Colicin Typing as an Epidemiological Tool in the Investigation of Outbreaks of Shigella sonnei

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Shigella sonnei has become the most frequently reported cause of shigellosis in the United States. Since Shigella subgroup D has no other serotypes, colicin production has been used as a basis for differentiating and identifying epidemiologically related strains. The results of colicin typing 115 cultures of S. sonnei from eight outbreaks of shigellosis occurring in widely separated regions of the United States support the usefulness of this technique. In each outbreak, the cultures were either of the same colicin type or were uniformly untypable. Unrelated cases yielded a variety of types. Definitions of the relative frequencies and geographic distributions of the various strains of S. sonnei in the United States await an accumulation of experience with the method.

Shigella sonnei has become the most frequently isolated and reported subgroup of the genus Shigella in the United States. In 1969, 60.9\% of all shigellae reported to the Center for Disease Control (CDC) were S. sonnei, as compared to 36.7\% in 1964. As S. sonnei isolations have progressively increased, there has been a corresponding decrease in the proportion of isolations of S. flexneri; S. dysenteriae and S. boydii have continued to be infrequently isolated (Fig. 1). The emergence of S. sonnei as the most common cause of shigellosis was documented earlier in the United Kingdom (6), France (4), elsewhere in Western Europe (8), and Japan (2).

Unlike the other subgroups of Shigella, which can be further divided into a number of serotypes, subgroup D contains only S. sonnei. In 1958, Abbott and Shannon (3) described a technique of differentiating strains of this organism by their capacity to produce colicins which inhibit the growth of selected indicator strains of other shigellae. Subsequently, Gillies modified the method and reported its usefulness as an epidemiological marker of S. sonnei (6).

The present paper presents the results of colicin typing in a series of eight outbreaks of S. sonnei reported to CDC from widely separated geographic areas of the United States.

Epidemiological investigations: outbreak 1—Ohio. In September and October 1968, a series of four separate common-source outbreaks of shigellosis in southwestern Ohio were associated with the ingestion of food from a single caterer (reference 9, p. 2-6). Of a total of 130 individuals known to have eaten food supplied by the firm, more than 98 persons became ill 12 to 70 hr later with symptoms characterized by severe diarrhea (many with mucus and blood), fever, abdominal cramps, and—less frequently—nausea and vomiting. Food histories implicated potato salad and chicken salad as vehicles of infection. Stool cultures from 29 patients and 1 food handler were positive for S. sonnei.

Outbreak 2—Vermont. During the months of September, October, and November 1968, 92 persons in Burlington, Vt., and a nearby suburb were known to have developed dysentery (reference 9, p. 6-7). Symptoms consisted of diarrhea, fever, abdominal cramps, occasionally vomiting, and less frequently, tenesmus and nausea. S. sonnei was cultured from the feces of 33 of those persons ill. Of the 28 index cases (first case of illness in a family), 18 were under 10 years of age. Despite a diligent and prolonged search, no common source for these cases could be found. However, some of these children could have played together, and most of the cases occurred in the lower socioeconomic section of the city.

Outbreak 3—Oregon. In March and April 1968, 31 of 36 residents in a housing development in central Oregon became ill with acute febrile gastroenteritis (reference 10, p. 11-12; Fig. 2). The illnesses lasted for 1 to 7 days (median 3 days) and were characterized by diarrhea (97\%), fever (71\%), nausea (65\%), cramps (48\%), headache (45\%), vomiting (42\%), and myalgia (19\%).

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man was hospitalized. *S. sonnei* was cultured from the stools of four persons with acute diarrhea, eight convalescent persons, and two of six visitors to the area. The epidemic curve was compatible with a common-source outbreak, and the epidemiological investigation suggested water as the vehicle of infection. The water from a shallow well shared by all residents was tested and cultures grew *S. sonnei* and coliform organisms.

**Outbreak 4—Texas.** In May 1969, four children with febrile diarrhea in a private home for mentally retarded and physically handicapped children had *S. sonnei* isolated from their stools. Subsequently, a total of 16 isolations of *S. sonnei* from children and one staff member at the home obtained over a 3-month period were sent to CDC for colicin typing. Person-to-person spread within the home was the implicated mode of transmission. No secondary cases in the community were reported.

**Outbreak 5—Oregon.** Between late July and the middle of August 1969, 37 persons in a city in southwestern Oregon developed an acute illness characterized by abdominal cramps, diarrhea, fever, and headache (reference 11, p. 6-7). Two of the children had febrile convulsions. Six persons were hospitalized; there were no fatalities. *S. sonnei* was recovered from the stools of 15 patients. Eight family groups were affected, and the index case in each of these families was always a child between the ages of 2 and 6 years. The only factor common to all of the children was their wading in a municipal pool in July 20 to 25. A water sample taken subsequently from the wading pool had a reported chlorine level of 0.5 ppm and yet was grossly contaminated with coliform organisms.

**Outbreak 6—New Jersey.** During September and November 1969, a biphasic outbreak of febrile diarrhea occurred in two of three wings of

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**FIG. 1.** Relative frequency of reported isolations of Shigella subgroups in the United States (1964 to 1969). Of the subgroups, *S. dysenteriae* and *S. boydii* each account for less than 1% of total Shigella isolations each year.

**FIG. 2.** Febrile gastroenteritis, Oregon (1969).
the pediatric nursery of a New Jersey school for the mentally retarded (reference 12, p. 6–7). The outbreak began abruptly on the A wing, in which 62 of 101 children (62%) developed diarrhea. The 86 children, aged 3 to 8 years, on B wing were spared. Affected later was the C wing, in which 43 of 63 (68%) children developed diarrhea. S. sonnei was isolated from stool specimens of 63 pediatric patients, four older inmates of the institution who work as aides in the nursery, and three attendants who are employed from the community. Transfer of personnel and patients between the A and C Wings was thought to account for the pattern of spread within the institution.

Outbreak 7—New York. In October and November 1969, an outbreak of shigellosis occurred in New York in an institution for homeless children (reference 12, p. 9–10). Figure 3 illustrates the time sequence of the dates of onset of symptomatic cases and dates of positive culture for asymptomatic persons. Two of three student nurses and 2 of 20 children were asymptomatic. Symptoms in the remaining persons included diarrhea (16 persons), fever (10), bloody stools (6), vomiting (2), and convulsions (1). The age range of affected children was 6 months to 2 years. There were 15 girls and 5 boys. S. sonnei was isolated from the stools of 18 of 35 infants and toddlers (51%) and 2 of 25 student nurses (8%) who lived or worked on a single ward.

Outbreak 8—foreign vessel. An outbreak of shigellosis occurred among the officers and crew of an oil supertanker enroute to Norfolk, Va., from Japan via Kuwait, Italy, Libya, and the Virgin Islands (13). Of a total of 42 persons abord, 28 (67%) developed febrile gastroenteritis. The index case was the chief steward—a food handler—who probably acquired his illness in Italy during the only shore leave of the voyage 2 days before his becoming ill. The abrupt onset of diarrhea in other crew members suggested a common-source outbreak; a salad hand-prepared by the chief steward was implicated when a strict vegetarian among the crew became ill. S. sonnei was isolated from the stool of the chief steward, and subsequent culture surveys revealed a total of 17 individuals with one or more isolations of S. sonnei.

MATERIALS AND METHODS

Producer strains. One-hundred-and-fifteen strains of S. sonnei forwarded to CDC by state public health laboratories were colicin typed. All but 11 of these strains were isolated from individuals involved in the eight previously described outbreaks.

Identification of strains. All strains of S. sonnei were identified biochemically and then by serological agglutination by the methods of Edwards and Ewing (5). Before typing, strains were checked for purity by streaking for isolation on MacConkey agar plates.

Indicator strains. The 15 indicator strains and 4 strains of recognized colicin type were kindly supplied by R. R. Gillies. They were stored on fresh nutrient agar in paraffin-sealed corked tubes at 25 C in the dark.

Culture media. The medium used in typing S. sonnei was freshly prepared Blood Agar Base (BBL), to which 5% rabbit or horse blood was added. Heart Infusion Broth (Difco) was used to grow the sets of indicator strains.

Colicin typing of S. sonnei. The method used was similar to the modification of the technique of Abbott and Shannon described by Gillies (6). Cultures of known stable colicin type were typed with each set of unknown test cultures to assure reliability of indicator strain susceptibility. The strain to be typed was streaked diametrically across the surface of blood-agar plates with a sterile cotton swab, making the width of the inoculum about 1 cm. Two plates for each test strain were incubated at 35 to 37 C for 24 hr.

The macroscopic growth was then removed with a glass slide. Microscopic remnants of the culture were killed by placing the inverted medium-containing portion of a 100-mm glass petri dish over its lid, which contained a circle of filter paper soaked with 3 ml of chloroform. After 15 min of exposure, the plate was opened, the filter paper was removed, and the plate was exposed to air in an exhaust hood to eliminate traces of chloroform vapor.

Cultures of the 15 indicator strains grown overnight in Heart Infusion Broth at 37 C were streaked across the full width of the surface of the chloroform-treated medium perpendicular to the line of the

![Fig. 3. Shigellosis outbreak, New York (1969).](image-url)
original test inoculum, seven strains on one plate and eight on another. A 0.01-ml quantitative loop was used to apply a uniform inoculum of each indicator strain. The plates were then incubated for 18 hr at 37 C, after which the plates were examined to record patterns of inhibition (Fig. 4).

RESULTS

A total of 115 cultures were colicin typed. Confirmation of the results in 91 cultures was kindly supplied by R. R. Gillies. Sixty-seven of these cultures were also phage typed in the laboratory of L. O. Kallings for corroborative purposes. Each outbreak will be discussed in the order that the epidemiological information was presented. The inhibition pattern results are summarized in Table 1.

Outbreak 1—Ohio. All 17 available cultures of individuals from each of the four epidemiologically related outbreaks were untypable, i.e., they did not produce colicins which inhibited any of the 15 indicator strains used. These cultures were of the same phage type.

Outbreak 2—Vermont. The nine isolations tested all corresponded to Gillies' type 4 except for variability of reactions with two of the 15 indicator strains. The phage type of eight of these specimens tested was uniform.

Outbreak 3—Oregon. Eleven S. sonnei cultures were received. Six were untypable; five of these were from patients involved in the waterborne outbreak described, and the other was from the well water incriminated. The other five cultures were obtained from persons not associated with the outbreak and were of three different colicin and phage types. Two were type 3a, and these were obtained from sisters with shigellosis in another region of the state.

Outbreak 4—Texas. All 14 cultures showed an identical pattern of inhibition of the indicator strains (Table 1, Fig. 4). However, this confirmed pattern does not correspond to that produced by any strain previously reported by Gillies (6).

Outbreak 5—Oregon. All 12 isolations from individuals epidemiologically associated with the wading pool were identical and, except for variable inhibition of indicator strain 9, correspond to Gillies' type 7 (Table 1, Fig. 4).

Outbreak 6—New Jersey. Nine of 10 cultures tested inhibited none of the 15 indicator strains. The other was unclassifiable according to recognized patterns of inhibition. Six of seven of these cultures could not be phage typed either.

Outbreak 7—New York. These 18 isolations were uniformly untypable except for one—a

TABLE 1. Patterns of inhibition of S. sonnei on indicator strains

| Indicator strain no. | 2 | 3a | 4 | 7 | Ohio (1)b | Vermont (2) | Oregon (3) | Texas (4) | Oregon (5) | New Jersey (6) | New York (7) | Ship (8) |
|----------------------|---|----|---|---|-------------|-------------|------------|-----------|------------|---------------|-------------|---------|
| 1                    | - | +  | +  | - | -           | +           | -          | +         | -          | -             | -           | +       |
| 2                    | + | +  | +  | + | -           | -           | +          | -         | -          | -             | -           | -       |
| 3                    | + | +  | +  | + | -           | +           | -          | +         | -          | -             | -           | +       |
| 4                    | - | -  | +  | + | -           | -           | +          | -         | -          | -             | -           | -       |
| 5                    | + | +  | +  | - | -           | -           | +          | -         | -          | -             | +           | +       |
| 6                    | - | +  | +  | - | +           | -           | -          | +         | -          | -             | -           | +       |
| 7                    | - | -  | -  | - | -           | -           | +          | -         | -          | -             | -           | +       |
| 8                    | + | +  | +  | - | -           | V           | +          | -         | -          | -             | -           | +       |
| 9                    | - | +  | +  | + | +           | -           | +          | -         | -          | -             | -           | +       |
| 10                   | - | -  | -  | - | -           | -           | +          | -         | -          | -             | -           | -       |
| 11                   | - | +  | +  | - | -           | -           | +          | -         | -          | -             | -           | +       |
| 12                   | - | +  | +  | - | -           | +           | -          | -         | -          | -             | -           | -       |
| 13                   | - | -  | -  | - | -           | -           | -          | -         | -          | -             | -           | +       |
| 14                   | - | -  | -  | - | -           | -           | -          | -         | -          | -             | -           | +       |
| 15                   | + | -  | -  | - | -           | +           | -          | -         | -          | -             | -           | +       |

- Symbols: +, inhibition of an indicator strain; V, variable reaction; -, no inhibition of an indicator strain; u/t, untypable strains, i.e., strains not producing colicins detectable; u/c, unclassifiable strains, i.e., strain giving patterns of inhibition differing from those of the 14 accepted colicin types.

b Numbers in parentheses refer to the outbreaks presented in the text.

c See text for explanation.
confirmed type 2. In reviewing the culture numbers with the referring laboratory, it was found that the individual from whom the type 2 culture was obtained was not related to the reported outbreak and resided in a different part of the city. The other 17 cultures were of a uniform phage type.

**Outbreak 8—foreign vessel.** These 18 cultures gave an identical pattern of inhibition. This pattern does not correspond to types heretofore recognized.

**Miscellaneous cultures.** Five *S. sonnei* isolations from Georgia, five from Oregon, and one from New York, yielded five different recognized patterns of inhibition. They were distributed among the cultures from known outbreaks during the typing procedure and were easily recognized as yielding patterns different from the uniform results within each outbreak group.

**DISCUSSION**

The reliability of colicin typing as an epidemiological marker can be assessed by two indexes. The first of these, which was not part of this study, is the constancy of type excreted by any one individual in serial isolations during clinical illness and convalescence. The second is uniformity of type in any one epidemic.

Cultures in all eight outbreaks reported in this study either demonstrated such uniformity or were untypable. Other cultures from the same states but which were unrelated epidemiologically were found to exhibit different patterns. Furthermore, in outbreak 2 (Vermont), although a common source could not be identified, the uniformity of those cultures typed substantiated the hypothesis that the spread of a single strain from person to person throughout the neighborhood was responsible for many of the cases. In outbreak 8, corroborative evidence linking the index case, who was also a food handler, to subsequent cases aboard the ship was shown by finding identical patterns of inhibition. In outbreak 1, laboratory support was provided for the hypothesis that a food handler contaminated a salad which served as a vehicle resulting in four separate but related outbreaks, when all cultures were found to be uniformly untypable.

The fact that no indicator strains are inhibited by a test organism limits but does not vitiate the usefulness of the technique, especially in areas where untypable strains are infrequent. The distribution of recognized colicine types differs in Scotland (6) and Japan (1); nonetheless, workers there have found only approximately 10% of strains are untypable. Additional indicator
strains may need to be developed for areas where the proportion of untypable strains is excessive.

It would be of interest to know the relative frequency of different colicin type of *S. sonnei* isolated in the United States. In this regard, the finding of inhibition patterns in outbreaks 4 and 8 not previously reported by Gillies (6) suggests that the distribution of colicin types in widely separated geographic areas may be quite different. Clarification of these questions must await an accumulation of experience with colicin typing of *S. sonnei* cultures in the United States.

The only alternative technique to colicin typing for differentiating strains within the subgroup D, *S. sonnei*, which has been adopted elsewhere as a routine procedure is phage typing. This technique has proved useful in Sweden in the past but currently is being reassessed (7). Cultures from outbreaks reported here, which were phage typed in Sweden, have supported the epidemiological hypotheses and the results of colicin typing.

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