

An Outbreak of Foodborne Illness Caused by *Escherichia coli* O39:NM, an Agent Not Fitting into the Existing Scheme for Classifying Diarrheogenic *E. coli*

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An outbreak of gastrointestinal illness with clinical and epidemiologic features of enterotoxigenic *Escherichia coli* (ETEC) occurred among patrons of a restaurant during April 1991. Illnesses among several groups of patrons were characterized by diarrhea (100%) and cramps (79%–88%) lasting a median of 3–5 days. Median incubation periods ranged from 50 to 56 h. A nonmotile strain of *E. coli* (*E. coli* O39), which was negative for heat-labile (LT) and heat-stable (STa, STb) ETEC toxins, was isolated only from ill patrons. This organism produced enteroaggregative *E. coli* heat-stable enterotoxin 1 and contained the enteropathogenic *E. coli* gene locus for enterocyte effacement; it did not display mannose-resistant adherence, but produced attaching and effacing lesions in the absence of mannose on cultured HEp-2 cells. *E. coli* that are not part of highly characterized but narrowly defined groups may be important causes of foodborne illness.

The increasing consumption of imported fruits and vegetables has increased the potential for outbreaks of enterotoxigenic *Escherichia coli* (ETEC) or other causes of “traveler’s diarrhea” to occur in the United States. Advances in microbiology have made it possible to identify an expanding array of virulence factors in strains of *E. coli* that are otherwise unremarkable by standard biochemical tests. The convergence of these trends will likely lead to the emergence and recognition of foodborne illness caused by an increasing number of diarrheogenic *E. coli* that have not yet been classified.

We describe an outbreak of diarrheal illness likely caused by *E. coli* O39:NM. We report our clinical and epidemiologic findings, describe the biologic characteristics of the causative agent, and briefly discuss the limitations of the currently accepted scheme for classifying diarrheogenic *E. coli*.

Methods

On 29 April 1991, local public health officials were notified of the occurrence of diarrheal illnesses among members of a group (index group) who ate at a local restaurant on 23 April. The restaurant served a large hotel and conference center and featured an elaborate buffet with a variety of fresh fruits, vegetables, salads, and gourmet food items that combined uncooked and cooked foods.

Epidemiologic studies. Members of the index group and 2 other groups of patrons were interviewed between 29 April and 2 May to ascertain illness and food consumption histories. Stool samples for bacterial culture were obtained from members of the index group on 30 April. For purposes of this investigation, a case was defined as a person who experienced onset of diarrhea within 5 days after eating at the restaurant. Diarrhea was defined as three or more loose stools in a 24-h period.

Environmental health inspections of the kitchen facilities were conducted on 30 April and 2 May. Food preparation procedures were reviewed and food-handlers were interviewed regarding the occurrence of recent diarrheal illnesses. Stool samples were collected from all food-handlers between 2 and 7 May.

After the voluntary closure of the restaurant on 3 May and subsequent news reports concerning the outbreak investigation, other restaurant patrons called to report illnesses. These patrons were interviewed and stool samples were requested from recently ill patrons, as well as from non-ill dining companions to serve as controls.

Laboratory studies. Stool samples were screened at a single laboratory for the presence of *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Vibrio*, *Aeromonas*, and *Plesiomonas* species and

Received 14 April 1997; revised 24 July 1997.

The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Departments of Defense or the Navy.

Financial support: US Naval Medical Research and Development Command, Work Unit 62787 A.001.01.E1X.1521.

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Escherichia coli O157:H7. Three isolates of each colonial morphology identified on desoxycholate agar were biotyped using conventional biochemical tests [1]. Direct examination of culture plates from members of the index group ($n = 10$) revealed a moderate to heavy growth of a single non-lactose-fermenting biotype ($n = 9$) while lactose-fermenting and other non-lactose-fermenting biotypes either were not present ($n = 3$) or were present in lesser numbers. Because this biotype was both predominant in cultures from each individual and common among members of this group, they were further characterized for somatic and flagellar antigens [1], tested for the presence of plasmids [2], and examined for antimicrobial susceptibility by disk diffusion [3].

Colonies of this *E. coli* biotype, identified as *E. coli* O39:NM and designated C7133, were immobilized on Whatman 541 filters and hybridized with [³²P]dCTP-labeled DNA probes that detect genes encoding the following *E. coli* virulence factors: ETEC heat-labile toxin (LT) and heat-stable enterotoxins a and b (STa, STb) [4]; enterohemorrhagic *E. coli* (EHEC) Shiga toxins I and II (Stx1, Stx2) [5]; enteroinvasive *E. coli* (EIEC) invasiveness (*ipaH*) [6]; enteropathogenic *E. coli* (EPEC) adherence factor (EAF) [7], bundle-forming pilus (*bfpA*) [8], and intimin (*eae*) [9]; diffuse adherence *E. coli* (DAEC) probe (*daaC*) [10]; and enteroaggregative adherence *E. coli* (EAaggEC) AA plasmid probe and heat-stable enterotoxin (*astA*) [11]. Plasmid and chromosomal DNA were prepared from strain C7133 by standard methods [11] and used for Southern hybridizations with the *eae* (intimin) and *astA* (EAST1, EAaggEC heat-stable enterotoxin 1) DNA probes. DNA restriction digestions were performed, followed by agarose gel electrophoresis, transfer of DNA to nitrocellulose, and hybridization under stringent conditions [11].

The ability to form attaching and effacing (AE) lesions was determined by the fluorescence-actin staining (FAS) assay, using HEP-2 cells in the absence of mannose but otherwise as described [12]. EAST1 production was assayed in Ussing chambers mounted with rabbit ileal tissue as previously described [13].

Blood samples were collected 2–4 weeks after onset of illness from members of the index group, other restaurant patrons, restaurant employees, and a control group of healthy persons, coworkers of the index group members, who had not recently eaten at the restaurant. These samples were tested for IgG antibody to *E. coli* O39 by EIA. *E. coli* O39 lipopolysaccharide was used as the coating antigen [14]. Serial dilutions were started at 1:20, and results were considered reactive if OD >0.1.

Results

Epidemiologic studies. Of 19 members of the index group, 17 (89%) developed an illness with diarrhea (100%) and cramps (88%), with onset of symptoms 11–122 h after the only meal they ate at the restaurant (table 1). The median incubation period was 56 h. Nausea, myalgias, fever, and vomiting were reported in fewer than half of the cases. Duration of illness ranged from 4 to 7 days (median, 5).

Fourteen (20%) of 71 members of 2 other groups and 91 other patrons developed a diarrheal illness 12–119 h after eating a meal at the restaurant from 19 April through 3 May. The median incubation period was 56 h after a meal. Symptoms

Table 1. Clinical characteristics of gastroenteritis associated with an outbreak of *E. coli* O39 infections.

Sign or symptom	Index group ($n = 17$), no. (%)	Other groups ($n = 14$), no. (%)	Other patrons ($n = 91$), no. (%)
Diarrhea*	17 (100)	14 (100)	91 (100)
Cramps	15 (88)	11 (79)	76 (84)
Nausea	6 (35)	7 (50)	46 (51)
Myalgia	4 (24)	3 (21)	29 (32)
Fever	3 (18)	2 (14)	32 (35)
Vomiting	3 (18)	2 (14)	9 (10)

* Part of case definition.

were similar to those reported by members of the index group (table 1). The duration of illness ranged from 1 to 7 days (median, 3).

The size and complexity of the buffet made it impractical to systematically assess food consumption histories among restaurant patrons. More than 50 food items ranging from “Provençale aromatics” to lime zest were available on the buffet.

Environmental health inspectors observed extensive hand contact with fresh fruits and vegetables as well as raw, cured, marinated, and smoked seafood and raw eggs during preparation of buffet items. Potential for cross-contamination of cooked and raw foods was observed within the kitchen and on the buffet. In addition, cold foods were being held at temperatures >4.5°C on the buffet. Five (15%) of 34 food-handlers reported experiencing a diarrheal illness with onset of symptoms on 23 April (2 food-handlers) or 29 April (3 food-handlers). Duration of illness ranged from 2 to 5 days (median, 3). Four (50%) of 8 food-handlers who worked in the cold food preparation area were ill compared with 1 (4%) of 26 food-handlers in other areas of the kitchen (odds ratio, 25.0; 95% confidence interval, 1.6–1280; $P = .007$).

Laboratory studies. Stool samples were collected from members of the index group ($n = 11$), other groups ($n = 3$), other ill restaurant patrons ($n = 8$), and non-ill restaurant patron controls ($n = 15$). No *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Vibrio*, or *Plesiomonas* species or *Escherichia coli* O157:H7 were isolated from these individuals. *Aeromonas* organisms were isolated from one ill person. A lactose-negative nonmotile *Escherichia coli* O39 was isolated from 9 members of the index group and 1 other ill patron who ate at the restaurant at the same time as did members of the index group. No *E. coli* O39 or other bacterial enteropathogens were isolated from 15 non-ill restaurant patrons or from 5 ill or 29 non-ill food-handlers.

The prototype *E. coli* O39:NM outbreak strain, C7133, was resistant to tetracycline, ampicillin, and carbenicillin. *E. coli* C7133 contained four plasmids with molecular masses of 55, 45, 5, and 2.6 MDa. It was negative by a battery of gene probes

Table 2. Results of serology for O39 antibody by clinical status and stool culture results for *E. coli* O39.

Status	n	GMT	No. (%) with <i>E. coli</i> O39 antibody titer		Odds ratio*
			≥160	<160	
Not ill, stool negative	18	28	1 (6)	17 (94)	Reference
Diarrhea, stool negative	5	53	2 (40)	3 (60)	11.3 [†]
Diarrhea, stool positive	9	173	6 (67)	3 (33)	34.0 [†]

NOTE. GMT, geometric mean antibody titer.

* % of cases with *E. coli* O39 antibody ≥160 vs. % of non-ill persons with *E. coli* O39 antibody.

[†] χ^2 test for trend = 11.1; *P* = .001.

for *E. coli* virulence factors including ETEC LT, STa, and STb, EHEC Stx1 and Stx2, EIEC *ipaH*, EPEC EAF and *bfpA*, and DAEC. Probes detected the presence of intimin (*eae*) and EAST1 (*astA*). C7133 contained at least one chromosomal and one plasmid copy of *astA*. In addition to *eae*, *E. coli* C7133 hybridized with a DNA probe derived from the end of the locus of enterocyte effacement (LEE) region opposite in location to *eae*, implying that this strain contains the entire LEE region [13].

Production of the gene products of both *eae* and *astA* genes were confirmed by functional assays. C7133 was positive in the FAS assay, demonstrating AE lesions in cultured HEp-2 cells in the absence of mannose. However, this strain did not exhibit mannose-resistant HEp-2 cell adherence. Enterotoxigenicity was demonstrated by an increase in short-circuit current (Isc). Isc increased $67.3 \pm 11.6 \mu\text{Amp}/\text{cm}^2$ (mean \pm SE, *n* = 4), compared with $-21.5 \pm 23.7 \mu\text{Amp}/\text{cm}^2$ for nontoxicogenic HB101 (pUC19) (*P* = .02). Results of serologic testing demonstrated a strong association between illness and having *E. coli* O39 isolated from stool and the presence of O39 antibody (table 2).

Discussion

Clinical and epidemiologic features of this outbreak were typical of previously described outbreaks of ETEC-associated diarrhea. However, the *E. coli* O39 associated with illness did not possess the LT, Sta, or Stb that defines ETEC. Two functional virulence factors were identified. The *eae* gene, characteristically harbored by EPEC and EHEC, is essential for expression of AE activity at the intestinal epithelial surface [9]. As with EPEC and EHEC strains, DNA probe results indicated that C7133 contains the entirety of the 35-kb LEE, which includes *eae* and all other determinants required for AE lesion formation [13]. EAST1 is a low-molecular-weight enterotoxin distinct from ETEC STa and Stb [11]. It seems probable that the combination of AE activity and EAST1 enterotoxin produc-

tion by this *E. coli* O39:NM strain caused the physiologic derangements leading to diarrhea in outbreak-related cases.

The combination of putative virulence factors ascribed to *E. coli* O39:NM may be seen as somewhat unusual. The presence of *eae* and absence of bundle-forming pilus-encoding genes provisionally lead to its being regarded as an atypical EPEC. Recently, a diverse group of atypical EPEC not expressing bundle-forming pilus has been isolated from children with diarrhea in Seattle, suggesting these organisms may be relatively common in the United States [15]. However, the absence of an appropriate control group precluded a formal evaluation of their etiologic role. Unlike *E. coli* O39:NM, EPEC principally cause infantile diarrhea. Of note, an outbreak of diarrhea due to an *eae*-positive *astA*-positive *E. coli* O111:B4 was reported in Finnish adults and schoolchildren [16] (H. Smith, personal communication). However, this strain also expressed a standard EPEC O antigen and exhibited the localized adherence phenotype in tissue culture. *E. coli* O39:NM may equally be regarded as an atypical ETEC strain, since it produces an adhesin (intimin) in combination with a heat-stable enterotoxin (EAST1), albeit distinct from the current defining ETEC toxins LT and STa. The one clear point that derives from this discussion is that the outbreak strain does not fit neatly into any of the recognized categories of diarrheogenic *E. coli*.

The terminology used to describe diarrheogenic *E. coli* is complex and by no means definitive. Since the first recognition some 50 years ago that *E. coli* could cause diarrhea, a wide variety of virulence factors have been discovered, and several categories of diarrheogenic *E. coli* have been distinguished. On the basis of current knowledge, these groups generally harbor nonoverlapping virulence factors, although both EPEC and EHEC possess *eae* and multiple categories possess *astA*. The majority of diarrheogenic *E. coli* virulence factors are encoded on pathogenicity islands, transmissible plasmids, bacteriophage, or transposons, and the consequent genetic plasticity could lead to emergence of other combinations of virulence factors.

While an extensive discussion of diarrheogenic *E. coli* nomenclature is beyond the scope of this report, our results have clear significance for investigations of diarrheal disease outbreaks. In particular, *E. coli* should always be considered a potential causative agent, even if detailed characterizations of potential virulence factors do not allow a suspected strain to be readily classified. Prompt and thorough epidemiologic investigation of outbreaks with evaluation of cases and appropriate controls will be needed to identify novel emerging foodborne pathogens and to further our understanding of their public health significance.

The Investigation Team

The investigation team included Wanda Boyer, Franklin Kairis, Linda Gabriel, and John Soler, Minnesota Department of Health, Minneapolis; Lisa Gyswyt, Brooklyn Park Environmental Manage-

ment and Enforcement Services, Brooklyn Park, Minnesota; Steve Bray, Roger Carlson, and Carol Hooker, Hennepin County Community Health Department, Hopkins, Minnesota; Alessio Fasano, Karen Jarvis, and Timothy McDaniel, University of Maryland School of Medicine, Baltimore; and Nadia Tornieporth, Division of Internal Medicine and Infectious Diseases, Cornell University Medical College, New York.

Acknowledgments

We thank the citizens of Minnesota and employees of the Northland Inn who participated in this investigation, Terry O'Brien, Minnesota Attorney General's Office, for legal counsel, and Jan Wiehle and David Determan for clerical assistance.

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