USE OF MOLECULAR SUBTYPING IN SURVEILLANCE FOR *SALMONELLA ENTERICA* SEROTYPE TYPHIMURIUM

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ABSTRACT

Background Because *Salmonella enterica* serotype typhimurium is the most common serotype isolated from persons with salmonellosis in the United States, it is difficult to detect unusual clusters or outbreaks. To determine whether molecular subtyping could be useful in public health surveillance for *S. enterica* serotype typhimurium, the Minnesota Department of Health initiated the routine use of pulsed-field gel electrophoresis (PFGE) of isolates.

Methods Beginning in 1994, all *S. enterica* serotype typhimurium isolates submitted by clinical laboratories to the Department of Health were subtyped by PFGE. A standard questionnaire was used to interview patients about possible sources of infection.

Results From 1994 through 1998, 998 cases of infection with *S. enterica* serotype typhimurium were reported to the Minnesota Department of Health (4.4 cases per 100,000 person-years). PFGE was performed on 958 of the isolates (96 percent), and 174 different patterns were identified. Sixteen outbreaks with a common source were identified, accounting for 154 cases. PFGE subtyping made it possible to confirm 10 outbreaks that involved small numbers of cases in institutional settings. Of six larger, community-based outbreaks, four would probably not have been recognized without PFGE subtyping. These four outbreaks accounted for 96 of the 154 culture-confirmed outbreak cases (62 percent). Fifty-six of 209 isolates tested for antimicrobial susceptibility (27 percent) were resistant to at least five antimicrobial agents. The multidrug-resistant isolates identified had unique PFGE patterns.

Conclusions Routine molecular subtyping of *S. enterica* serotype typhimurium by PFGE can improve the detection of outbreaks and aid in the identification of multidrug-resistant strains. Combining routine molecular subtyping with a method of rapid communication among public health authorities can improve surveillance for *S. enterica* serotype typhimurium infections. (N Engl J Med 2001;344:189-95.)

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SALMONELLA *enterica* serotype typhimurium is the most common salmonella serotype isolated from humans and animals in the United States. From 1994 to 1998, this serotype made up 24 percent of salmonella isolates from humans and 19 percent of isolates from animals in the United States.[1] During the same period, 29 percent of salmonella isolates from Minnesota residents were of the serotype typhimurium. Currently, little is known about the molecular epidemiologic features of this organism. Salmonella surveillance efforts have focused on the serotype, location, and date of infection, but this traditional approach is not sensitive enough to detect outbreaks caused by common serotypes, such as typhimurium. In the past several years, molecular subtyping of salmonella has been used to distinguish between outbreak-associated infections and sporadic infections.[2-9] To evaluate the usefulness of routine molecular subtyping as part of surveillance for the typhimurium serotype, the Minnesota Department of Health began in 1994 to perform routine molecular subtyping of all such isolates submitted to its public health laboratory.

METHODS

Ascertainment of Cases

Salmonella infections are reportable to the Minnesota Department of Health, and the public health laboratory of the department is the only laboratory that confirms and serotypes salmonella isolates from clinical laboratories within Minnesota. In October 1995, the Minnesota rules governing communicable diseases were amended to require all clinical laboratories to send salmonella isolates obtained from infected patients to the public health laboratory for the purposes of public health surveillance. In 1995, a standard questionnaire was developed and routinely administered to these patients.

A patient was defined as a Minnesota resident whose stool or blood had been found to be positive for *S. enterica* serotype typhimurium. Patients were identified as a result of submission of isolates from clinical laboratories or reports from health care providers. After a salmonella isolate had been confirmed as having the typhimurium serotype at the Minnesota Department of Health, the patient was interviewed by telephone with the use of the questionnaire to ascertain the history of his or her illness and potential sources of exposure to the organism during the seven days before the onset of illness (e.g., day-care attendance, drinking untreated water or unpasteurized milk, work-related exposure, and eating in a restaurant and other food-related exposure). When they were being interviewed, most patients did not know what subtype of salmonella they had been infected with.

Epidemiologic Analysis

Age-specific rates of infection were determined from 1996 population estimates obtained from Minnesota Health Statistics.[10] Descriptive analyses were performed on Epi Info software (version 6.01, Centers for Disease Control and Prevention [CDC], Atlanta). An outbreak was defined as two or more cases in different households with a common exposure. Investigations of restaurant-associated outbreaks involved interviewing employees and collecting stool specimens from them for culture. A cluster was defined as two or more cases in different households with the same subtype, occurring within two weeks of each other, for which a common source

could not be determined. Outbreaks and clusters were defined as mutually exclusive.

To determine how outbreaks were detected, we divided the method of detection into two categories: subtype and interview. "Subtype" refers to investigations of outbreaks that were initiated by identification of a common cluster of subtypes on pulsed-field gel electrophoresis (PFGE). Interview-initiated investigations were identified by routine interviewing or reports from the public, health care providers, or infection-control practitioners.

To evaluate the usefulness of the Salmonella Outbreak Detection Algorithm (SODA) for the identification of *S. enterica* serotype typhimurium outbreaks, we compared the outbreaks that we identified with those identified by this algorithm. SODA is a laboratory-based system established in 1995 by the CDC, which compares recently reported cases of salmonella infection with a five-year mean number of cases for the same serotype and calendar week of report.[11] If a statistically significant difference is found, the state health department is notified.

Molecular Subtyping

In using the PFGE method for the isolation and restriction of DNA, we followed the standard protocol of the CDC for *Escherichia coli* O157:H7, with minor differences in PFGE conditions.[12] DNA fragments were separated with an electrophoresis apparatus (CHEF Mapper or a CHEF-DR III, Bio-Rad Laboratories, Hercules, Calif.). A strain of *S. enterica* serotype typhimurium (MN#E97000847) was chosen as a standard. Gels were run with

the use of 0.5× TBE buffer (45 mmol TRIS base, 45 mmol boric acid, and 1 mmol EDTA) at 14°C, a linear increase in switching times (from 10.3 to 64.0 seconds) over a period of 22 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, St. Louis), digitized (Gel Doc 1000 system, Bio-Rad), and photographed with ultraviolet illumination from a fixed camera position.

Gel Analysis and Interpretation of PFGE Results

The PFGE profiles were compared by using the Jaccard coefficient and Molecular Analyst Plus software (Bio-Rad). To be considered a match, the DNA patterns could not differ from each other by more than 1 percent with respect to molecular weight. Matches were confirmed visually on the basis of the finding of exact matches of all bands in the range of 30 to 1000 kb (Fig. 1). For the identification of outbreaks and clusters and for comparison of multidrug-resistant isolates, we used the guidelines of Tenover et al. to interpret DNA-restriction patterns generated by PFGE.[13]

Antimicrobial-Susceptibility Tests

Beginning in 1995, a program monitoring antimicrobial resistance was established. Every fifth salmonella isolate received by the public health laboratory was tested for antimicrobial-drug susceptibility. Isolates from nonresidents of Minnesota, duplicate isolates, and all but the first isolate from investigations of outbreaks were removed from the analysis. Disk-diffusion tests were performed on
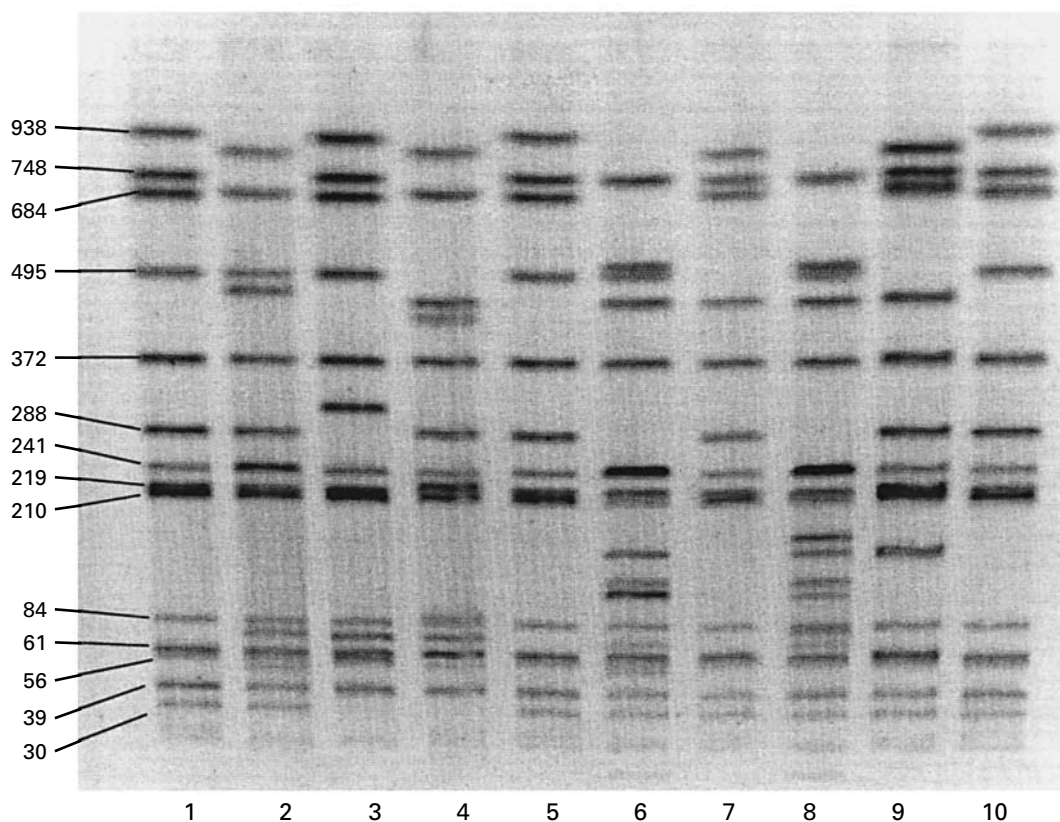
Figure 1. Patterns on Pulsed-Field Gel Electrophoresis (PFGE) of Selected Isolates of *Salmonella enterica* Serotype Typhimurium.

Lanes 1, 5, and 10 show a standard strain used as a molecular-weight standard (as shown on the left). Lanes 2, 3, and 7 show isolates from three separate outbreaks. Lane 4 shows a common PFGE pattern associated with isolates that were resistant to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline. Lane 6 shows the most common PFGE pattern associated with isolates that were resistant to ampicillin, kanamycin, streptomycin, sulfisoxazole, and tetracycline. Lanes 8 and 9 show strains associated with sporadic infections.

Mueller–Hinton agar with standard zone diameters established by the National Committee for Clinical Laboratory Standards.[14] The antimicrobial-susceptibility panel included ampicillin, cefotaxime, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, tetracycline, and trimethoprim–sulfamethoxazole. Sulfisoxazole was added to the panel in 1998, and the susceptibility of selected multidrug-resistant isolates was evaluated. Selected multidrug-resistant isolates were sent to the CDC for bacteriophage typing.

RESULTS

Characteristics of the Isolates

From 1994 through 1998, 998 cases of *S. enterica* serotype typhimurium were reported among Minnesota residents (4.4 cases per 100,000 person-years). The highest age-specific rate was among children younger than five years of age: 13.5 cases per 100,000 person-years. A total of 450 of the 998 cases (45 percent) were identified from specimens collected during the four months from June through September in any given year. From 1995 through 1998, 771 of 864 patients (89 percent) were interviewed. The remaining patients were lost to follow-up because they could not be contacted, they declined to be interviewed, or there were language barriers.

Isolates were received from 958 reported cases (96 percent) from 1994 through 1998 (Table 1). One hundred seventy-four PFGE patterns were identified, of which 106 (61 percent) were represented by only a single case isolate. The five most common patterns accounted for 448 isolates (47 percent). These five patterns were identified each year during the five-year period.

The number of isolates received per week by the public health laboratory ranged from 0 to 23, with a median of 3. The median number of isolates received per week was consistent from year to year, but there were marked seasonal differences. From January through March, a median of two isolates was received per week, as compared with six from July through September. The median number of PFGE patterns ob-

served per week was 3 (range, 0 to 11). During the 5-year period, there were 47 weeks (22 percent) in which six or more isolates were received. A median of 5 different PFGE patterns were observed during these weeks (range, 3 to 11).

Detection of Outbreaks

No outbreaks were detected in 1994. From 1995 through 1998, a total of 16 common-source outbreaks and 63 clusters were identified (Table 2). These accounted for 154 and 241 cases, respectively. Of the 16 common-source outbreaks, 10 occurred in institutional settings and were detected by either telephone interviews of patients by staff members of the Minnesota Department of Health or telephoned reports from the public, physicians, or infection-control practitioners (Table 3). These included six outbreaks among children in a home child-care setting; two among nursing-home residents; one among students, faculty, and horses at a veterinary teaching hospital; and one involving two patients in a renal dialysis unit. The 10 outbreaks involved small numbers of cases, which PFGE subtyping confirmed as outbreak-associated rather than unrelated sporadic cases. This prompted a review of infection-control practices in each setting.

Within each outbreak, the PFGE patterns of isolates from humans were indistinguishable. In the dialysis unit, the two patients shared a hospital room, and the index patient had diarrhea the week before the second patient became ill. No illness was documented among hospital staff. In the outbreak in a veterinary teaching hospital, some isolates obtained from ill horses and the hospital environment differed by a single band from the human isolates. In two of the child-care settings, routine PFGE subtyping identified ongoing transmission. Symptomatic cases were separated by at least three weeks, and without PFGE subtyping they might have been dismissed as unrelated sporadic cases.

Six outbreaks were in community settings. Four in-

TABLE 1. SUMMARY OF *SALMONELLA ENTERICA* SEROTYPE TYPHIMURIUM ISOLATES SUBMITTED TO THE MINNESOTA DEPARTMENT OF HEALTH, 1994 THROUGH 1998.*

YEAR	NO. OF CASES REPORTED	NO. (%) SUBTYPED BY PFGE	NO. (%) INTERVIEWED	TOTAL NO. OF PFGE PATTERNS IDENTIFIED	NO. (%) OF PFGE PATTERNS REPRESENTED BY A SINGLE ISOLATE
1994	134	116 (87)	—	32	17 (53)
1995	287	278 (97)	255 (89)	53	32 (60)
1996	198	193 (97)	178 (90)	47	27 (57)
1997	178	171 (96)	164 (92)	67	45 (67)
1998	201	200 (>99)	174 (87)	70	42 (60)
Total	998	958 (96)	771 (89)	174†	106 (61)

*PFGE denotes pulsed-field gel electrophoresis.

†Some patterns were observed in more than one year, but each subtype was counted only once.

TABLE 2. DISTRIBUTION OF CLUSTERS AND
OUTBREAKS OF *SALMONELLA ENTERICA*
SEROTYPE TYPHIMURIUM INFECTIONS
IN MINNESOTA, 1995 THROUGH 1998.

NO. OF CASES*	NO. OF CLUSTERS	NO. OF OUTBREAKS
2	20	10
3	20	—
4	9	—
5	4	—
6	4	—
>7	6	6
Total	63	16

*The number of cases of culture-confirmed *Salmonella enterica* serotype typhimurium infection is given.

volved restaurants, one involved contaminated pork served at a wedding reception, and the other involved a commercial microwavable chicken product sold in grocery stores.[15] These food-borne outbreaks accounted for 128 of the 154 outbreak-related cases (83 percent). PFGE subtyping was instrumental in initiating four of these six outbreak investigations, which accounted for 96 of the 154 culture-confirmed outbreak cases (62 percent). For example, over a 15-week period in 1998, 79 cases of *S. enterica* serotype typhimurium infection were reported to the Minnesota De-

partment of Health. Initial interviews of patients identified only two outbreaks, each consisting of two cases, one outbreak associated with a wedding reception and the other with a child-care setting. However, an indistinguishable PFGE pattern was observed among 32 of the 79 isolates (41 percent). More focused interviews were performed with patients with this PFGE pattern, and an outbreak associated with a commercial microwavable chicken product was identified, leading to the recall of the product and changes in the label to identify the product more clearly as uncooked.[15]

PFGE subtyping helped us to set priorities and focused efforts when increases in multiple patterns were concurrently observed, especially during the peak summer months. From 1995 through 1998, 10 outbreaks overlapped temporally. In eight outbreaks, cases were identified over a period of at least four weeks, with a median of two cases identified per week (range, zero to seven). In 1995, three restaurant-associated outbreaks occurred simultaneously (Fig. 2). Two of these outbreaks were identified through subtype-specific surveillance, and the other was reported to the Minnesota Department of Health by a hospital infection-control practitioner. During a 16-week period, 157 cases of *S. enterica* serotype typhimurium infection were reported. The outbreak-associated PFGE patterns from these restaurants accounted for 55, 20, and 24 cases. In initial interviews of the patients infected during the two restaurant outbreaks identified through subtype-specific surveillance, fewer than half

TABLE 3. CONFIRMED OUTBREAKS OF *SALMONELLA ENTERICA* SEROTYPE TYPHIMURIUM
IN MINNESOTA, 1995 THROUGH 1998.

YEAR	MONTHS	SETTING	NO. OF CASES IN WHICH *SALMONELLA ENTERICA* SEROTYPE TYPHIMURIUM WAS ISOLATED	IMPLICATED SOURCE OR METHOD OF TRANSMISSION	METHOD OF DETECTION*
1995	Jan.–Feb.	Nursing home	8	Food worker	Interview
1995	June–Aug.	Restaurant	46	Food worker	Subtyping
1995	July–Aug.	Restaurant	10	Food worker	Subtyping
1995	Aug.–Sept.	Restaurant	30	Food worker	Interview
1995	Aug.–Jan.	Veterinary school	2	Horses	Interview
1996	April–July	Restaurant	8	Food worker	Subtyping
1996	April	Home child-care setting	2	Person-to-person	Interview
1996	Sept.	Home child-care setting	2	Person-to-person	Interview
1997	Sept.	Home child-care setting	2	Person-to-person	Interview
1997	Sept.	Nursing home	2	Person-to-person	Interview
1997	Nov.	Home child-care setting	2	Person-to-person	Interview
1998	Feb.	Dialysis unit	2	Unknown	Interview
1998	June–July	Home child-care setting	2	Person-to-person	Interview
1998	Aug.–Dec.	Commercial food product	32	Chicken Kiev	Subtyping
1998	Oct.	Wedding reception	2	Pork	Interview
1998	Oct.	Home child-care setting	2	Person-to-person	Interview
Total			154		

*Methods of outbreak detection were divided into subtype and interview. "Subtyping" refers to outbreak investigations initiated by the routine subtyping of isolates. "Interview" refers to outbreaks initiated by routine interviews by the Minnesota Department of Health staff or reports from the public, health care providers, or infection-control practitioners.

of the patients reported eating at the restaurant ultimately associated with infection.

During the investigation of the four outbreaks in restaurants, 545 stool samples were collected from 162 restaurant employees. Thirty-three infected employees were identified. Ten of the 33 infected employees (30 percent) reported that they had not had diarrhea in the 30 days preceding the outbreak. During the exclusion of restaurant employees, stool samples were obtained from each employee until two consecutive negative cultures were obtained. PFGE patterns from the stool samples of restaurant employees were stable over time, with occasional single-band differences.

From 1996 through 1998, the SODA system of the CDC identified nine instances in which there was a statistically significant increase in the number of cases of *S. enterica* serotype typhimurium infection occurring in a given week and notified the Minnesota Department of Health. In six of the instances, there was no evidence of an outbreak; rather, increases were due to an increase in the number of isolates with multiple, unrelated PFGE patterns. In one instance, two outbreaks occurred simultaneously. The remaining two notifications were for a single extended outbreak involving a commercial food product.[15] From 1996 through 1998, the Minnesota Department of Health
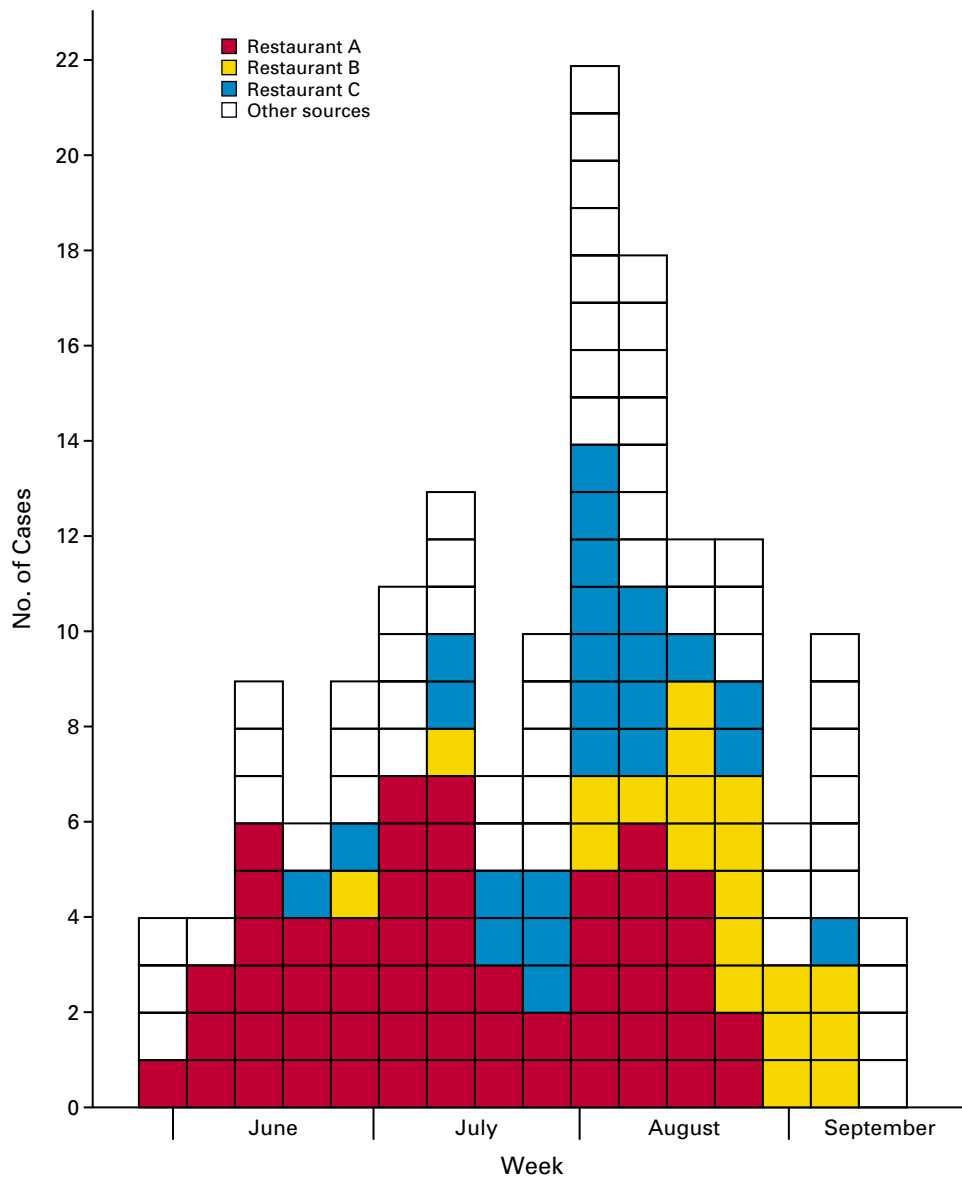

Figure 2. Patterns on Pulsed-Field Gel Electrophoresis of Isolates of *Salmonella enterica* Serotype Typhimurium, According to the Week of Specimen Collection in Minnesota and the Source of the Isolates, June through September 1995.

detected 11 outbreaks of infection due to *S. enterica* serotype typhimurium. The SODA system notified the Minnesota Department of Health of three of these outbreaks two to four weeks after the Minnesota Department of Health had already identified them.

Antimicrobial-Susceptibility Profiles

Two hundred nine isolates from Minnesota residents were tested for antimicrobial susceptibility. All isolates were sensitive to cefotaxime and ciprofloxacin. Fifty-six of the 209 isolates (27 percent) were resistant to five or more antimicrobial agents. Twenty-eight (13 percent) were resistant to at least ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (R-type ACSSuT). These were represented by 13 different PFGE patterns, of which 11 (85 percent) were clonally related. The 11 PFGE patterns accounted for 26 of the 28 isolates (93 percent). Two PFGE patterns accounted for 17 of the 28 isolates (61 percent) (Fig. 1). One of these two patterns was consistently observed over the five-year period (lane 4 in Fig. 1). Nineteen isolates that were resistant to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline underwent phage typing, and 18 (95 percent) were part of the definitive type 104 (DT104) complex.

Of the remaining 28 isolates resistant to five or more antimicrobial agents, 21 (75 percent) were resistant to ampicillin, kanamycin, streptomycin, sulfisoxazole, and tetracycline (R-type AKSSuT). Fifteen of the 21 isolates (71 percent) were represented by a single PFGE pattern, which was observed each year (lane 6 in Fig. 1). This single pattern accounted for 16 isolates during this time, of which 94 percent were resistant to ampicillin, kanamycin, streptomycin, sulfisoxazole, and tetracycline. The results of phage typing of selected isolates with this pattern did not conform to any known phage types.

DISCUSSION

Routine molecular subtyping of isolates of *S. enterica* serotype typhimurium greatly enhanced our surveillance for this common serotype of salmonella. With knowledge of the PFGE pattern, we were able to assign priorities for treating patients with *S. enterica* serotype typhimurium infection, focus investigations, and avoid unnecessary investigations of concurrent increases in unrelated patterns. Routine subtyping allowed us to detect outbreaks that would not have been detected by traditional surveillance methods, as well as to confirm outbreaks reported by traditional methods, since we could identify cases as outbreak-associated rather than as unrelated sporadic cases. This approach allowed us to intervene to stop transmission from a variety of sources of infection and to rapidly identify specific multidrug-resistant strains of importance to public health.

We identified 174 unique PFGE patterns of *S. enterica* serotype typhimurium over a five-year period. The diversity of PFGE subtypes probably indicates that infections were derived from many different sources. These PFGE patterns were stable over time. There were common subtype patterns over the entire five-year period, with homogeneity of serial stool cultures from individual patients and among outbreak-related strains. PFGE patterns were also associated with antimicrobial-resistance profiles and phage-type results.

The routine use of PFGE and the epidemiologic follow-up of individual patients were crucial to identify and confirm the 16 outbreaks detected between 1995 and 1998. Four of six community-based outbreaks would not have been detected without subtype-specific surveillance. In one instance, subtype-specific surveillance was instrumental in the recall of a contaminated commercial product. Before the introduction of PFGE surveillance, 563 cases of *S. enterica* serotype typhimurium infection were reported to the Minnesota Department of Health from 1990 through 1993, but no outbreaks of this serotype were recognized. In half the outbreaks in our study, we identified a median of two cases per week. The onset of illness during these outbreaks occurred over a period of weeks or months. We believe these cases resulted from low-level, intermittent transmission of *S. enterica* serotype typhimurium through contact with contaminated food, infected food workers, and environmental contamination in restaurants and licensed home child-care settings.

Conventional surveillance systems look for unusual temporal increases in serotypes and are likely to miss many outbreaks of common serotypes, such as *S. enterica* serotype typhimurium, *S. enterica* serovar enteritidis, and *S. enterica* serotype heidelberg. Conversely, conventional surveillance leads to unnecessary investigations of unrelated cases that are temporally clustered. These systems lack the sensitivity to detect outbreaks involving common serotypes, especially when they involve few cases occurring over a period of several weeks. This is also true for automated algorithms, such as SODA. Twenty-seven percent of clusters identified by the SODA system in 1997 that prompted notification to states were serotype typhimurium.[16] For surveillance of *S. enterica* serotype typhimurium infections in Minnesota, this algorithm was neither sensitive nor specific in detecting outbreaks. One way to increase specificity of this algorithm would be to include information on molecular subtypes for common serotypes in each state. Because the usefulness of the SODA system is dependent on the timeliness of data entry, states should be encouraged to update this information daily.

Surveillance systems for *S. enterica* serotype typhimurium should include routine molecular subtyping together with the epidemiologic support to investigate subtype clusters. The routine use of PFGE subtyping requires isolates to be sent to the state public health laboratory promptly and individual patients to

be interviewed. Isolates should be typed as soon as possible after receipt rather than in batches, and retrospective summaries detailing PFGE patterns according to the week, month, and year should be provided. In Minnesota, isolates were received at the Minnesota Department of Health a median of five days after stool collection, and the PFGE results were generally available two to seven days later.

Surveillance of molecular subtypes was also useful in identifying specific multidrug-resistant strains of *S. enterica* serotype typhimurium. Previous investigators have highlighted the use of plasmid analysis, PFGE, and phage typing for differentiating multidrug-resistant *S. enterica* serotype typhimurium.[17-20] We found that isolates with certain unique PFGE patterns consistently had common phage types (e.g., DT104) and antimicrobial-resistance profiles (e.g., they were resistant to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline [R-type ACSSuT] or resistant to ampicillin, kanamycin, streptomycin, sulfisoxazole, and tetracycline [R-type AKSSuT]). These characteristics have also been observed by Besser et al.,[21] and we recently found this association in an outbreak of *S. enterica* serotype typhimurium DT104 in a home child-care setting.[22] Phage typing has been the primary method of subtyping for *S. enterica* serotype typhimurium, but it requires access to special reagents and can only be performed at a few institutions. Many state public health laboratories can perform PFGE, and in the absence of timely phage typing, PFGE is a simple way to detect and monitor multidrug-resistant strains.

We have previously demonstrated that PFGE is a crucial tool for public health surveillance and disease-prevention efforts for *E. coli* O157:H7.[23] Our results support the same conclusion with respect to surveillance for *S. enterica* serotype typhimurium and most likely other common salmonella serotypes. Recently, the CDC suggested that all state public health laboratories should have the capacity to perform molecular subtyping of food-borne pathogens in a timely manner. PulseNet, the CDC's national molecular-subtyping network for food-borne disease surveillance, currently uses PFGE to characterize *E. coli* O157, *Listeria monocytogenes,* shigella, and salmonella.

Continued efforts are needed to increase the number of states in which routine rapid molecular subtyping is performed. Routine subtyping and epidemiologic follow-up will also demonstrate to clinical laboratories the value of submitting isolates to public health laboratories. Without the participation of clinical laboratories, on either a voluntary or a mandatory basis, public health surveillance for food-borne pathogens will be inadequate. As more states are included in the PulseNet system, detection of regional outbreaks of infection with *S. enterica* serotype typhimurium and identification of contaminated food products will increase.

REFERENCES

1. *Salmonella* surveillance: annual tabulation summary, 1993-1998. Atlanta: Centers for Disease Control and Prevention, 1996-1999.
2. Roels TH, Frazak PA, Kazmierczak JJ, et al. Incomplete sanitation of a meat grinder and ingestion of raw ground beef: contributing factors to a large outbreak of *Salmonella typhimurium* infection. Epidemiol Infect 1997;119:127-34.
3. Andrews R, Feldheim J, Carman R, et al. Concurrent outbreaks of *Salmonella typhimurium* in South Australia. Commun Dis Intell 1997;21:61-2.
4. Davies A, O'Neill P, Towers L, Cooke M. An outbreak of *Salmonella typhimurium* DT104 food poisoning associated with eating beef. Commun Dis Rep CDR Rev 1996;6:R159-R162.
5. Corbett-Fenney G, Riain U. The use of pulsed-field gel electrophoresis for subdivision of *Salmonella typhimurium* in an outbreak situation. J Infect 1998;36:175-7.
6. Cody SH, Abbott SL, Marfin AA, et al. Two outbreaks of multidrug-resistant *Salmonella* serotype typhimurium DT104 infections linked to raw-milk cheese in northern California. JAMA 1999;281:1805-10.
7. Fey PD, Safranek TJ, Rupp ME, et al. Ceftriaxone-resistant salmonella infection acquired by a child from cattle. N Engl J Med 2000;342:1242-9.
8. Mølbak K, Baggesen DL, Aarestrup FM, et al. An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype typhimurium DT104. N Engl J Med 1999;341:1420-5.
9. Villar RG, Macek MD, Simons S, et al. Investigation of multidrug-resistant *Salmonella* serotype typhimurium DT104 infections linked to raw-milk cheese in Washington State. JAMA 1999;281:1811-6.
10. Minnesota Health Statistics, 1996. Minneapolis: Center for Health Statistics, Minnesota Department of Health, 1998.
11. Hutwagner LC, Maloney EK, Bean NH, Slutsker L, Martin SM. Using laboratory-based surveillance data for prevention: an algorithm for detecting *Salmonella* outbreaks. Emerg Infect Dis 1997;3:395-400.
12. Standardized molecular subtyping of *Escherichia coli* by pulsed-field gel electrophoresis, training course. Atlanta: Centers for Disease Control and Prevention, 1996.
13. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233-9.
14. Performance standards for antimicrobial disk susceptibility tests. 6th ed. Approved standard. Document M2-A6. Wayne, Pa.: National Committee for Clinical Laboratory Standards, 1997.
15. Smith KE, Bender JB, Baker SB, Boxrud DJ, Besser JM, Hedberg CW. Outbreak of Salmonella typhimurium infections due to a microwaveable chicken Kiev product. Clin Infect Dis 1999;29:1064. abstract.
16. Hutwagner L, Villar R, Bishop R, et al. *Salmonella* outbreak detection algorithm (SODA): reviewing the first year in operation. In: Program and abstracts of the International Conference on Emerging Diseases, Atlanta, March 8–11, 1998:117.
17. Vahaboglu H, Dodanli S, Eroglu C, et al. Characterization of multiple-antibiotic-resistant *Salmonella typhimurium* strains: molecular epidemiology of PER-1-producing isolates and evidence for nosocomial plasmid exchange by a clone. J Clin Microbiol 1996;34:2942-6.
18. Threlfall EJ, Hampton MD, Schofield SL, Ward LR, Frost JA, Rowe B. Epidemiological application of differentiating multiresistant *Salmonella typhimurium* DT104 by plasmid profile. Commun Dis Rep CDR Rev 1996;6:R155-R158.
19. Carraminana JJ, Humbert F, Ermel G, Colin P. Molecular epidemiological investigation of *Salmonella typhimurium* strains related to an egg-borne outbreak. Res Microbiol 1997;148:633-6.
20. Ridley A, Threlfall JE. Molecular epidemiology of antibiotic resistance genes in multiresistant epidemic *Salmonella typhimurium* DT 104. Microb Drug Resist 1998;4:113-8.
21. Besser TE, Gay CC, Gay JM, et al. Salmonellosis associated with *S. typhimurium* DT104 in the USA. Vet Rec 1997;104:75.
22. Bender JB, Smith KE, Boxrud D, et al. Outbreak of multidrug-resistant *Salmonella typhimurium* DT104 in a home day care. In: Program and abstracts of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, September 26–29, 1999. Washington, D.C.: American Society for Microbiology, 1999:695. abstract.
23. Bender JB, Hedberg CW, Besser JM, Boxrud DJ, MacDonald KL, Osterholm MT. Surveillance for *Escherichia coli* O157:H7 infections in Minnesota by molecular subtyping. N Engl J Med 1997;337:388-94.