

Short Paper

Cite this article: Kashima K *et al* (2021). An outbreak of food poisoning due to *Escherichia coli* serotype O7:H4 carrying *astA* for enteroaggregative *E. coli* heat-stable enterotoxin1 (EAST1). *Epidemiology and Infection* **149**, e244, 1–3. <https://doi.org/10.1017/S0950268821002338>

Received: 14 July 2021

Revised: 10 October 2021



Accepted: 13 October 2021

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An outbreak of food poisoning due to *Escherichia coli* serotype O7:H4 carrying *astA* for enteroaggregative *E. coli* heat-stable enterotoxin1 (EAST1)

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Abstract

In June 2020, a large-scale food poisoning outbreak involving about 3000 elementary and junior high school students occurred in Yashio, Saitama, Japan. A school lunch was the only food stuff ingested by all of the patients. *Escherichia coli* serotype O7:H4 carrying the *astA* gene for enteroaggregative *E. coli* (EAggEC) heat-stable enterotoxin 1 (EAST1) was detected in faecal specimens from the patients, and sample inspection revealed its presence in a seaweed salad and red seaweed (*Gigartina tenella*) as one of the raw materials. Analysis of the antibiotic sensitivity of the isolates revealed resistance to ampicillin and cefotaxime. All isolates were confirmed to be of the same origin by pulsed-field gel electrophoresis after digestion with the restriction enzyme *Xba*I, and single nucleotide polymorphism analysis using whole genome sequencing. To our knowledge, this is the first report of a large-scale food poisoning caused by *E. coli* O7:H4, which lacks well-characterized virulence genes other than *astA*.

In Japan, since 2012, diarrheagenic *Escherichia coli* have been classified into six categories: enterohemorrhagic/Shiga toxin-producing *E. coli* (EHEC/STEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAggEC) and ‘other diarrheagenic *E. coli*’. ‘Other diarrheagenic *E. coli*’ is defined as follows: not categorized by the above five definitions but is considered a pathogen of gastroenteritis, such as *E. coli* strains isolated from many patients involved in an outbreak and characterized as the same by biochemical analysis [1, 2].

EAggEC heat-stable enterotoxin 1 (EAST1) was originally found as an enterotoxin of EAggEC [3]. Although its virulence is not clear, food poisoning cases have occurred where *E. coli* carrying the *astA* for EAST1 was commonly detected in patients. In such cases, it is classified as ‘other diarrheagenic *E. coli*’. In Osaka, Japan, in 1996, the first case of an outbreak caused by *E. coli* that did not have well-characterized virulence genes other than *astA* was reported [4]. Since then, outbreaks caused by *E. coli* carrying *astA* have been reported in various places, with many due to the O166:H15 serotype, and others have been attributed to O169:HNM, OUT, etc. [4–6]. The number of patients per outbreak was less than 200.

From 27 to 28 June 2020, many elementary and junior high school students in Yashio, Saitama, Japan presented with gastroenteritis symptoms such as diarrhoea and abdominal pain. Patients with symptoms were distributed in all 15 public elementary and junior high schools in Yashio and the Yashio Board of Education. A school lunch supplied by a private school meal supply facility was the common food stuff ingested by all the patients.

As many as 6732 students, teachers and other staff had consumed the school lunch, and 2958 of them developed symptoms. The major symptoms were diarrhoea (2716, 91.8%), abdominal pain (2581, 87.3%), fever (671, 22.7%) and other symptoms. The subsequent investigation involved the collection of faecal specimens from 19 patients from 8 of 15 schools and the Yashio Board of Education who were not receiving antibiotics. In addition, 27 food samples (lunch served on 24, 25 and 26 June) stored by meal type at the Yashio Board of Education and the private school meal supply facility, and nine faecal specimens from the food preparation staff were collected. Also, four swab samples of the kitchen facilities were collected.

Faecal specimens were examined for the presence of salmonella, shigella, vibrios, campylobacter, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens* and Norovirus.

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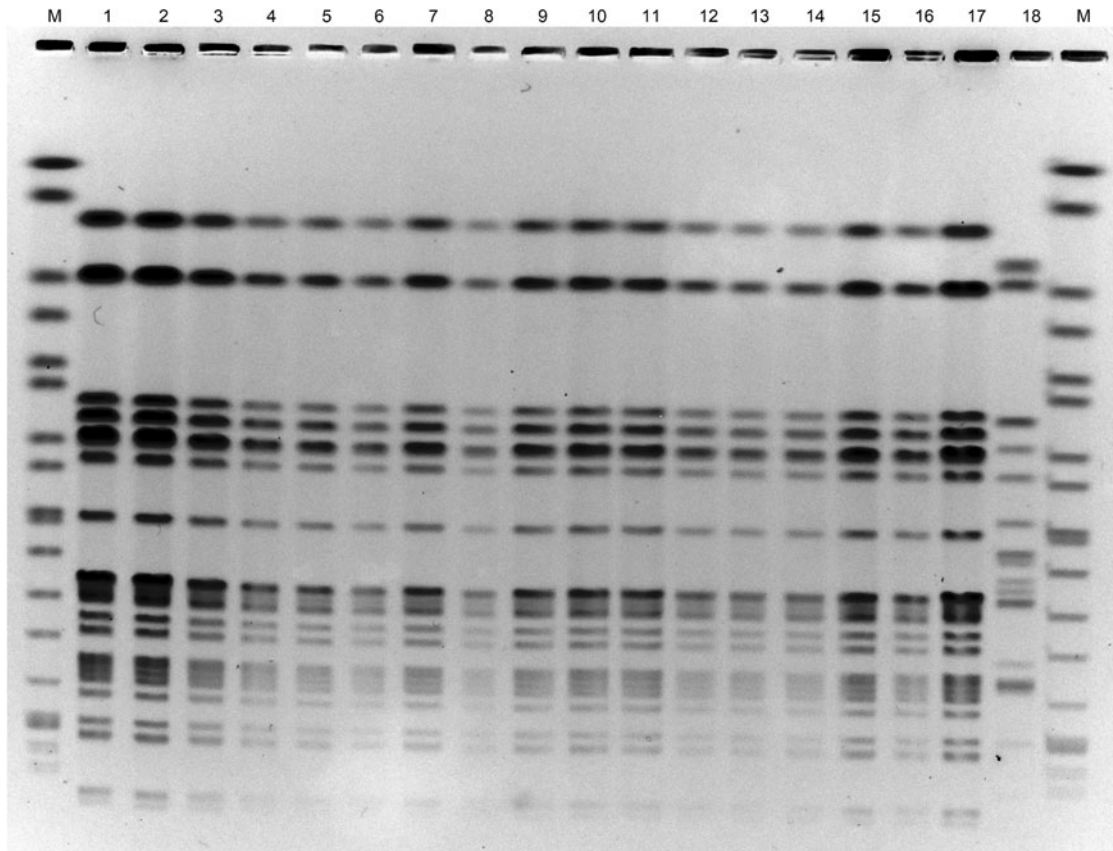


Fig. 1. Pulsed-field gel electrophoresis of DNA of *E. coli* O7:H4 strains after treating with the restriction enzyme *Xba*I. Lanes: M, size marker; 1–14, strains from patient's faecal specimens; 15–16, strains from seaweed salad; 17, strain from red seaweed; 18, *E. coli* O7:H15 strain having no *astA*.

Direct examination of culture plates from 18 of 19 patients' samples and five of nine food workers' samples showed growth of lactose-fermenting colonies that appeared to be *E. coli* on modified Drigalski agar (Eiken Chemical, Tokyo, Japan). No food poisoning-associated bacteria or virus were detected other than *E. coli*. The colonies were confirmed for the presence of virulence-associated genes for diarrheagenic *E. coli* and tested for biochemical characteristics. Individual colonies were suspended in distilled water and heated at 100 °C for 10 min, then centrifuged at 20 000 × *g* for 5 min. The supernatant was used as a template DNA solution for multiplex ExEC (*elt*, *estA1*, *estA2*, *stx1*, *stx2*, *stx2f*, *invE*) and EpAll (*eae*, *aggR*, *astA*, *afaD*) PCR [7, 8]. The thermocycling conditions were 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 50 °C (for EpAll; 55 °C) for 30 s and 72 °C for 1 min, followed by 72 °C for 10 min. The PCR products were mixed with MIDORI GREEN DIRECT (Nippon Genetics, Tokyo, Japan) and subjected to 2% agarose gel electrophoresis. The PCR products were detected under blue/green LED light. The *astA* amplicon was detected in 14 isolates from 14 patients' faecal specimens. No pathogenic genes were detected in the faecal specimens of four patients and five food workers. The 14 isolates in which *astA* was detected showed the same characteristics as typical *E. coli*: glucose and lactose fermenting, indole reaction positive, Voges-Proskauer negative, motility positive, lysine decarboxylase positive and citrate utilization negative. The serotype of the 14 isolates was evaluated with *E. coli* antisera (Denka, Tokyo, Japan and SSI Diagnostica, Hillerød, Denmark) and *E. coli* O-genotyping PCR [9]. All isolates

were confirmed as *E. coli* O7:H4. Based on the above results, the 14 isolates from 14 patients were identified as *E. coli* serotype O7:H4, which lacks well-characterized virulence genes other than *astA*.

To investigate the cause of this outbreak, 27 stored food samples and four swab samples were tested for *E. coli* serotype O7:H4 carrying *astA*. Twenty-five grams of individual food samples were homogenized in 225 ml m-EC Broth (Oxoid, Basingstoke, Hampshire, UK), and each swab was placed in 10 ml m-EC Broth, incubated statically at 42 °C for 18 h, then plated onto modified Drigalski agar, sorbitol MacConkey agar (Oxoid) and CHROMagar STEC (CHROMagar, Paris, France). Five colonies similar to the isolates from patients were tested using PCR assay, biochemical characteristics and serotype. *Escherichia coli* serotype O7:H4 carrying *astA* was detected in two seaweed salad samples served on 26 June of the 27 food samples collected from the Yashio Board of Education and the private school meal supply facility. At the same time, a quantitative test for *E. coli* in the seaweed salad was performed; however, *E. coli* could not be quantified. No *E. coli* was detected in food samples other than the seaweed salad samples, or in the swab samples.

The seaweed salad was made from six kinds of seaweed rehydrated with water and boiled vegetables with dressing. Coliforms were detected in the red seaweed (*Gigartina tenella*) by the manufacturer during self-inspection. Thus, we investigated whether *E. coli* carrying *astA* was detected in the red seaweed, and a positive result was obtained. When we traced back the red seaweed, it turned out to be the product imported from overseas in 2017. Coliforms were not detected by inspection at the time of import

nor at the time of sale to the manufacturer. In addition, no complaints about the same lot of seaweed, besides this case, have been identified. Therefore, the source of the *E. coli* contamination of red seaweed could not be identified.

Pulsed-field gel electrophoresis (PFGE) was performed to compare the 17 isolates obtained from 14 patients, two seaweed salads and one red seaweed [10]. All isolates showed the same DNA banding pattern in PFGE after digestion with the restriction enzyme *Xba*I (Roche Diagnostics, Mannheim, Germany) (Fig. 1). The sensitivity of the 17 isolates to the following antibiotics was examined using Sensi Discs (Becton Dickinson, Franklin Lakes, NJ, USA) according to the performance standards for antimicrobial disk susceptibility tests of the Clinical and Laboratory Standards Institute: amikacin, ampicillin, ceftazidime, cefotaxime, cefoxitin, chloramphenicol, ciprofloxacin, colistin, fosfomycin, gentamicin, imipenem, kanamycin, meropenem, nalidixic acid, norfloxacin, streptomycin, sulfamethoxazole/trimethoprim and tetracycline. All isolates were resistant to ampicillin and cefotaxime.

Furthermore, the whole genomes of *E. coli* O7:H4 isolates carrying *astA* from two patients (Fig. 1, lanes 2 and 9), seaweed salad (lane 15) and red seaweed (lane 17) were sequenced using MiSeq (Illumina, San Diego, CA, USA). Core genome single nucleotide polymorphisms (cgSNPs) were analysed as previously described [11]. Only one locus of cgSNP was detected between the isolates of patients and food samples. Therefore, it was concluded that all isolates were the same clone. Based on the above results, this outbreak of food poisoning was concluded to be due to *E. coli* serotype O7:H4 carrying *astA*, and originated from the red seaweed in the seaweed salad.

Food poisoning cases due to *E. coli* do not necessarily result in the detection of pathogens in food during the follow-up investigation. This outbreak was rare and important, as the same pathogen obtained from patients was detected in food samples and the raw material. While the virulence of EAST1 is not clear, food poisoning cases due to *E. coli* carrying *astA* for EAST1 show relatively mild symptoms. Therefore, it is often difficult to identify *E. coli* carrying *astA* as a cause of food poisoning in instances with small numbers of patients. However, this outbreak of gastroenteritis occurred in about 3000 people. In order to elucidate the pathogenicity of EAST1, it is desirable to document outbreaks and perform pathogenic studies of *E. coli* strains carrying *astA*/EAST1. Since this particular outbreak was attributed to seaweed,

it is recommended that marine products be carefully monitored for contamination.

Conflict of interest. None.

Data availability. Short reads and draft genomes of *E. coli* O7:H4 isolates were deposited in DDBJ/ENA/GenBank under the accession number (PRJDB11800).

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