

## Outbreak of *Salmonella typhimurium* Gastroenteritis Due to an Imported Strain Resistant to Ampicillin, Chloramphenicol, and Trimethoprim-Sulfamethoxazole in a Nursery

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Received 1 June 1984/Accepted 20 August 1984

An outbreak caused by a highly resistant strain of *Salmonella typhimurium* occurred in a nursery at a university medical center. The outbreak strain, which was resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole, was apparently imported from the Far East by a Cambodian refugee. The five patients involved had severe underlying diseases, and bacteremia and meningitis developed in one of these patients. The only reservoir identified was the gastrointestinal tracts of the infected patients, and infection was probably transmitted by the contaminated hands of hospital personnel. The outbreak was rapidly brought under control by isolating cases outside of the nursery and by instituting enteric precautions for infants who remained in the nursery. When compared by disk diffusion susceptibility tests with 353 strains of *S. typhimurium* tested at the Centers for Disease Control, the imported strain had a unique antibiogram. Bacteriophage typing of the strains revealed that all were untypable; this, in itself, was a good marker, because only 5 to 10% of *S. typhimurium* isolates in this country have this property. Agarose gel electrophoresis of isolates from the five patients revealed an identical plasmid banding pattern consisting of three large and three small plasmids. Highly resistant strains of *S. typhimurium* imported from the Far East may spread rapidly when introduced into a hospital nursery. Prompt institution of control measures may limit the outbreak and prevent systemic infections for which there are few effective therapeutic agents.

In the last 30 years, several reports of outbreaks of *Salmonella* gastroenteritis in hospitalized patients have been published (1, 6, 10, 12, 15, 16, 22, 25, 27). Some of the reported outbreaks have been due to multiply antibiotic-resistant *Salmonella* strains. Drug resistance in *Salmonella* strains has been reported since 1958, when Huey and Edwards described tetracycline resistance in *S. typhimurium* (14). According to data from the Centers for Disease Control (CDC), nontyphoidal *Salmonella* strains developed increasing resistance to multiple antibiotics between the mid-1960s and mid-1970s (24). In this report, we describe an outbreak in a nursery due to a strain of *S. typhimurium* resistant to many antibiotics, including ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-SMX). The index case was in a 7-month-old Cambodian refugee who was born in a hospital in the Philippines and who emigrated, at less than 1 month of age, to the United States in September 1981.

### MATERIALS AND METHODS

**Epidemiological investigation.** The outbreak occurred in the Sick Baby Nursery (SBN) at the Medical College of Virginia. The SBN was a 16-bed unit, with all patients cared for in a single room without partitions. The age range of infants admitted to this unit was from birth to 9 months.

A case was defined as illness in a patient with a stool culture positive for the outbreak *Salmonella* strain. The following information was extracted from the medical record of each case: age, sex, date of admission, diagnoses, instrumentation, medications, diet, symptoms, results of cultures

with antibiograms, and hospital staff who cared for the patients.

Stool specimens or rectal swabs were collected from all patients in the SBN and transported to the laboratory for processing. Rectal swabs for cultures were also taken from hospital staff; these swabs were collected in tubes of selenite F broth (Difco Laboratories) for transport to the laboratory. Cultures were taken from environmental surfaces with sterile cotton swabs moistened in sterile water.

**Microbiological methods.** Rectal swabs were incubated overnight at room temperature in selenite F broth and were then subcultured to salmonella-shigella agar (Difco) and xylose-lactose-glucose agar (Difco). Swabs from environmental surfaces were plated on blood agar. Fluids for culture were added to an equal volume of brain heart infusion broth (Difco). All cultures were incubated at 37°C for 48 h. *Salmonella* organisms were identified by the method of Edwards and Ewing (9). Isolates of *S. typhimurium* were tested for their susceptibility to antibiotics by a disk diffusion technique (5) at CDC and by an agar dilution technique (21) at the Medical College of Virginia. Tube dilution susceptibility tests were performed by a previously described technique (21). The antibiotic resistance pattern of the outbreak strain was compared with strains of *S. typhimurium* studied at CDC from 1977 to 1984.

Bacteriophage susceptibility of *Salmonella* isolates was determined on phage agar by the semiautomated procedure described by Farmer et al. (11). The bacteriophage set used to type *S. typhimurium*, which was described by Anderson (3), consists of 31 phages.

Plasmid DNA was isolated by a modification of the

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method of Macrina et al. (18) and by the method described by Crosa and Falkow (8). Crude lysates obtained by both methods were either frozen for future analysis or analyzed immediately by agarose gel electrophoresis. Electrophoresis was done through a vertical 0.7% agarose gel by the method of Meyers et al. (20) and was carried out at 35 mA (~80 V) for 2 to 3 h.

**RESULTS**

**Description of outbreak.** On 25 March 1982, a 7-month-old Cambodian infant was admitted to the SBN with a diagnosis of otitis media, infantile spasms, and severe neurological damage. The patient also had a history of intracranial bleeding and meningitis due to *Escherichia coli*. The infant had been a known carrier of *S. typhimurium* since 3 weeks of age. Because there was no private room available on the floor, the infant was placed on enteric isolation in the SBN and transferred to a private room the next day. Despite brief exposure, two cases of secondary infection and two cases of tertiary infection developed with the same organism and antibiogram as that of the index case. Although neither of the secondary cases occupied beds close to the index case, each of the tertiary cases occupied a bed next to a secondary case. Tertiary cases were exposed to the secondary cases during a period when the latter were shedding the epidemic isolate in stool and before their isolation. Once identified, each case was placed on enteric isolation in a private room outside of the SBN. All infants present at that time in the SBN were placed on enteric precautions until three stool cultures were negative for *Salmonella* isolates. The last case was diagnosed on 15 April 1982, and no multiply resistant *Salmonella* strains have been isolated at the Medical College of Virginia since then. The attack rate of *Salmonella* infection during the outbreak was 10.5%.

**Epidemiological investigation.** On 12 April 1982, an epidemiological investigation was initiated. All patients (including the index case) had green or yellow loose to watery stools. Two of the patients were noted to have gross blood in their stools, and two of the remaining three had occult blood in their stools. One patient had fever, one patient had vomiting, and one had both fever and vomiting. One of the tertiary cases developed bacteremia and meningitis due to the outbreak strain.

The four cases (two secondary and two tertiary) ranged in age from 17 days to 6 months, with a mean age of 3.5 months. Three were female and one was male. All four infants had one or more underlying diseases, including

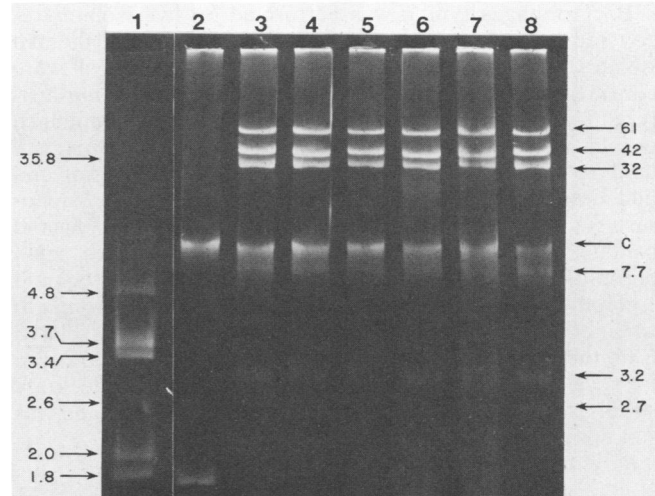


FIG. 1. Composite of the agarose gel electrophoresis of crude lysates of six strains of *S. typhimurium* from five infected patients (lanes 3 through 8) and of a *Salmonella* C<sub>2</sub> strain from an asymptomatic house officer (lane #2). The numbers on the left represent the molecular sizes (in megadaltons) of marker plasmids in reference strain *E. coli* V517 (18), and the numbers on the right represent the molecular sizes (in megadaltons) of plasmids seen in the study strains (lanes 3 through 8). C represents chromosomal DNA. Migration was from top to bottom.

bronchopulmonary dysplasia, bronchiolitis, Goldenhar's syndrome, ventricular septal defect, patent ductus arteriosus, coarctation of the aorta, congestive heart failure, and failure to thrive. Two had been treated with ampicillin before diagnosis of *Salmonella* infection. Three of the cases received only commercially prepared formula; the fourth infant received both commercially prepared formula and commercially prepared baby food. Three cases had nasogastric tubes, and in three cases the respiratory tract was suctioned. Two infants had had esophagograms and esophagomanometry.

One or more rectal cultures were taken from 51 (65%) of 78 medical personnel who had contact with the patients in the SBN. All 116 cultures were negative for the epidemic isolate, but a *Salmonella* group C<sub>2</sub> strain was recovered from one doctor. Environmental cultures were taken from a heat lamp, towel rack, oxygen flow meter, doppler, oximeter, laryngoscope handle and blade, and an Ambu bag in the SBN and from the manometer domes and pump in the gastrointestinal endoscopy suite. Cultures were taken from the immediate environment of one of the cases after she was transferred to the pediatric intensive care unit. None of the cultures yielded *Salmonella* isolates.

**Microbiology.** By the disk diffusion technique, the outbreak organisms were resistant to sulfadiazine, streptomycin, kanamycin, tetracycline, chloramphenicol, penicillin, ampicillin, carbenicillin, and cephalothin. They were susceptible to colistin and intermediate in response to nalidixic acid. Results of agar dilution susceptibility tests are shown in Table 1. Tube dilution susceptibility tests were performed with the isolates from stool and blood of the patient with sepsis. Both isolates required MICs and MBCs of greater than 100 µg of ampicillin and chloramphenicol per ml. The MIC and MBC of TMP-SMX were both greater than 16-304 µg/ml. The MIC and MBC of moxalactam were both equal to 0.78 µg/ml.

TABLE 1. Results of agar dilution susceptibility tests with the epidemic strain of *S. typhimurium*

Antibiotic	MIC (µg/ml)
Moxalactam.....	1.0
Amikacin.....	2.0
Cefoxitin.....	5.0
Gentamicin.....	5.0
Penicillin.....	>5.0
Ampicillin.....	>25.0
Nafcillin.....	>10.0
Carbenicillin.....	>250.0
Cefazolin.....	25.0
Tetracycline.....	>5.0
Chloramphenicol.....	>25.0
Erythromycin.....	>5.0
TMP-SMX.....	>8-152

Bacteriophage typing was performed on five isolates recovered from patients during the outbreak. None of the five isolates was lysed by any of 31 bacteriophages, and all were considered untypable. Ten isolates of *S. typhimurium* analyzed for plasmid content included a stool isolate from each of four patients and several stool, blood, and cerebrospinal fluid isolates from the infant with disseminated infection and underlying congenital heart disease. Agarose gel electrophoresis of 10 study isolates revealed an identical plasmid banding pattern consisting of three large and three small plasmids. Of 10 isolates from the five patients, 6 (5 stool and 1 blood) showed similar patterns (Fig. 1, lanes 3 through 8) when compared with a *Salmonella* group C<sub>2</sub> strain isolated from the stool of an asymptomatic house officer who cared for several of these infants (lane 2). In contrast to the study strains, the latter strain contained one small plasmid and was susceptible to most of the antibiotics tested.

### DISCUSSION

Although the outbreak was limited by rapid institution of control measures, serious systemic infection that was difficult to treat developed in one patient (2). The frequency with which highly resistant *Salmonella* isolates are introduced into the United States is unknown, but such strains may spread rapidly in hospitals and cause serious infections with limited possibilities for effective antimicrobial therapy. Only one nosocomial outbreak due to a *Salmonella* species resistant to ampicillin, chloramphenicol, and TMP-SMX has been reported previously, and this outbreak occurred in an adult hospital population (17). Thus, this is the first nursery outbreak reported due to a *Salmonella* strain resistant to ampicillin, chloramphenicol, and TMP-SMX. Strains of *S. typhimurium* that are this antibiotic resistant are quite rare in the United States. Among 353 *S. typhimurium* strains (sent to the Enteric Reference Laboratories, CDC for bacteriophage typing), only 6 were resistant to chloramphenicol (by disk diffusion method), and 4 of these were also resistant to ampicillin. None of the 353 strains was resistant to 10 of the 12 drugs tested unlike the present strain, but 2 were resistant to 9 drugs. Thus, the antibiogram provided a unique marker in these cases.

All patients in this outbreak had serious underlying diseases, which may have predisposed them to infection with the outbreak microorganism. Two of the patients had received ampicillin before infection, and this, as reported by others (1), may have been a risk factor for acquisition of the epidemic isolate. Three of four outbreak cases had had manipulations of their oropharynx by insertion of nasogastric tubes or by suctioning. These manipulations may have provided opportunities for inoculation of the infants by contaminated hands of personnel. Two of the patients had esophageal instrumentation, but all environmental cultures in the endoscopy suite were negative for *Salmonella* isolates.

There was no evidence of food-borne transmission. All formulas and food were commercially prepared, and infants fed the same preparations in other nursing units did not become infected with *S. typhimurium*. Since no carriers were identified among hospital staff and since all environmental cultures were negative, the most likely mode of transmission was person-to-person via the contaminated hands of hospital personnel.

Bacteriophage typing indicated that all the isolates were the same strain. Although all the strains were untypable, this, in itself, is a good marker, because only 5 to 10% of *S.*

*typhimurium* strains in this country share this property. Plasmid pattern analysis of the isolates of *S. typhimurium* revealed an identical banding pattern in all outbreak isolates, including multiple blood and cerebrospinal fluid isolates from the patient with systemic infection. A *Salmonella* C<sub>2</sub> strain isolated from the stool of an asymptomatic house officer who cared for several of these children revealed a dissimilar plasmid pattern (Fig. 1). Plasmid pattern analysis has proven useful for characterizing both gram-positive and gram-negative organisms (4, 19, 26) and, when applied to investigation of outbreaks of infections due to *Salmonella* serotypes, compares favorably with bacteriophage typing (13), biotyping, and antibiotic resistance pattern analysis (7, 23).

The strain of *S. typhimurium* that caused the outbreak appeared to have been imported from the Far East by a Cambodian refugee. Such isolates may be multiply antibiotic resistant and spread rapidly when introduced into a hospital nursery. Rapid detection and prompt institution of control measures may limit the outbreak due to such an organism and prevent systemic infections which are difficult to treat because of the few antibiotics available to which the organism is susceptible.

### ACKNOWLEDGMENTS

We thank the personnel from the microbiology laboratory of the Medical College of Virginia for laboratory assistance, Frances Hickman-Brenner for the phage and antibiogram data (disk method), and Julie Rhodes and Mary Ann Mundie for typing the manuscript.

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