Ruth Ann Dunn, MD William N. Hall, MD, MPH Jaime V. Altamirano, MD, MPH Steven E. Dietrich, MS Barbara Robinson-Dunn, PhD David R. Johnson, MD, MPH

All the authors are with the Michigan Department of Public Health. Dr. Dunn is Chief of the Immunization Section. Dr. Hall is chief of the Disease Surveillance Section. Dr. Altamirano serves as a Medical Epidemiologist within the Disease Surveillance Section. Mr. Dietrich is a Microbiologist in the Molecular Epidemiology Unit. Dr. Robinson-Dunn is Chief of the Microbiology Section. Dr. Johnson is Chief of the Disease Control Division. At the time of this study, Dr. Dunn was a Preventive Medicine Resident with the Division of Field Epidemiology, Centers for Disease Control and Prevention.

Tearsheet requests to Dr. Ruth Ann Dunn, Michigan Department of Public Health, 3500 North Martin Luther King Jr. Blvd., P.O. Box 30035, Lansing, MI 48909; tel. 517-335-8159.

Outbreak of *Shigella flexneri* Linked to Salad Prepared at a Central Commissary in Michigan

SYNOPSIS

IN AUGUST 1992, the Michigan Department of Public Health was notified of a cluster of persons with *Shigella flexneri* infections, all of whom had eaten at different outlets of a single restaurant chain. The chain prepared many foods at a central kitchen.

A matched case-control study to determine risk factors for illness among patrons of the restaurant chain was undertaken. An inspection of the commissary and a review of commissary inspection and employee records were conducted. Of the 46 patients identified, 44 had eaten tossed salad versus 33 of the 71 controls (matched odds ratio = 56.9; 95 percent confidence interval 5.0, 648.1). Improper salad preparation techniques were used, and the salad preparation area had not been inspected in several years. Some salad preparers had been ill shortly before the outbreak but continued to work.

The greater use of central kitchens could lead to larger outbreaks of illness related to improper food handling. Raw vegetables are a potential vehicle for transmission of shigellosis. Coordination of all agencies responsible for inspecting commissaries should be assured. Assuring restriction of ill food handlers will require management to take an active role in identification of ill employees and in the development of incentives to report illness.

higella is responsible for a small proportion of reported foodborne disease outbreaks in the United States; from 1983 to 1987, only 2.7 to 7.2 percent of foodborne disease outbreaks were attributed to shigellosis (1). Common-source foodborne outbreaks of shigellosis typically involve the species S. sonnei. The contaminated item is often a salad containing a food rich in protein or carbohydrate, such as potato, shrimp, tuna, or chicken (2).

In previous reports, the place of preparation of the implicated food was usually a noncommercial setting, such as a home, church, or school. Commercial food preparation sites like restaurants, caterers, or food processing facilities were implicated in only about 25 percent of the outbreaks on which information was available (2). The proportion of reported outbreaks of shigellosis linked to commercial food preparation sites appears to have grown, however. In outbreaks that occurred between 1983 and 1987 where the food preparation site was identified, 49 percent were linked to commercial food preparation (1). Although selective investigation of outbreaks could account for this trend, it is also possible that commercial food preparation sites are playing a greater role in foodborne shigellosis outbreaks. Commercial and institutional food preparation in a central kitchen or commissary is becoming more common (3,4), because it is an efficient, cost-effective, standardized way to prepare food for multiple, distant sites. Restaurant trade magazines contain many articles that feature the use of the latest machines and computer systems in central food preparation facilities. Some food handling is still necessary, however, (3,4)and improper handling in a central preparation facility may lead to a widespread outbreak of illness through the distribution of foods prepared there.

On August 4 and 5, 1992, the Michigan Department of Public Health (MDPH) was notified of three cases of *S. flexneri*. The only factor that the three infected persons apparently had in common was eating at one of two outlets of a restaurant chain shortly before becoming ill. By the evening of August 6, MDPH had received reports on a total of 10 persons with *Shigella* infection who had eaten at one of five locations of the restaurant chain that had 37 outlets in Michigan, two in Indiana, 18 in Ohio, and 11 in Florida. Most foods for all of the Michigan, Indiana, and four of the Ohio restaurants were prepared at a central commissary in Michigan.

The investigation that followed revealed an outbreak of a less common *Shigella* species, *S. flexneri* type 2b, that was associated with eating tossed salad containing only raw vegetables. The vegetables had been cut or shredded, assembled, and distributed to three States from the central commissary in Michigan.

Materials and Methods

Epidemiologic investigation. Persons with culture-confirmed shigellosis were identified through reports by local health departments, laboratories, and hospitals. A message was sent via a computer network to all 50 local health departments in Michigan, informing them of the possible association of shigellosis with the restaurant chain and requesting reports of persons with shigellosis. State departments of health in Ohio and Indiana were notified and similarly requested to report cases of shigellosis.

We interviewed persons with shigellosis by telephone and asked them the dates of illness onset, symptoms, where they had eaten in the week before illness, and whether they had eaten at the restaurant chain. If they had eaten at the chain, they were asked for the date and time of the meal, the description of the meal, who else had eaten with them, and whether those eating partners had become ill.

We began a matched case-control study on August 6 to determine possible risk factors for illness among patrons of the restaurant chain. A case was defined as any person from whom a stool sample grew *S. flexneri* or a yet-to-be-determined species of *Shigella* and who dined at one of the chain's restaurants within 4 days prior to illness onset. Controls were people who ate with a case person at the restaurant but who did not become ill. Cases and controls were asked about the food items they consumed at the restaurant. Matched odds ratios were calculated using the Mantel-Haenszel method (5). Confidence intervals for matched odds ratios were calculated using the estimator for the variance described by Robins and coworkers (6). All calculations were performed using Epi Info, Version 5.01b, computer software (7).

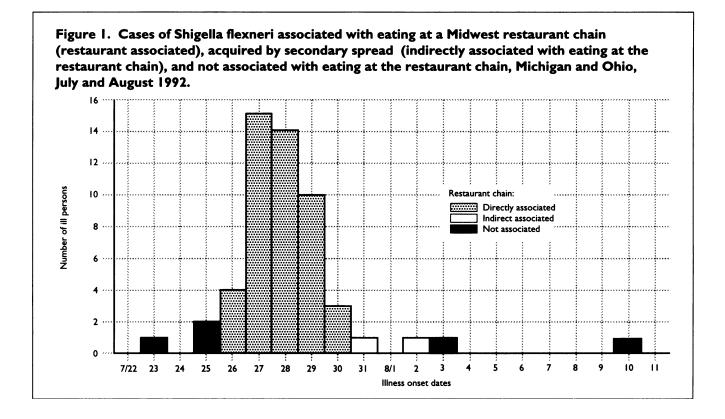
To ascertain whether other clusters or outbreaks of S. *flexneri* were occurring, we solicited reports of all S. *flexneri* cases in the United States for July and August 1992 from the Enteric Diseases Branch of the Centers for Disease Control and Prevention (CDC) in Atlanta, GA.

Environmental investigation. Past inspection reports from the local health department on the central commissary were reviewed. The commissary was inspected on August 6, 1992, by the local department and again on August 11 jointly by MDPH and the local department. Samples of all salads and dressings were taken during the August 6 inspection for bacteriological analysis in the MDPH laboratory. These foods were made on or around August 6. Salads prepared between July 22 and July 24 were not available for analysis.

Employee investigation. Employees of the central commissary were interviewed on August 8 for a history of any illness that occurred after July 1. Those who reported illness were further questioned about their symptoms, their work location, and meals they had eaten at the commissary cafeteria. Stool samples were collected from all employees who had gastrointestinal illness and from those who worked in salad and salad dressing preparation. Any household members of ill employees who also had been ill submitted stool samples.

Laboratory investigation. Stool samples from employees, their ill family members, and one case person and food items from the commissary were cultured for *Shigella* at the MDPH laboratory using standard methods (8-10). Stool samples from all other cases were cultured in other laboratories. *Shigella* isolates from these cultures were speciated and typed at the MDPH laboratory (10). At least one outside laboratory performed antibiograms on *Shigella* isolates that they identified. Three case isolates were sent to CDC for subtyping. Isolates of *S. flexneri* from July and August 1992 were solicited from five other States.

To distinguish the outbreak strain from other strains of S. *flexneri* type 2, techniques to characterize the plasmid and chromosomal DNA were performed on the out-of-State isolates and both Michigan case and non-case isolates of S. *flexneri* type 2. Plasmid DNA was prepared by alkaline lysis (11) and by electrophoresis done in 0.7 percent agarose gels. For chromosomal restriction analysis, agarose-embedded DNA was prepared (12) and digested with the low frequencycutting restriction endonucleases NotI and XbaI. The restriction fragments were resolved by pulsed field gel electrophoresis (CHEF-DRII, BioRad Laboratories) using pulse times of 30 and 60 seconds (NotI) or 15 and 35 seconds (XbaI).



Results

Epidemiologic investigation. We identified 46 persons who met the case definition. All 46 had culture-confirmed *S. flexneri*. We identified seven other persons with culture-confirmed *S. flexneri* who had not eaten at the restaurant chain. Two of these 7 reported recent close contact with a person who had become ill after eating at one of the chain's restaurants, suggesting that their illnesses occurred by secondary spread.

Of the 46 cases, 24 (52 percent) were female. The median age was 40 (range, 5 to 83 years). Symptoms experienced by most cases included diarrhea (100 percent), fever (88 percent), abdominal cramping (70 percent), and vomiting (63 percent). Of 34 cases for whom information was available, the mean duration of illness was 16 days (range, 7 to 27 days). Of 42 cases for whom information was available, 21 (50 percent) were hospitalized. There were no outbreak-associated deaths.

Case persons had dates of illness onset between July 26 and July 30, with a peak on July 27 (figure 1). The mean incubation period was 53 hours (range, 14 to 78 hours).

Case persons reported eating at 21 of 37 restaurants in the chain in Michigan and one restaurant in Ohio (figure 2). Numbers and percentages of case and control persons who ate various foods at the restaurant chain are shown in table 1.

In the matched analysis, food histories of the 71 control persons were compared with the 46 case persons. Shigellosis was associated with the tossed salad mix [matched odds ratio (OR) = 56.9; 95 percent confidence intervals 5.0, 648.1; P = 0.0002]. No other food items were significantly associated with illness.

The national *shigella* surveillance system revealed no recent clusters or outbreaks of *S. flexneri* other than the

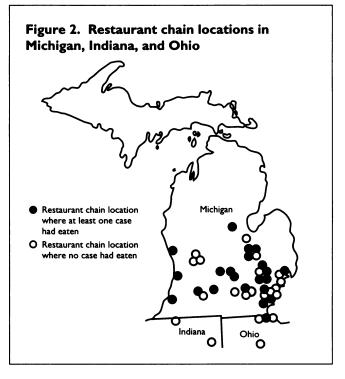


Table I. Foods consumed by 46 patrons of a restaurant chain in Michigan and Ohio who became ill (cases) and 71 who did not (controls) by number and percentage, July 24–28, 1992

	Cases		Controls	
Foods	Number	Percent	Number	Percent
Entrees:				
Beef	7	15	12	17
Chicken	22	4 8	25	35
Fish	7	15	14	20
Other	4	9	12	17
Salads:				
Tossed'	44	96	33	46
Caesar	0	0	2	3
Salad dressings:				
Ranch	18	39	9	13
Blue cheese	12	26	8	11
Other ²		24	16	23
Other fresh vegetables		7	5	7
Fresh fruits		20	14	20
Desserts:				
Pie	7	15	5	7
Cake		20	14	20
Ice cream		13	9	13
Other	2	4	6	8

Includes tossed, taco, and chef salads

²Includes french, italian, thousand island, vinegar, and caesar salad dressings, and hot sauce for taco salads.

Michigan outbreak. Similarly, health officials in California (the source of the lettuce) reported no increases in *S. flexneri* infections.

Environmental investigation. The U.S. Department of Agriculture (USDA) regularly inspected the meat and poultry operations in the commissary and had not reported problems. The local health department had also conducted inspections there. At the last inspection in January 1992, no violations were found, but only the commissary cafeteria had been inspected. Salad production had not been inspected for at least 7 years.

From the August 6 inspection, the local health department reported that many of the food preparation procedures required a large amount of direct food handling by employees. At the August 11 inspection, MDPH and the local health department learned that salad preparation at the commissary had been suspended beginning August 8.

Prior to the suspension, salad was prepared in the following steps: Salad components—iceberg and romaine lettuce, carrots, and red cabbage—were held in a central cold storage area until ready for processing. The iceberg lettuce was cored and the outer leaves removed before chopping; it was not washed or rinsed unless visibly dirty. The leaves of romaine lettuce were soaked in a chlorine solution before chopping. Carrots were rinsed, peeled, and shredded by machine. Red cabbage was cored by hand then shredded by machine. Shredded carrots and cabbage were then mixed by hand in large bowls. The salad mix—used for tossed, chef, and taco salads was prepared into 8.5 pound bags in an assembly line. The iceberg lettuce was first chopped by machine, which blew the chopped lettuce into a bag. Employees added or subtracted lettuce by hand to meet a weight standard. Other employees added by hand an ascorbic acid solution, ice, shredded carrots, cabbage, and romaine lettuce. The bags of salad were stored in coolers until they were loaded onto refrigerated trucks for distribution. These bags were labeled to be consumed no later than 4 days after the date of production.

Based on company records, the date of distribution was within 2 days after production, with older supplies going out before newer ones. A comparison of exposure dates among case persons with the production dates and the distribution patterns of salad revealed that most cases could be explained by contamination of salad made on July 22 and 23.

The commissary received produce for salads through a local wholesaler. The iceberg and romaine lettuce came from fields in Salinas Valley, CA; the carrots from Central Valley, CA; and the red cabbage from west central Michigan. Codes on the packed produce were not recorded by the wholesaler to identify more specifically the field and the date of harvest.

Employee investigation. Of 199 employees at the central commissary, 15 reported a nonspecific diarrheal illness between July 1 and August 8. The dates of onset ranged from July 6 to August 8. The median duration of illness was 24 hours (range, 1 hour to 14 days). All but one ill employee ate at the commissary cafeteria where food items prepared in the commissary were served. Tossed salad was offered daily; 12 (86 percent) of the 14 ill employees who ate at the cafeteria consumed tossed salad regularly.

 Table 2. Plasmid and chromosomal analysis results of isolates of Shigella flexneri type 2, July and August 1992

llsolate number	State	Plasmid profile	Chromosomal pattern
In restaurant ch	ain		
1–21	Michigan	Α	I
22	Michigan	A	I
23-24 ²		Α	I
Not in restauran	-		
25	Michigan	Α	2
26	•	D	4
27	•	E	3
28	•	B	12
29		B	ND
30		č	ND
31		D	II.
32		Ā	8
33		F	ND
34		G	ND
35		Ă	7
36		B	ND
37		č	10
38		c	ND
	South Dakota	В	9
• • • • • • • • • • • • • • • • • • • •	South Dakota	B	ND
70-72	Jouur Dakota	D	ND

¹Missing largest band.

²Infection acquired by secondary spread from a case. NOTE: ND = not done.

Shigella flexneri

С

Plasmid profile

Lane

Of the 15 ill employees, 4 worked in salad preparation. Two became ill in August, after the exposure dates for the cases. One salad preparer had onset of illness on July 6 but terminated her employment on July 15. The fourth estimated her date of illness to be between July 19 and August 8. This employee and two of the other three salad preparers worked while they were ill. It was not possible to determine what part of salad preparation was performed by an employee on a given day. Further, salad preparation was often one of several duties of an employee.

Laboratory investigation. Of the 46 case isolates of S. *flexneri*, 34 were typed by the MDPH laboratory; all 34 were S. *flexneri* type 2. The three isolates that were evaluated by CDC were type 2b. The antibiogram of one isolate showed resistance to ampicillin and sensitivity to the other antibiotics tested for, including trimethoprim-sulfamethox-azole. None of the stool samples collected from employees grew Shigella. Similarly, none of the salads or dressings cultured at MDPH grew Shigella. Five States—California, Colorado, Connecticut, Hawaii, and South Dakota—sent 34 S. flexneri isolates. Of these 34, 15 were S. flexneri type 2.

The results of the plasmid and chromosomal analyses are shown in table 2 and in figures 3 and 4. Case isolates (isolates no. 1-22, table 2) had an identical plasmid profile (plasmid profile A) and chromosomal restriction pattern

Figure 3. Agarose gel electrophoresis of plasmids from isolates of

9 10

11 12 13 14

- 212 Kb/140 Mdal

7 Kb/9.4 Mdal

– 5.1 Kb/3.2 Mdal

- 4.0 Kb/2.5 Mdal

- 3.1 Kb/1.9 Mdal

- 1.6 Kb/0.9 Mdal

E D G

В

(pattern 1), as did the two isolates (no. 23 and no. 24) from persons infected by secondary spread from cases. One isolate from a Michigan resident without a history of exposure to the restaurant chain (no. 25) and two out-of-State isolates (no. 32 and no. 35) also had plasmid profile A; however, these three isolates had chromosomal patterns that were different from the outbreak strain and from each other.

Interventions. On August 6, the local health department restricted ill or recently ill commissary employees from food handling until a negative stool culture was obtained from them. That evening, MDPH issued the following instructions to the restaurant chain:

1. At the commissary and restaurant outlets served by the commissary, immediately dispose of any salads and other foods not requiring further cooking.

2. Perform special cleaning of food preparation equipment at the commissary.

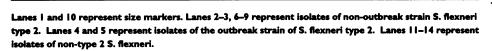
3. Institute emergency hygienic practices at the commissary.

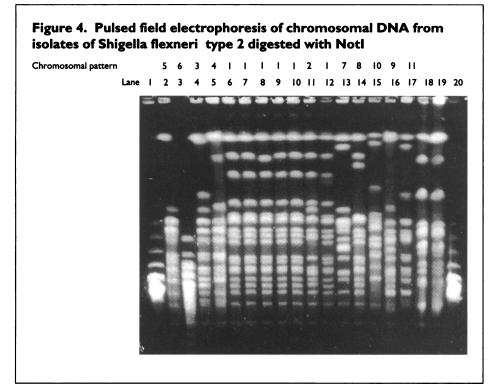
The commissary management voluntarily stopped the production of salads containing lettuce, including the tossed salad mix. The restaurant chain's central commissary in Ohio began providing lettuce salads for the outlets served by the Michigan commissary.

Discussion

A two-State outbreak of Shigella flexneri type 2 infections occurred in July and August 1992. Almost all of these infections were associated with eating tossed salad prepared at the central commissary of a restaurant chain in Michigan. Using an estimate of 20 infections for every reported case of Shigella infection, approximately 920 persons were infected. Plasmid analysis suggested that persons who had eaten at the restaurant chain had the same strain of S. flexneri type 2. Chromosomal restriction analysis further differentiated the outbreak strain from other isolates with the same plasmid profiletwo from other States and one from Michigan. No epidemiologic link to the restaurant chain was found for these three persons.

This investigation yielded several important findings. First, S. *flexneri* is uncommonly identified in Michigan; in 1989, only 53 (7 percent) of 772 Shigella isolates reported in Michigan were S.





Pulse time is 30 seconds. Lanes | and 20 represent size markers. Lanes 2-5, ||, |3-17 represent non-outbreak strains of S. flexneri type 2. Lanes 6-10 and 12 represent isolates of the outbreak strain of S. flexneri type 2. Lanes 18-19 represent restriction enzyme digest controls.

flexneri. Second, three S. flexneri isolates that were not epidemiologically linked to the outbreak had an identical plasmid profile with the outbreak strain. This finding reinforces the potential limitation of using only one molecular analytic technique to identify isolates of the identical strain (13). Third, tossed salad, without a protein or carbohydrate component, was implicated as the probable vehicle for infection. Tossed salad and iceberg lettuce alone have been implicated recently in foodborne outbreaks of S. sonnei (14,15). S. sonnei has been shown to survive on iceberg lettuce at temperatures used for refrigeration and to grow well at room temperature (14).

In this outbreak, the component of the salad that was contaminated could not be determined with certainty because (a) the salad was served pre-mixed to the consumer; (b) we did not ask case persons which salad components they had eaten (many could not recall all of the ingredients when asked to list them); and (c) each component was handled at many steps in salad production.

The source of the contamination was most likely an infected food handler at the commissary. Salad was handled by workers with bare hands, which provided multiple opportunities for contamination. Four salad preparers reported diarrheal illness. At least one and possibly two of these persons were ill in the period before the outbreak when food contamination likely occurred, and they continued to work around the time of illness. Stool cultures from these two salad preparers did not grow *Shigella*. If either of these salad preparers had been infected with *Shigella*, the delay between illness onset and culture was sufficient to explain the absence of *Shigella* in their stool.

Alternatively, these persons did not have Shigella, and we did not identify the infected food handler. Either he or she had an asymptomatic infection or did not admit to or remember working in salad preparation during the period of interest. Similarly, the difference in mean duration of illness of case persons compared with that of employees (16 days versus 24 hours) may be due to selective identification of mildly symptomatic employees who could continue to work, underreporting of illness by employees, or some employees having an infection other than Shigella.

A positive stool culture from an employee may not have been helpful to the investigation because most ill employees had eaten tossed salad prepared at the commissary. It would have been difficult to determine whether the

infected employee had contaminated the salad or had become infected after eating it.

Contamination of a salad component in the field was considered, but this was believed to be a less likely explanation for the outbreak. No other contemporaneous *S. flexneri* outbreaks were reported from elsewhere in the United States. In addition, plasmid and chromosomal analysis of isolates from other States, including the area of California where the lettuce was harvested, showed that these infections were caused by strains of *S. flexneri* other than the one that caused the Michigan outbreak.

Inspections of the commissary had not been carried out in food preparation areas that did not involve meat and poultry or bakery items for several years because local health officials assumed another agency was conducting them. Confusion may have occurred in part because three agencies had jurisdiction over different food preparation procedures at the commissary. After this outbreak occurred, health officials checked to be sure that similar commissaries in Michigan were being completely inspected. When several agencies need to conduct inspections within a facility because of the different food items prepared, health departments should verify that all areas are being inspected. Regular inspections of the salad preparation areas may have resulted in earlier remediation of improper foodhandling, including not regularly washing the iceberg lettuce.

Preventing contamination of food products with *Shigella* is still the best means of preventing foodborne transmission

of this organism. Despite the mechanization of many of the food processing steps in this commissary, our investigation illustrated the continued importance of proper personal hygiene to prevent the spread of infection. In this instance, reducing hand contact of salad components by using utensils whenever possible would be of additional benefit.

Ill employees should be restricted from food handling activities, and the management of food preparation facilities should undertake an active role in identifying ill employees. Management may further encourage employees to report illness by developing incentives that minimize the adverse consequences of reporting, such as allowing sick food handlers to work in areas of the facility not involving food preparation or offering some amount of paid sick leave.

The Calhoun and Genesee County, Michigan, Health Departments and other local health departments in Michigan and Ohio investigated reports of shigellosis. The health departments of California, Colorado, Connecticut, Hawaii, and South Dakota contributed Shigella flexneri isolates for analysis. Frances P. Downes, DrPH, Laboratory Services Division of the Michigan Department of Public Health, directed the plasmid and chromosomal analyses. Gerard N. Stelma, PhD, U.S. Environmental Protection Agency, provided data from studies of Shigella inoculation onto iceberg lettuce. Thomas Hoogerhyde, Phil Kirkwood, and Flint C. Watt of the Michigan Bureau of Environmental and Occupational Health, supervised the investigation of the central commissary. Paul R. Cieslak, MD, and Robert E. Quick, MD, MPH, Centers for Disease Control and Prevention, served as consultants during the investigation.

References

1. Foodborne disease outbreaks, 5-year summary, 1983–1987. MMWR Morb Mortal Wkly Rep 39: 15–59, March 1990.

- Black, R. E., Craun, G. F., and Blake, P. A.: Epidemiology of common-source outbreaks of shigellosis in the United States, 1961–1975. Am J Epidemiol 108: 47–52 (1978).
- 3. King, P.: Giving foodservice its own space. Food Management 25: 80-84, October 1990.
- 4. King, P.: Food production on a grand scale. Food Management 23: 60-69, April 1988.
- Mantel, N., and Haenszel, W.: Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 22: 719-748 (1959).
- Robins, J., Greenland, S., and Breslow, N. E.: A general estimator for the variance of the Mantel-Haenszel odds ratio. Am J Epidemiol 124: 719-723 (1986).
- Dean, A. G., Dean, J. A., Burton, A. H., and Dicker, R. C.: Epi Info, Version 5.0: a word processing, database, and statistics program for epidemiology on microcomputers. Centers for Disease Control, Atlanta, GA, 1991.
- Food and Drug Administration: Bacteriological analytical manual. Ed.
 Association of Official Analytical Chemists Arlington, VA, 1984, pp. 9.01-9.05.
- İsenberg, H. D., Washington, J. A., Doern, G. V., and Amsterdam, D.: Specimen collection and handling. *In* Manual of clinical microbiology. Ed. 5, edited by A. Balows, et al. American Society of Microbiology, Washington, DC, 1991, pp. 19–21.
- Farmer, J. J., and Kelly, M. T.: Enterobacteriaceae. In Manual of clinical microbiology. Ed. 5, edited by A. Balows, et al. American Society of Microbiology, Washington, DC, 1991, pp. 370-371.
- Birnboim, H. C., and Doly, J.: A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 7: 1513-1523 (1979)
- 12. Smith, C. L., Klco, S. R., and Cantor, C. R.: Pulsed-field gel electrophoresis and the technology of large DNA molecules. *In* Genome analysis: a practical approach, edited by K. Davies. IRL Press, Oxford Press, England, 1988, pp. 41-72.
- Prado, D., Murray, B. E., Cleary, T. G., and Pickering, L. K.: Limitations of using the plasmid pattern as an epidemiological tool for clinical isolates of *Shigella sonnei*. J Infect Dis 155: 314–316 (1987).
- Davis, H., et al.: A shigellosis outbreak traced to commercially distributed shredded lettuce. Am J Epidemiol 128: 1312–1321 (1988).
- Martin, D. L., et al.: Contaminated produce—a common source for two outbreaks of *Shigella* gastroenteritis. Am J Epidemiol 124: 299–305 (1986).