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Outbreak of *Shigella sonnei* in a Clinical Microbiology Laboratory

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Laboratory technologists (22%) developed infections with *Shigella sonnei*. The isolates had the same antibiogram and pulse-field gel electrophoresis pattern as an unknown isolate handled by a laboratory student. Covering faucet handles with paper towels during hand washing in the laboratory was protective. No further cases occurred after the laboratory was cleaned with a phenolic agent and a handle-free faucet was installed.

The incidence of infection acquired in hospital microbiology laboratories is approximately 4.0 per 1,000 person-years (9). *Shigella* species are among the many pathogens acquired in this setting (2–5, 9), and the risk of medical technologists acquiring shigellosis in clinical microbiology laboratories is approximately 0.84 per 1,000 person-years (4). This report describes an outbreak of *Shigella sonnei* infection among medical technologists in a hospital microbiology laboratory.

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MATERIALS AND METHODS

The outbreak. The 719-bed university-affiliated hospital microbiology laboratory employs 19 medical technologists and processes 137,000 specimens per year. Three medical technology students were also working in the laboratory in January 1996. Between 23 and 28 January 1996, 6 of the 19 medical technologists but none of the 3 medical technology students developed diarrhea.

Laboratory and statistical methods. Culturing of stool specimens from the six ill technologists was performed on xylose-lysine-deoxycholate and MacConkey agar plates at 35°C. *S. sonnei* was identified by using the BBL Crystal E/NF System (Becton Dickinson, Cockeysville, Md.) and the *Shigella* Serogroup Kit (Becton Dickinson). The Kirby-Bauer method was used to determine the antimicrobial susceptibilities of the isolates.

The *S. sonnei* isolates were characterized by preparing total genomic DNA in agarose plugs and carrying out pulsed-field gel electrophoresis (PFGE) (1, 7). After growth overnight in Trypticase soy broth (Difco Laboratories, Detroit, Mich.), 2 ml of each culture was washed in cell suspension buffer and resuspended in 0.5 ml of the same buffer. An equal volume of 2% low-melting-point agarose (Sigma Chemical Co., St. Louis, Mo.) was added to each of the cell suspensions, and 100-μl portions were pipetted into plug molds (Bio-Rad, Hercules, Calif.). Hardened plugs were pushed out into lysis buffer containing 1 mg of lysozyme (Sigma Chemical Co.) per ml and incubated overnight at 37°C. The lysis buffer was then replaced with digestion buffer containing 0.1 mg of proteinase K (Sigma Chemical Co.) per ml, and the plugs were incubated overnight at 55°C. The plugs were then washed four times in Tris-EDTA buffer (TE) (10:1 Tris/EDTA; pH 8.0), at least 1 h for each of the first three washes and then overnight for the final wash. Plugs were then stored in TE at 4°C. The DNA in the plugs was digested with *Xba*I overnight at 37°C according to the manufacturer's directions (New England BioLabs, Beverly, Mass.). PFGE was carried out in 0.25× Tris-boric acid-EDTA buffer (pH 8.0) with a CHEF-DR II PFGE apparatus (Bio-Rad). The gel contained 1% pulsed-field-certified agarose (Bio-Rad) in 0.25× Tris-boric acid-EDTA buffer. The gel was maintained at 14°C while being electrophoresed at 200 V with switch times increasing from 5 to 35 s. Lambda concatemers (Sigma Chemical Co.) were used as molecular weight markers. After being electrophoresed for 17 h, the gel was stained with ethidium bromide, destained, and viewed under UV light.

The EPI Info program (USD, Inc., Stone Mountain, Ga.) was used for statistical analyses (Fisher exact test and exact 95% confidence intervals).

RESULTS

All six of the affected individuals had abdominal cramps, diarrhea, back pain, chills, and headache; five also had myalgia; four also had either vomiting, fever, or malaise; and two also had hematochezia. Cultures of stool specimens from the six technologists grew *S. sonnei*, and all isolates had the same antibiogram (resistant to ampicillin, mezlocillin, and piperacillin; sensitive to ampicillin-sulbactam, cefazolin, cefotetan, cefuroxime, ceftriaxone, ciprofloxacin, gentamicin, imipenem, and trimethoprim-sulfamethoxazole). Cultures of stool specimens from four of the 16 asymptomatic technologists and students, including the student who worked with the *S. sonnei* isolate, were performed. No shigellae grew on any of these cultures. The most recent *S. sonnei* clinical isolate had been processed in the laboratory on 1 January 1996, 22 days before the first medical technologist became ill, and this isolate had a different antibiogram (sensitive to all 12 of the antimicrobial agents tested). However, a medical technology student had been given a locally stored control strain of *S. sonnei* as an unknown to identify and had been handling the isolate from 16 to 24 January 1996. The antibiogram of this isolate matched that of the *S. sonnei* isolates from the six ill technologists. The *S. sonnei* isolates from the six ill technologists were all identical by PFGE and matched the unknown isolate handled by the student (Fig. 1).

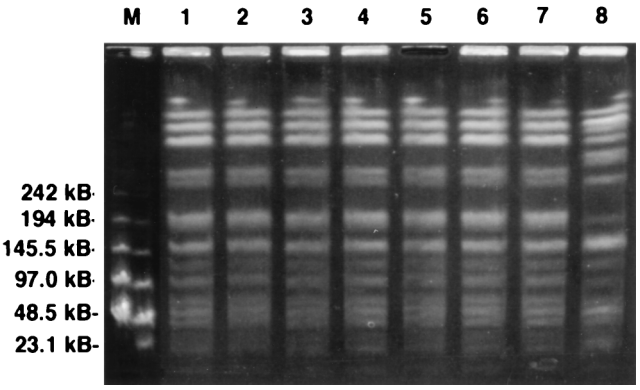

FIG. 1. Molecular fingerprinting of *Shigella* isolates by pulsed-field gel electrophoresis. The image of the gel was scanned into the Adobe Photo Shop software program (Adobe Systems Inc., San Jose, Calif.). Lanes: M, lambda DNA size markers; 1, *S. sonnei* specimen given to a medical technology student as an unknown isolate to identify; 2 to 7, *S. sonnei* isolates from stool cultures of the six infected medical technologists; 8, the last *S. sonnei* clinical isolate processed in the microbiology laboratory before the epidemic.

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TABLE 1. Risk factors for acquisition of shigellosis by medical technologists

Risk factor or lab bench[a]	No. of culture-positive cases (n = 6)	No. of asymptomatic controls (n = 15)[b]	P
Bench			
1	2	5	0.68
2	1	3	0.68
3	1	3	0.68
4	1	2	0.66
5	1	1	0.50
6[c]	2	1	0.20
7	0	1	0.71
8	0	1	0.71
9	1	0	0.28
10	0	2	0.50
11	0	1	0.71
12[d]	1	0	0.28
Worked evening shift	1	2	0.66
Assisted a student with identification of unknown isolate	1	0	0.28
Shared equipment with a student	2	1	0.20
On duty with a student	6	11	0.22
Routinely washed hands before leaving lab	5	15	0.28
Washed hands before eating	5	15	0.28
Shared food with a student	0	0	1.0
Went on break with a student	0	0	1.0
Used a hand washing sink in work area[e]	6	11	0.22
Used a paper towel to turn off faucet	1	11	0.03[f]
Used processing sink 1	0	2	0.50
Used processing sink 2	0	2	0.50
Used processing sink 3	0	1	0.71

[a] Represents the location of each medical technologist's workbench in the lab.
[b] Excludes index student.
[c] The workbench used most often by the medical technology student who handled the *S. sonnei* unknown isolate.
[d] The workbench occasionally used by the medical technology student who handled the *S. sonnei* unknown isolate.
[e] Four of the 15 asymptomatic technologists and students used a sink outside of the work area for hand washing.
[f] Odds ratio, 0.07; 95% confidence interval, 0 to 1.07.

This PFGE pattern was different from that of the last known clinical isolate processed in the laboratory (Fig. 1). The student did not develop diarrhea or any of the other symptoms described above. He was the only technologist in the laboratory to routinely wear gloves. The workbench (bench 6) where the student spent most of his time identifying the unknown isolate was the bench closest to the sink used for hand washing. He admitted to having had heavy glove contamination while working with the *Shigella* specimen on 21 January. On 15 December 1995, the sink used for hand washing had been moved from outside the medical technologist's work area to inside this area and control of the water flow had been changed from foot pedal to faucet handle operation.

A case control study was done to assess 26 possible risk factors associated with the cases of shigellosis (Table 1). The use of paper towels over the faucet handles as a barrier had a protective effect (odds ratio, 0.07 [95% confidence interval, 0.00 to 1.07]; P = 0.03). Cultures of sites in the laboratory environment were negative for *Shigella* species; however, the laboratory had already been thoroughly cleaned with a phenolic agent.

Control measures included a mandatory leave of absence for each affected technologist until resolution of diarrhea, the use of a phenolic agent to clean environmental surfaces in the work area of the laboratory, educating laboratory personnel about transmission of enteric pathogens, and, based on the case-control study findings, the benefit of using paper towels over faucet handles as barriers until the sink could be refitted with an infrared-activated faucet (Fig. 2), and the institution of a new policy stating that students were not to be given a *Shigella* isolate as an unknown to identify. Since the implementation of these interventions, no further laboratory-acquired cases of shigellosis have been documented. Among the six technologists, the total time lost from work was 73 days (range, 1 to 54 days), representing approximately $10,000 in wage value.

DISCUSSION

This is one of the largest reported outbreaks of shigellosis in a hospital laboratory. In a recent survey of 166 British clinical laboratories (5), *Shigella* infections were the most common of the laboratory-acquired infections; however, the mechanism(s) of transmission was not elucidated. Interestingly, three of the *Shigella* infections reported in this survey originated from quality control specimens. The same authors have also provided data suggesting that *Shigella flexneri* is more easily transmitted in the laboratory setting than is *S. sonnei* (4).

A number of events may have culminated in this outbreak. Namely, a *Shigella* isolate was subjected to prolonged handling by a student over a period of several days at a time when the sink used for hand washing in the laboratory had been changed from foot pedal to faucet handle operation. The *Shigella* isolate given to the student was a quality control strain stocked in the laboratory. Transmission appears to have resulted from contamination of the faucet handles of the single sink used for hand washing by technologists in the work area. The student working with the unknown isolate admitted that heavy glove contamination had occurred when he placed his gloved finger in a titer well containing a high concentration of *S. sonnei* during the typing process. This occurred at the bench closest to the hand washing sink and farthest from the processing sink (bench 6). It is believed that the student broke laboratory protocol and used the hand washing sink, rather than the sink that he had been instructed to use for processing, to discard the concentrated *Shigella* suspension in the titer wells and to wash off the reusable titer wells following disinfection with Lysol I.C. (National Laboratories, Montvale, N.J.). This activity likely led to contamination of the sink and faucet handles. We have no evidence suggesting that the epidemic we investigated was ini-

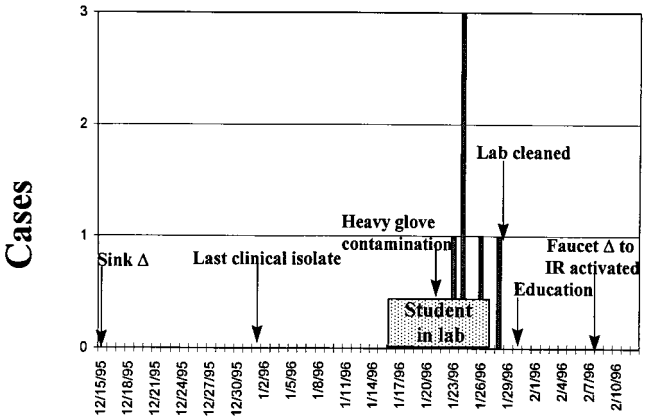

FIG. 2. Epidemic curve. See the text for details. Δ, change; IR, infrared.

tiated intentionally. However, a recent outbreak of *Shigella dysenteriae* among 12 of 45 laboratory technologists apparently resulted from intentional contamination of food with the microbiology laboratory's stock strain (6).

For the last 20 years, medical technology students, after undergoing the same training in good laboratory practice, have been working in the laboratory at which the outbreak described in this paper occurred. Despite working with *Shigella* species and other pathogens, none of these students was known or suspected to have acquired a laboratory-associated infection. Upon further questioning, the involved student was also noted to have had other breaches in technique during his rotation through the microbiology laboratory. As with so many areas of infection control, written protocols and appropriate training in sterile technique may not ensure good practice. This report emphasizes the fact that despite the rigorous training of students and new personnel, close supervision of these individuals is of paramount importance.

Although *S. sonnei* has been previously recovered from environmental surfaces of a clinical microbiology laboratory (8), cultures of environmental surfaces in the present study were unrevealing. This was probably due to widespread cleaning of the laboratory prior to the culturing of environmental sites.

Design of sinks in clinical microbiology laboratories is not mentioned in some recent texts dealing with optimal design requirements (2, 3), possibly because there is no mention of transmission of infection by this route in these otherwise authoritative texts. However, in one text (10) it is recommended that dry paper towels be used to turn off faucets after hands are cleaned and that knee- or foot-controlled sinks be installed in laboratories handling moderate- to high-risk microbes. The findings in the present report suggest that the risk of transmission of microbial pathogens in the clinical microbiology laboratory can be minimized by replacement of work area sinks equipped with faucet handles by those equipped with foot pedals or with infrared-activated systems. Thorough cleaning of environmental surfaces cannot be overemphasized. For those laboratories with faucet handle controls on sinks, the use of paper towels as barriers may reduce transmission of microbial pathogens in this high-risk environment.

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