in the later stages of the outbreak, which contributed to the second peak (D11–D13).

During the second peak of this outbreak, the supply of DDW sourced from WSS-S was discontinued, but the use of "rinsing water" sourced from WSS-S

continued. Waterborne infections caused by HuSaV and *C. coli* may occur through exposure to "rinsing water," and HuSaV may also be transmitted from human to human. This could explain the second peak observed in the epidemic. On D11, all patients were transferred to quarantine, effectively blocking both waterborne and human-to-human transmission. The decrease in the number of new patients following this measure indicates its effectiveness.

The results of aerobic plate counting and coliform detection indicated that the water samples from the WSS-S were poor of quality. But neither *C. coli* nor HuSaV were identified in the water samples. This was the limitation in this study. Waterborne outbreaks often occur on a large scale and involve complex processes of pathogens examination and tracing. Therefore, effective methods for pathogen identification were very critical.

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