Fatal Salmonellosis Originating in a Clinical Microbiology Laboratory

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Laboratory-acquired infections concern all microbiologists. During ongoing surveillance of laboratory-acquired enteric infections, salmonella infections in the wife and son of a laboratory worker were documented; the first case was fatal. Neither patient had had any contact with the laboratory. The infecting organisms were Salmonella typhi and a multiply resistant strain of Salmonella agona that were being worked with in the laboratory; both strains had been received 1 to 2 years previously for proficiency testing exercises. This report documents the transmission of enteric pathogens beyond the confines of the laboratory, with its tragic outcome, and suggests measures to prevent the recurrence of this problem.

Laboratory-acquired infections have been recognized since the advent of microbiology as a science (8), and safety in the laboratory is an issue which affects all microbiologists. Although those working with etiological agents are at greatest risk for acquiring infection, on occasion there has been transmission of these agents beyond the confines of the laboratory (10).

Recently we reported that the laboratory is becoming a significant reservoir for Salmonella typhi in the United States (1). Most of the laboratory-acquired cases of typhoid fever are due to exposure to strains of S. typhi used for proficiency testing or educational purposes rather than isolates from ill patients.

In the years 1977 through 1979, S. typhi isolates were sent to about 4,500 laboratories in the United States by three national proficiency testing organizations. Use of S. typhi strains in statewide proficiency testing programs and in the teaching of microbiology and undergraduate students may have been considerable, but the magnitude of that usage is not known. During the same 3-year period, 672 cases of typhoid fever were reported to the Centers for Disease Control (CDC) (D. N. Taylor and M. J. Blaser, unpublished data). In total, the number of individuals in the United States exposed to S. typhi from voluntary sources (proficiency testing, educational purposes) probably far exceeded the number of individuals exposed to a strain isolated from a clinical specimen during this 3-year period. During the period from 1977 to 1979, laboratory-acquired cases represented 11.2% of the sporadic typhoid cases reported in the United States (1).

Continuing surveillance of this problem brought the following cases to our attention.

CASE REPORTS

Case report 1. On 24 June 1980 a 35-year-old woman who previously had been in excellent health developed diarrhea, abdominal cramping, nausea, and vomiting. Three days later her stools became bloody, and she began having high fevers, headaches, and myalgias. She was hospitalized on 30 June after having a temperature up to 102°F (ca. 38.9°C); the leukocyte count was 3,700/µl with 68% segmented neutrophils and 23% juvenile forms. Reciprocal agglutination titers to S. typhi O and H antigens were 640 and 2,560, respectively, and intravenous ampicillin (1 g every 4 h) was started. Three blood cultures grew S. typhi susceptible to ampicillin and chloramphenicol. The patient continued to have a fever up to 105°F (ca. 40.6°C), and treatment with chloramphenicol (50 mg/kg per 24 h) was also begun. On 1 July S. typhi was isolated from stool cultures. On 2 July the patient continued to be febrile, became apprehensive and hypotensive (blood pressure, 60/40), and had cooling of her extremities. Gentamicin (3 mg/kg) was given as a loading dose. Over the next 8 h she became increasingly hypotensive, she developed a metabolic acidosis, and her blood pressure could not be supported with fluids, pressors, or steroids. Pulmonary edema, disseminated intravascular coagulation, hemolysis, coma, and death ensued in rapid progression. There was no post-mortem examination. Three blood cultures drawn on 1 July later grew Salmonella agona which was shown to be resistant to chloramphenicol, ampicillin, tetracycline, gentamicin, kanamycin, tobramycin, carbenicillin, and cephalothin and susceptible only to amikacin and cefoxitin.

Case report 2. On 5 July 1980, the 14-year-old son of the deceased woman became ill, complaining of a temperature up to 105°F (ca. 40.6°C), malaise, and diarrhea. He was hospitalized on 8 July, and an en-
larged spleen was noted on examination. His leukocyte count was 4,800/μl with 48% immature neutrophils. *Salmonella* H titer was 40, but all of four stool cultures were negative for salmonellae. After nine blood cultures were obtained, he was treated with chloramphenicol and amikacin, with gradual defervescence over the next 4 days. When *S. typhi* was grown from all nine blood cultures, the amikacin was discontinued. On 15 July he had *Salmonella* O and H titers of 80 and 5,420, respectively. A 14-day course of chloramphenicol was completed, and the boy had a full recovery. On 24 August his *Salmonella* H titer was 640.

**Epidemiological investigation.** The husband of patient 1 and father of patient 2 was a 37-year-old man (Mr. A) who was Chief of the bacteriology laboratory in a 500-bed hospital in Arkansas. He had had 11 years of experience as a microbiologist and had never had a laboratory-acquired infection as far as he knew. He last had a typhoid vaccination in 1964 while in the military service.

The laboratory was staffed by five microbiology technologists, including himself and one student completing her internship for a degree in medical technology. The laboratory had participated in microbiology proficiency testing surveys for more than 10 years, including those given by the Arkansas State Department of Health, the CDC, and the College of American Pathologists. A collection of 40 stock strains of pathogenic organisms was kept on agar slants in the laboratory for quality control testing and teaching purposes.

Since early May 1980, Mr. A had prepared the evening meal for his wife and two children (14-year-old boy and 6-year-old girl). He prepared the complete meal which frequently included a meat course, vegetables, salad, and cake. All family members partook of the meals, although on occasion missed a meal.

Beginning in early June, Mr. A subcultured the laboratory stock strains. There were 13 slants of shigellae (including 3 of *Shigella dysenteriae*), 6 of *S. typhi*, 3 of *S. agona*, strains of 3 other *Enterobacteriaceae*, and 2 slants of *Yersinia enterocolitica*. The procedure used was to subculture the strains to blood agar and MacConkey plates, perform biochemical testing using API 20E strips (Analytab Products, Plainview, N.Y.) and Micro-ID strips (General Diagnostics, Morris Plains, N.J.), and then, after the identity of the strain was confirmed, reculture to a slant. Several of the strains, including *S. typhi*, were given as unknowns for the student to identify. Mr. A subcultured three to five stock strains each working day. On about 17 June he subcultured six slants of *S. typhi* and two slants of *S. agona*. He believes that the source of the *S. typhi* was from a College of American Pathologists proficiency test received in March 1979. The *S. agona* was sent as part of the voluntary CDC proficiency testing program on 14 April 1978. He and the student worked on the organisms until at least 23 June (a total of five working days). Neither knew of any breaks in their techniques.

Neither Mr. A, nor the student, nor anyone else in the laboratory was ill in the next month with diarrhea or fever. Two stool cultures obtained from Mr. A on 7 and 8 July were negative for *Salmonella*. After he had a fever to an unknown degree on 11 July, a blood culture and leukocyte count were obtained, both of which were normal.

**History of the *S. agona* strain.** The movements of the *S. agona* strain were traced since 1976. In August 1976, an outbreak of salmonellosis occurred at an orphanage in Colombia, South America. Two infants from that orphanage were brought to New York and, after developing severe diarrhea, were hospitalized in early September. One month after the isolation of multiply resistant *S. agona* from these infants, two nosocomial infections with the same organism were reported. Because of the pattern of multiple antibiotic resistance, in November 1976 the isolates were sent to the Epidemiologic Investigations Laboratory Branch, CDC. In response to a request for a *Salmonella* strain with an unusual susceptibility pattern, a culture of the *S. agona* isolate was given to the Proficiency Testing Branch, CDC, in November 1977. In April 1978, it was sent to 754 laboratories as part of an antimicrobial proficiency testing exercise. After the strain was identified at the Arkansas hospital, a culture was included in the stock collection where it remained until June 1980 when it was subcultured.

**RESULTS**

**Laboratory findings.** At CDC, bacteriophage typing of the College of American Pathologists proficiency testing strain, the stock culture of *S. typhi* and the *S. typhi* isolates from patients 1 and 2 showed all to be type E-1. All four strains were susceptible to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole. Bacteriophage typing results of the *S. agona* strain from the proficiency exercise, the laboratory's stock culture, and the isolate from patient 1 were identical, and all showed the same pattern of resistance to multiple antibiotics. Review of Enteric Diseases Laboratory Section (Center for Infectious Diseases, CDC) records of antimicrobial susceptibility showed that none of 33 other strains of *S. agona* received since June 1979 have shown this resistance pattern to antibiotics. The titration of Mr. A's serum from 24 August and 24 October to *S. typhi* O, H, and Vi antigens (4, 6) showed H titers of less than 20 and Vi titers of less than 10; the first O titer was 80, and the second was 160.

**DISCUSSION**

Based on bacteriophage typing for the *S. typhi* and *S. agona* and the unusual antibiogram pattern for the *S. agona*, there is little doubt that the immediate source of both of these strains was the laboratory stock cultures. Mr. A, who worked with the strains and then prepared food for the affected patients, was the most obvious intermediary. Several cultures from him were negative, his H and Vi titers were low, and his O titer was moderately elevated, although that
result is nonspecific (9). These findings suggest that he may have passively transferred the organisms from the laboratory to his family, without having become infected himself.

These cases illustrate that not only are laboratory workers at risk for laboratory-acquired infections but that others with no direct contact to the laboratory may also be at risk. In the past year, three other cases of laboratory-acquired typhoid fever reported to CDC have occurred in persons who had not worked in a microbiology laboratory. One patient visited the clinical microbiology laboratory next door to the clinical chemistry laboratory in which she worked. Two other patients were college students attending an afternoon class in blood coagulation held in the same laboratory in which S. typhi had been handled in the morning. In these latter cases, exposure to contaminated counter tops may possibly have occurred.

Another situation possibly similar to that in Arkansas occurred in 1974, when a child whose mother was a microbiology laboratory technologist developed typhoid. His mother was in the habit of eating her lunch in the laboratory, and after working with S. typhi cultures (from a proficiency test), ate her lunch and then brought her half-eaten sandwich home for her son to finish. Both the proficiency test strain and the isolate from the child were phage type C1 (R. Waldman, personal communication).

Laboratory-acquired S. typhi infection can be a serious illness. In the past 3.5 years, 32 laboratory workers have been hospitalized due to laboratory-acquired typhoid; one patient had a partial colectomy (5). This report illustrates that laboratory-acquired salmonella infections can be fatal, not only for the laboratory worker but for family members or friends.

A laboratory proficiency test was the source of the S. typhi strain involved in these cases. These two cases are the second and third cases reported in association with that particular exercise, after an interval of more than 1 year after the exercise. The source of the S. agona strain was also a laboratory proficiency exercise for identification and antimicrobial susceptibility testing. The strain that had been sent out in April 1978 was an organism with multiple antibiotic resistance which had caused an outbreak previously and which was believed to be of enhanced virulence (2). Other laboratories may still have this strain among their stock cultures. Virulent organisms maintained on artificial media can retain pathogenic properties indefinitely (7).

Although proficiency testing of practicing microbiologists is desirable, the strains selected should meet several minimal criteria before dissemination. Whenever possible, strains should be selected that are of low virulence and that are sensitive to the antibiotics most commonly used for treatment of infections due to that organism. When this is not possible, laboratories should be advised as to the organism's susceptibility pattern shortly after the proficiency testing exercise. Whenever possible, strains for proficiency testing should have an unusual serotype, bio-type, or other marker so that laboratory-acquired infections due to that organism could be traced. The use of a marker strain would permit tracing the transmission when infection occurs in individuals not immediately connected with a laboratory. Perhaps the S. agona strain which may have been kept as a stock culture by some of the 754 laboratories to which it was sent, should be replaced with a Salmonella strain without resistance and with an unusual serotype.

Proficiency tests per se do not cause laboratory accidents; carelessness and poor techniques are the major causes for this problem (3). The use of human pathogens of high virulence for the testing or the education of students who have little experience in microbiology should be avoided. Because of the risk of infection not only to themselves but to others, microbiologists must be especially scrupulous about adherence to recognized standards of safety (11).

The continuing occurrence of laboratory-acquired infections demonstrates the need for each laboratory's management to reaffirm its responsibility for safe laboratory practices, for training of personnel in safe practices, and for maintaining on-going surveillance of these practices. Safe practices should encompass not only the handling of S. typhi but that of other bacterial, fungal, or viral agents. Care should be exercised in the acquisition and testing of patients' specimens, proficiency testing samples, and stock cultures. Except in certain situations, for example, when large quantities of a known pathogen are handled for which special precautions are needed, there should be no conscious distinction in the handling of microbiological materials on the basis of conjecture as to whether or not the materials contain a pathogen; safe practices should be applied uniformly in the microbiology laboratory.

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LITERATURE CITED