

SURVEILLANCE FOR *ESCHERICHIA COLI* O157:H7 INFECTIONS IN MINNESOTA BY MOLECULAR SUBTYPING

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ABSTRACT

Background *Escherichia coli* O157:H7 is a leading cause of diarrhea and the hemolytic-uremic syndrome. Current public health surveillance for *E. coli* O157:H7 requires considerable resources; traditional methods lack the sensitivity and specificity to detect outbreaks effectively.

Methods During 1994 and 1995, the Minnesota Department of Health requested that all clinical isolates of *E. coli* O157:H7 be submitted to our laboratory. Isolates were subtyped by pulsed-field gel electrophoresis (PFGE), and patients were interviewed about potential sources of infection.

Results In 1994 and 1995, 344 cases of *E. coli* O157:H7 infection were reported to the Minnesota Department of Health; 317 (92 percent) were subtyped by PFGE, and 143 distinct PFGE patterns were identified. Ten outbreaks of *E. coli* O157:H7 were identified; these accounted for 56 (18 percent) of the 317 subtyped cases. Four outbreaks were detected solely as a result of subtype-specific surveillance. In 11 two-week periods, the number of reported cases of *E. coli* O157:H7 doubled from the previous two weeks. In eight of these instances, the patterns identified were dissimilar and there were no outbreaks. Two of the remaining three increases resulted from multiple simultaneous outbreaks.

Conclusions Subtype-specific surveillance for *E. coli* O157:H7 can identify outbreaks that are not detected by traditional methods and can ascertain whether sudden increases in reported cases are due to sporadic isolated cases or to one or more outbreaks. (N Engl J Med 1997;337:388-94.)

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SINCE *Escherichia coli* O157:H7 was identified as an enteric pathogen in 1982, it has been the subject of multiple-outbreak and other epidemiologic investigations.¹ However, the molecular epidemiology and ecology of this organism in humans and animals still need to be defined more fully. For example, in 1994 and 1995, 64 outbreaks of *E. coli* O157:H7 were reported to the Centers for Disease Control and Prevention (CDC) (Sparling PH: personal communication). These outbreaks accounted for only 998 cases during this two-year period, as compared with the estimated 20,000 *E. coli* O157:H7 infections that occur annually in the United States.² The remaining cases are probably sporadic or part of unrecognized outbreaks.

Molecular-subtyping methods have been developed to discriminate various strains of *E. coli* O157:H7.³⁻⁷ These methods have been applied primarily when there have been outbreaks, to distinguish between outbreak-associated and sporadic or unrelated infections. When there was a sudden increase in reported cases, subtyping was used to discriminate among isolates of *E. coli* O157:H7, helping the epidemiologic investigators to identify a common vehicle of infection.⁸ However, the value of isolate subtyping as a surveillance tool on a population basis is not known. We began routine molecular subtyping of *E. coli* O157:H7 isolates submitted to the Minnesota Department of Health in 1994 and 1995. The objectives of our study were to improve our understanding of the epidemiology of *E. coli* O157:H7 and to determine whether routine molecular subtyping of *E. coli* O157:H7 isolates is useful in public health practice.

METHODS

Case Ascertainment

The reporting of *E. coli* O157:H7 infections to the Minnesota Department of Health became compulsory in 1987. In 1994 and the first three quarters of 1995, the department requested that all clinical laboratories in the state submit any *E. coli* O157:H7 isolates from Minnesota residents to the state public health laboratory for confirmation and molecular subtyping. Beginning in October 1995, submission of *E. coli* O157:H7 isolates was required under the Minnesota rules governing communicable diseases.

Patients were defined as sick people who resided in Minnesota and had *E. coli* O157:H7 isolated from a stool culture. They were identified through the submission of isolates from clinical laboratories or through direct reports from health care providers. Once isolates were confirmed as being *E. coli* O157:H7, the patients were interviewed by telephone; a standardized questionnaire was used to ascertain their history of diarrheal illness and any potential common exposures (e.g., attending a day-care center, drinking untreated water, consuming undercooked hamburger, and eating in restaurants). Interviews were conducted before the results of subtyping by pulsed-field gel electrophoresis (PFGE) became available, by interviewers who were not aware of the results.

The reporting of the hemolytic-uremic syndrome was required in Minnesota beginning in 1987. Patients were defined as Minnesota residents with the triad of microangiopathic hemolytic anemia (hematocrit <30 percent, with evidence of intravascular erythrocyte destruction on a peripheral-blood smear), thrombo-

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cytopenia (platelet count, $\leq 150,000$ per cubic millimeter), and evidence of renal involvement (blood urea nitrogen, ≥ 20 mg per deciliter or abnormal urinary sediment). Patients were identified through an active-passive reporting system. The active component was based on routine contact with specialists in pediatric intensive care and infection-control practitioners at major tertiary care centers in the state. In addition, Minnesota physicians were encouraged to obtain stool cultures from all patients with the hemolytic-uremic syndrome for isolation of *E. coli* O157:H7.

Epidemiologic Analysis

Information collected for each patient included his or her name, age, sex, and place of residence; the molecular-subtype designation of the isolate; the "event date"; the presence or absence of the hemolytic-uremic syndrome; and whether the case was part of a recognized outbreak. The event date was defined as the earliest date for which recorded information was available about the onset of symptoms, the date of stool collection, or the date the specimen was received at the laboratory. To determine overall and age-specific rates of infection, population estimates were obtained from *Minnesota Health Statistics, 1994*.⁹ Descriptive analyses were conducted by using EpiInfo, version 6.01 (CDC, Atlanta).

An outbreak was defined as two or more cases in separate households with a common epidemiologic exposure. A case cluster was defined as two or more cases involving the same subtype, occurring within two weeks of each other, for which no common source could be determined by epidemiologic investigation. Temporal increases in the number of cases were evaluated by comparing the number of cases detected during the current two-week period with the number of cases detected during the previous two-week period.

Molecular Subtyping

DNA Isolation and Restriction

Bacterial suspensions were made by emulsifying the growth from 24-hour sheep's-blood agar cultures (BHI, Becton Dickinson, Cockeysville, Md.) in physiologic saline and standardizing to 55 to 65 percent transmittance at 590 nm. The suspension was concentrated by centrifugation, resuspended in EET (100 mM EDTA, 10 mM ethylene glycol-bis[beta-aminoethyl ether]N,N,N',N'-tetraacetic acid, and 10 mM TRIS; pH 8.0), and immobilized in 1.6 percent agarose (SeaPlaque, GTG; FMC Bioproducts, Rockland, Me.). Plugs were then treated with lysozyme (EET with 0.05 percent N-lauroylsarcosine [Sigma, St. Louis] and 200 μ g of lysozyme per milliliter [Boehringer Mannheim, Indianapolis]) for four hours at 30°C, followed by proteinase K (EET with 1.0 percent sodium dodecyl sulfate and 1 mg of proteinase K per milliliter; Boehringer Mannheim) for at least six hours at 50°C and were washed four times with TE buffer (10 mM TRIS, pH 8.0, and 1 mM EDTA) for 30 minutes. All the washing steps were conducted with rotation at 170 cycles per minute by using a 1.0-in.-diameter orbital stroke. Inserts were cut into lengthwise slices and restricted with *Xba*I endonuclease in 100 μ l of buffer H (Boehringer Mannheim; 30 units) for at least four hours at 37°C. Restricted slices were washed in 0.5 \times TBE (which consists of 5.4 g of TRIS base per liter, 2.75 g of boric acid per liter, and 0.645 g of EDTA per liter) and loaded directly onto a 1 percent agarose gel (FastLane, FMC Bioproducts).

PFGE Conditions

DNA fragments were separated with a CHEF Mapper (Bio-Rad Laboratories, Hercules, Calif.). Lambda concatemers and *Saccharomyces cerevisiae* DNA (Boehringer Mannheim) were used as standards. Gels were run with the use of 0.5 \times TBE buffer at 14°C, a linear ramp of 12.56 to 40.09 seconds over a period of 24 hours, a 120-degree switch angle, and a gradient of 6.0 v per centimeter. The gels were then stained with 0.01 percent ethidium bromide solution (Sigma) and photographed with ultraviolet illumination from a fixed camera position.

Gel Analysis and Interpretation

Restriction-fragment patterns were initially matched by visual comparison and measurement of physical band migration relative to lambda and yeast standards. Gel photographs were subsequently digitized with a Photodoc 1000 System (Bio-Rad), and PFGE profiles were compared by using the Jaccard coefficient and Molecular Analyst Plus software (Bio-Rad). DNA patterns were screened with a 3 percent molecular weight-matching tolerance, and matches were confirmed visually. PFGE strains were defined as having exact matches of all bands in the 147-to-700-kb range. A single isolate representing each PFGE pattern was retested to verify its uniqueness and to establish run-to-run reproducibility. On each gel, an *E. coli* O157:H7 reference strain was used in three lanes as a process control (Fig. 1).

RESULTS

Three hundred forty-four cases of *E. coli* O157:H7 infection were reported among Minnesota residents during 1994 (147 cases) and 1995 (197). The annual rate of reported infections among Minnesota residents over the two-year period was 3.8 per 100,000 person-years (Table 1). The age-specific rate of infection for children under the age of five years was 9.3 per 100,000 person-years.

Isolates were received from 135 (92 percent) and 182 (92 percent) of the 1994 and 1995 patients, respectively (Table 2). Patients with isolates available for subtyping and those without were similar with respect to age (mean age, 28.3 and 25.7 years, respectively) and sex (48 percent and 46 percent male, respectively). One hundred forty-three distinct PFGE patterns were identified during the two-year period.

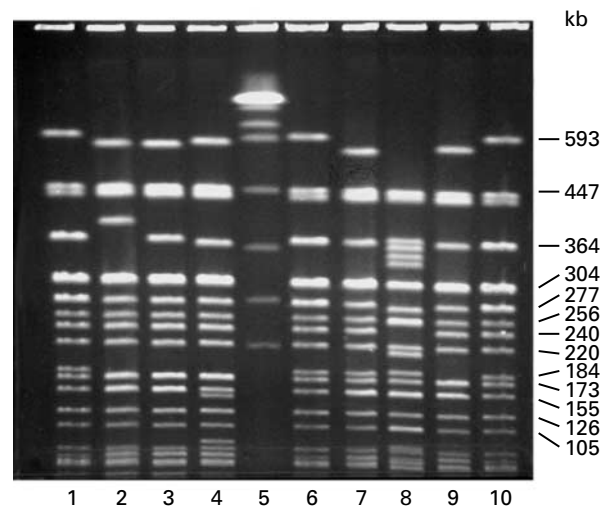


Fig. 1. Patterns on Pulsed-Field Gel Electrophoresis of *E. coli* O157:H7.

Lanes 1, 6, and 10 show isolate G5244, which is a standard strain used to characterize molecular size. Lane 5 shows *Saccharomyces cerevisiae*, used as an additional molecular-size standard. Lanes 3 and 9 show isolates from cases involved in a single cluster. Lanes 2, 4, 7, and 8 show isolates from other sporadic cases of disease.

TABLE 1. NUMBER OF REPORTED CASES AND AGE-SPECIFIC INCIDENCE RATES FOR *E. COLI* O157:H7 INFECTION AND THE HEMOLYTIC-UREMIC SYNDROME IN MINNESOTA, 1994 AND 1995.

AGE (YR)	POPULATION	NO. OF <i>E. COLI</i> CASES	ANNUAL INCIDENCE OF <i>E. COLI</i> O157:H7 INFECTION*	NO. OF ISOLATES SUBTYPED (%)†	NO. OF HUS CASES‡	ANNUAL INCIDENCE OF HUS*‡
<5	353,151	66	9.3	61 (92)	15	2.1
5-9	362,542	38	5.2	35 (92)	7	1.9
10-19	639,465	62	4.8	57 (92)	1	0.2
20-64	2,650,090	133	2.5	123 (92)	1	0.0
≥65	565,107	37	3.3	36 (97)	1	0.1
Unknown		8	—	5 (63)	—	—
Total	4,570,355	344	3.8	317 (92)	25	0.3

*Values are per 100,000 person-years.

†Percentages are of the total isolates for the age group.

‡HUS denotes the hemolytic-uremic syndrome.

TABLE 2. SUMMARY OF SUBMISSIONS OF *E. COLI* O157:H7 ISOLATES TO THE MINNESOTA DEPARTMENT OF HEALTH, 1994 AND 1995.

YEAR	NO. OF CASES REPORTED	NO. SUBTYPED (%)	NO. OF PATTERNS IDENTIFIED	NO. OF PATTERNS REPRESENTED BY A SINGLE ISOLATE (%)
1994	147	135 (92)	72	52 (72)
1995	197	182 (92)	86	59 (69)
Total	344	317 (92)	143*	98 (69)†

*Fifteen subtypes were identified in both 1994 and 1995.

†Five subtypes had a single isolate each in one year and two or more each in the other year. Four subtypes had one isolate each identified in 1994 and one each in 1995.

Ninety-eight of these patterns (69 percent) were unique and occurred in only a single case isolate. Seventy-two different patterns were identified in 1994; 52 (72 percent) were unique. In 1995, 86 different patterns were identified; 59 (69 percent) were unique. The five most common patterns identified in 1994 and 1995 accounted for 109 of the total of 317 isolates (34 percent). Only 15 of the patterns (10 percent) identified in 1994 were also observed in 1995.

The date of the onset of illness (266 patients) or of stool collection (44) was obtained for 310 (98 percent) of the 317 patients with available isolates. The date of receipt of the specimen at the laboratory was available for the remaining patients. In 53 percent of the 1994 cases and 60 percent of the 1995 cases, the onset of illness was between July and Sep-

tember (Fig. 2 and 3). In both 1994 and 1995, the number of cases reported according to the week of onset varied sufficiently to suggest the possibility that some cases represented communitywide common-source outbreaks. During the two years of observation, there were 11 occasions when the number of *E. coli* O157:H7 cases reported within a two-week period was double the number in the previous two-week period. In eight of these instances, PFGE analysis demonstrated that the increases consisted of isolates with different PFGE patterns. Two of the remaining three increases were caused by multiple outbreaks with isolates of different PFGE patterns occurring simultaneously.

Ten outbreaks with a common source were identified (Table 3). These 10 outbreaks accounted for only 56 (18 percent) of the 317 cases whose isolates were subtyped. Four outbreaks were associated with the consumption of hamburger from specific retail markets. Three outbreaks were associated with the consumption of contaminated water. The other outbreaks involved person-to-person transmission in a children's day-care facility, the consumption of contaminated coleslaw during a faculty meal at a college, and the consumption of contaminated roast beef at a church banquet. Four outbreaks were identified on the basis of subtyping data only, when a temporal cluster of identical subtypes initiated an epidemiologic investigation. Three were identified after notification by community agents such as county health departments or hospital infection-control practitioners. The remaining outbreaks were detected through a combination of reviews of subtyping data and concurrent interviews of patients with reported cases.

In addition to the 10 outbreaks, 35 case clusters

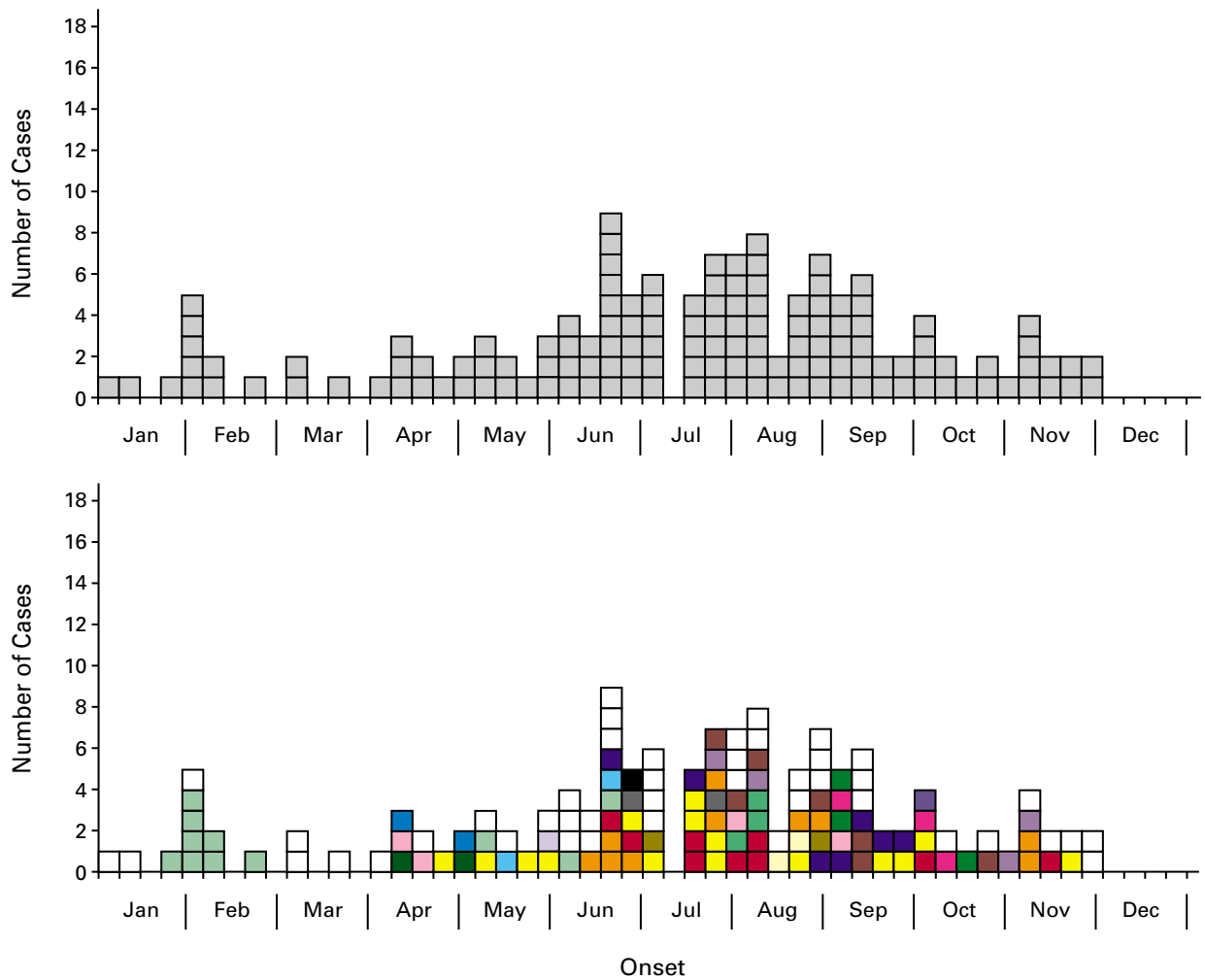


Figure 2. Cases of *E. coli* O157:H7 Infection According to the Week of Onset (Top Panel), and According to the Pattern on Pulsed-Field Gel Electrophoresis (PFGE) (Bottom Panel), Minnesota, 1994. In the bottom panel, each white box represents a single, unrelated PFGE pattern, and each color represents multiple isolates of a unique PFGE pattern.

without recognized common sources were identified. These 35 clusters accounted for 94 (30 percent) of the 317 cases subtyped. Thirty (86 percent) of the 35 clusters involved three or fewer patients. In eight clusters, two patients resided in the same household.

Of the 10 outbreaks and 35 case clusters, 9 involved five or more isolates of an identical subtype pattern; a common source was identified in 6 (67 percent) of those. Conversely, of the 36 clusters involving two to four isolates with a single PFGE pattern, a common source was identified in only 4 (11 percent).

A high level of homogeneity was noted among isolates obtained from epidemiologically linked cases. Of the 56 cases identified as part of the 10 out-

breaks recognized in 1994 and 1995, only 2 cases in 2 different outbreaks involved a PFGE pattern different from the recognized outbreak strain. Although these isolates differed from the outbreak strain by one band, they were closely related to it when evaluated according to the interpretive criteria proposed by Tenover et al.¹⁰

Twenty-five cases of the hemolytic-uremic syndrome were identified during the two-year period — 9 in 1994 and 16 in 1995. Fourteen patients (56 percent) had *E. coli* O157:H7 isolated from stool specimens. Eleven different *E. coli* O157:H7 patterns were recognized among these patients. No enteric pathogen was isolated from the other 11 patients. Twenty-two patients with the hemolytic-uremic syndrome (88 percent) were under 10 years of age,

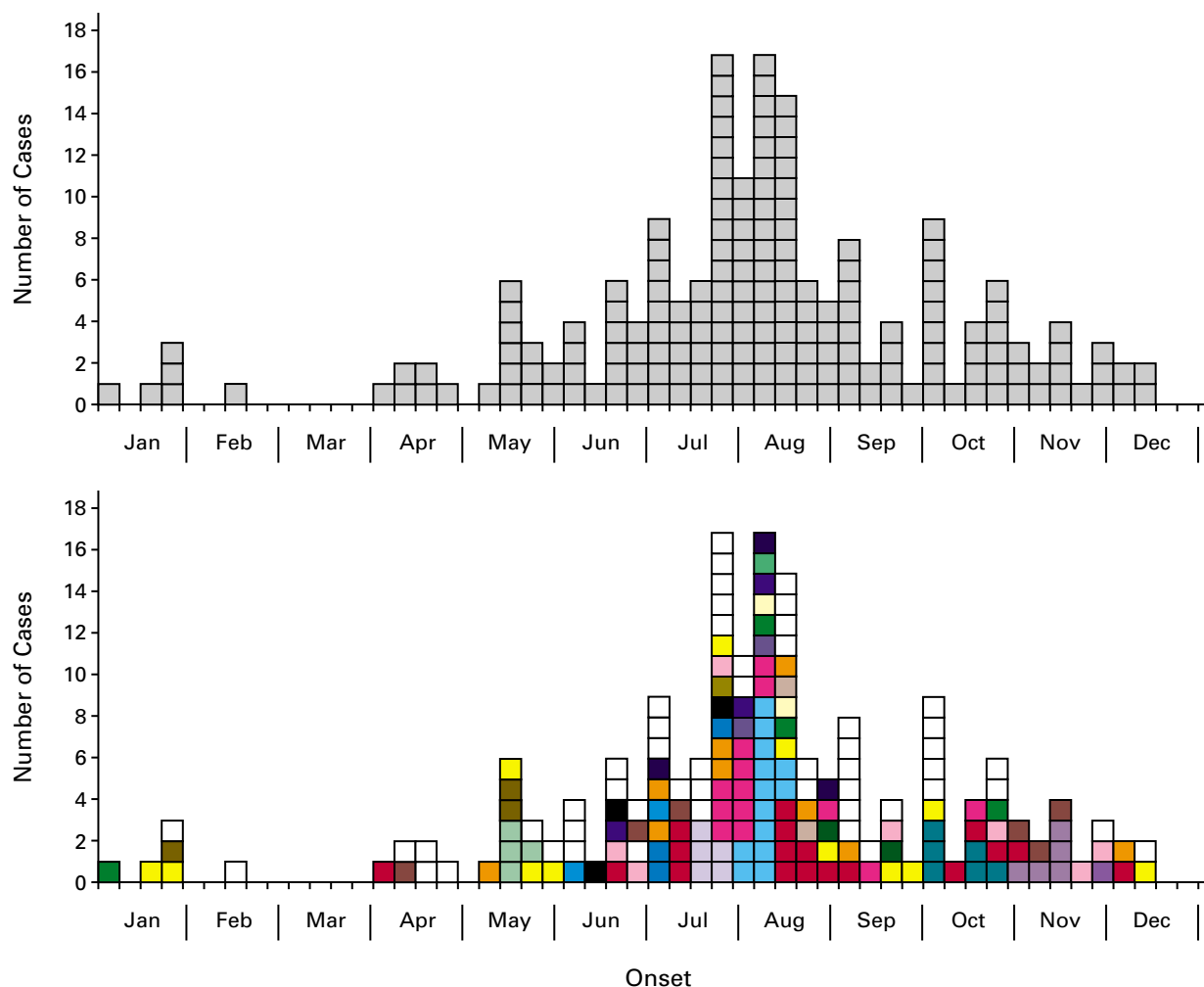


Figure 3. Cases of *E. coli* O157:H7 Infection According to the Week of Onset (Top Panel) and According to the Pattern on Pulsed-Field Gel Electrophoresis (PFGE), Minnesota, 1995.

In the bottom panel, each white box represents a single, unrelated PFGE pattern, and each color represents multiple isolates of a unique PFGE pattern.

and 76 percent (19) were male. The annual age-specific rate of the hemolytic-uremic syndrome in children less than 10 years of age with documented *E. coli* O157:H7 infection was 1.2 per 100,000 person-years. Fifteen cases of the syndrome (60 percent) occurred in July, August, and September, reflecting the same seasonal trend as was documented for *E. coli* O157:H7 infections overall. The ratio of *E. coli* O157:H7 infections to cases of the hemolytic-uremic syndrome among children less than 10 years of age did not vary significantly according to the geographic area within the state. In the Minneapolis-St. Paul metropolitan area, there were 4.3 *E. coli* O157:H7 infections reported for each case of the hemolytic-uremic syndrome. Outside the metropolitan area, 5.2 cases of *E. coli* O157:H7 infection were reported for each case of the syndrome.

DISCUSSION

We report here the results of molecular subtyping of *E. coli* O157:H7 isolates and the usefulness of this method in public health practice. Our major findings include the following. First, there was a marked heterogeneity of isolates, with 143 distinct PFGE patterns identified during the two-year study period. Second, subtyping provided a tool for determining whether or not an outbreak or multiple outbreaks were present when the number of cases increased. For most of the sudden increases in numbers of reported cases, there was no evidence of an outbreak when isolates were subtyped. Without the benefit of subtyping, investigations of similar increases in other areas of the country have led to unnecessary use of limited public health resources.^{8,11} In two instances in Minnesota, what originally appeared to be a single

TABLE 3. CONFIRMED *E. COLI* O157:H7 OUTBREAKS IN MINNESOTA, 1994 AND 1995.

OUTBREAK No.	YEAR	SETTING	No. OF CASES WITH <i>E. COLI</i> O157:H7 ISOLATED	No. OF PATIENTS IDENTIFIED	SOURCE IMPLICATED	METHOD OF DETECTION*
1	1994	College	3	11	Coleslaw, soup	Interview
2	1994	Market	8	8	Hamburger	Subtype
3	1995	Private party	2	2	Hamburger	Subtype
4	1995	Market	4	4	Hamburger	Combined
5	1995	Church banquet	13	31	Roast beef	Interview
6	1995	Public beach	2	2	Lake	Subtype
7	1995	Camp	9	45	Drinking water	Combined
8	1995	Public beach	6	6	Lake	Combined
9	1995	Day care	5	5	Person-to-person	Interview
10	1995	Market	5	5	Hamburger	Subtype

*Subtype denotes subtype surveillance, interview denotes interview of patients after case notification, and combined denotes subtype surveillance and interview.

outbreak was subsequently recognized through subtyping to be multiple outbreaks occurring within a defined period. Third, subtype-specific surveillance allowed us to identify outbreaks that would not have been detected by traditional surveillance methods. Almost half the outbreaks documented (4 of 10) were identified by subtype-specific surveillance alone.

The heterogeneity of the *E. coli* O157:H7 patterns identified in our study has implications for efforts to prevent and control infections. Since most case clusters were small and many cases were sporadic, primary-prevention strategies must focus on reducing contamination of raw foods and changing behavior, particularly behavior related to food preparation and general hygiene.^{1,12,13}

During the two-year period, over half the patients identified harbored *E. coli* isolates that had unique PFGE patterns. However, 30 percent of the patients had an *E. coli* isolate with a PFGE pattern that was isolated from at least one other person during the same two-week period but for which no epidemiologic association could be identified. Because cases involving *E. coli* isolates with similar PFGE patterns often clustered temporally, the data suggest that different *E. coli* strains may contaminate a common product and subsequently appear in the community where the product is distributed. Such outbreaks go unrecognized because relatively few cases are detected. Even if molecular subtyping is used on a state-wide basis and multiple isolates with common PFGE patterns are identified, it may be difficult to determine the source if the numbers of cases are low, unless amplification occurs through other community exposures, such as the contamination of swimming

beaches or transmission within child-care settings.¹⁴⁻¹⁷ For example, when a cluster of five or more cases of a single PFGE pattern was recognized, we found a common source 67 percent of the time. In contrast, when only two to four cases with the same pattern were identified, a common source was found only 11 percent of the time. These results suggest that the identification of outbreaks is unlikely when commercial products are distributed over a multistate area and few cases are identified in each state. Active subtype surveillance on a regional basis may be necessary to increase the likelihood of detecting such outbreaks, identifying the source, and taking appropriate public health action.

The large number of PFGE patterns found in this study can be attributed in part to the stringency of the definition of a strain. Strain relatedness, determined according to the interpretive criteria proposed by Tenover et al.,¹⁰ was not used to differentiate among PFGE patterns in this population-based study. As Tenover et al. state, their criteria for determining strain relatedness were designed for the evaluation of outbreaks of limited size and duration. Conversely, we believe that the interpretive criteria for strain matching of isolates obtained through population-based surveillance should be more restrictive to provide the specificity needed to relate apparently sporadic infections over a large geographic area. This approach is critical to the detection of outbreaks associated with widely distributed food products. Furthermore, our data indicate that PFGE patterns tend to be stable in outbreaks, although single-band differences were found in two instances among isolates from epidemiologically associated cases.

Using PFGE subtyping (or other subtyping methods as they become available) as a routine surveillance tool requires, first, a system for submitting and receiving isolates. In Minnesota, clinical laboratories are required to notify the Minnesota Department of Health of any pathogenic *E. coli* isolated within 24 hours of the completion of microbiologic cultures and to submit isolates to the state public health laboratory. Second, laboratory staff must be available at a central location to characterize isolates rapidly as they are submitted. Generally, results were available within five days when there was one full-time PFGE technician. It is also essential to have the epidemiologic capacity to investigate cases rapidly when identical PFGE patterns are identified. Although this system may seem expensive, much of the cost can be offset by not initiating epidemiologic investigations when the number of cases increases unless subtyping data support the possibility of a common source. Increasing the specificity of outbreak case definitions by the use of molecular subtyping can conserve resources by decreasing the amount of time spent on unnecessary and labor-intensive case-control studies.

Molecular subtyping of *E. coli* O157:H7 has revolutionized population-based surveillance for this organism in Minnesota. We now routinely subtype all *E. coli* O157:H7 isolates and consider this technique to be an integral part of disease prevention and control in our state. We are currently exploring similar subtyping approaches for other enteric and invasive bacterial pathogens, and we believe that such techniques will substantially improve other public health surveillance efforts, particularly at the state and regional levels.

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