Colicin Typing of Shigella sonnei

GEORGE K. MORRIS AND JOY G. WELLS

Bureau of Epidemiology, Center for Disease Control, Atlanta, Georgia 30333

Received for publication 6 November 1973

The colicin typing method for differentiating epidemic strains of Shigella sonnei was evaluated. Forty percent of the strains were untypable, but the method divides 60% of the strains into 15 established colicin types. The method has reasonably good reproducibility, but should not be interpreted with the same degree of confidence as some other characteristics such as serotypes. For this reason, known type strains should be included as controls in each analysis to ensure uniformity of results.

Over the past decade Shigella sonnei has replaced Shigella flexneri as the most prevalent species isolated from epidemics of shigellosis in the United States, now accounting for about 80% of all Shigella isolates reported to the Center for Disease Control (7). S. sonnei is unique among the shigellae in that it is the only species in the genus that does not have serological subgroups. Therefore, it is very important to be able to differentiate epidemic strains of S. sonnei. A method of differentiating epidemic strains by colicin typing was introduced in Great Britain in 1958 by Abbott and Shannon (2). The method of Abbott and co-workers (1, 2) was further modified by Gillies (10). Reller, in 1970, evaluated the colicin typing method with isolates from eight epidemics in the United States and found the method to be a useful epidemiological tool (16). Hettiaratchy et al. modified this system for typing Escherichia coli. However, only one-third of the E. coli strains were typable (12).

Since 1969, we have been evaluating the colicin typing method of differentiating strains of S. sonnei isolated in outbreaks in the United States. This paper describes the results of these studies.

MATERIALS AND METHODS

The indicator strains used in our laboratory were the same as those described by Abbott and co-workers (1, 2). Both indicator strains and colicin type strains were obtained from R. R. Gillies, University of Edinburgh, Scotland, Joan Davies, Public Health Laboratories, Guildford, England, and T. F. Elias-Jones, The City Laboratory, Glasgow, Scotland. Strains of S. sonnei to be typed were collected during outbreaks of shigellosis by our laboratories and identified as previously described (13). Strains isolated by other laboratories in the United States and sent to our laboratory for colicin typing were streaked onto MacConkey agar plates to test for purity and were tested for agglutination with S. sonnei antiserum.

 Cultures were typed by the method of Abbott and co-workers (1, 2) with the modifications of Gillies (10) and Reller (16). These methods were briefly as follows. The strain of S. sonnei to be typed was inoculated in a diametric streak on two blood agar plates and incubated at 36°C for 18 to 24 h. The macroscopic growth was scraped off the agar surfaces by using a glass slide (2.5 × 7.6 cm). The remaining culture on the plates was killed by adding 1 to 2 ml of chloroform into the lid of the petri dish containing filter paper and then by inverting the dish in the lid. After approximately 15 min, the plates were aerated by exposing the agar surface for 15 min. The 15 indicator strains were then applied at right angles to the original line of growth. Indicator strains 1 to 8 were applied to the first plate and indicator strains 9 to 15 to the second plate. The plates were then incubated at 36°C for 18 to 24 h. The patterns of inhibitions were recorded as follows: thinning of growth, +; zones of inhibition less than 2 cm in width, ++; and zones of inhibition greater than 2 cm in width, ++++. The presence of a few resistant colonies was recorded, but for the purposes of describing patterns of inhibition, any detectable inhibition was considered positive. When colicin typing results were not compatible with epidemiological data, the antibiogram determined by the method of Bauer et al. (5) was used as an aid in resolving the discrepancy.

RESULTS

In order to determine the reproducibility of the colicin typing method, known colicin type strains were repeatedly tested. During the initial studies, indicator strains 11 and 14 gave the opposite reaction to that reported by British workers on type strains 1A, 1B, 8, 10, and 12. These indicator strains were originally obtained from Gillies and were those strains used by Reller (16). Indicator strain 11, obtained from Elias-Jones, and indicator strain 14, obtained
from Davies, were found to yield reactions closer to those reported by British workers and were used in subsequent tests.

The frequency with which the various indicator strains were inhibited by the known colicin type strains can be seen in Table 1. Most of our results agreed reasonably well with results of the British workers, but it is clear that variations in the method exist.

Of all types tested in our laboratory, types 5 and 6 showed the poorest correlation with patterns reported by British workers. Type 6 has been shown even in very early work to have a variable pattern. Aoki et al. (3) reported that they were not able to distinguish type 6 and type 11. We did not experience any problems in distinguishing between these types with freshly isolated cultures. Some other major discrepancies were type 1B with indicator 10, types 3 and 8 with indicator 4, and type 13 with indicators 5 and 8. Indicators 5 and 8 also responded differently with type strains 5 and 6 than was reported previously by British workers. The colicin typing patterns of types 1A, 3A, 7, 9, 10, 11, and 13 agreed very closely with previously reported results. A Gillies type 14 strain (10) could not be obtained for study, and a Gillies type 15 strain (11) gave a pattern of inhibition identical to type 8.

The poor correlation between our results with type 5 and those previously reported by other workers may be partly due to our not having isolated type 5 from the United States. The culture used in these studies was an old strain. Freshly isolated type 9 and 11 strains were compared with old strains previously held as stock culture for more than 4 and 3 years, respectively, to test the effect of aged cultures.

### Table 1. Inhibition of indicator strains by known type strains

| Type strain | Data source | Indicator strain no.* |
|-------------|-------------|------------------------|
|             | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 1A          | CDC* | 10 | 10 | 10 | 0 | 0 | 10 | 10 | 0 | 0 | 10 | 0 | 1 | 0 | 10 |
|             | British† | + | + | + | + | - | - | + | - | - | - | - | - | - | + |
| 1B          | CDC | 8 | 10 | 9 | 0 | 0 | 9 | 10 | 0 | 9 | 5 | 0 | 0 | 0 | 10 |
|             | British | + | + | + | + | + | + | + | + | - | - | - | - | - | + |
| 2           | CDC | 0 | 10 | 7 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 10 |
|             | British | - | + | + | - | - | - | - | - | - | - | - | - | - | + |
| 3           | CDC | 10 | 10 | 10 | 4 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
|             | British | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 3A          | CDC | 10 | 10 | 10 | 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
|             | British | + | + | + | + | + | + | + | + | - | - | - | + | + | + |
| 4           | CDC | 9 | 10 | 9 | 0 | 8 | 0 | 0 | 8 | 10 | 0 | 9 | 8 | 10 | 10 |
|             | British | + | + | + | + | + | + | + | - | - | + | + | + | - | + |
| 5           | CDC | 10 | 10 | 10 | 0 | 3 | 10 | 10 | 3 | 6 | 10 | 5 | 3 | 7 | 10 |
|             | British | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 6           | CDC | 0 | 10 | 10 | 0 | 3 | 10 | 10 | 3 | 6 | 10 | 5 | 3 | 7 | 10 |
|             | British | + | + | + | + | + | + | + | + | - | - | - | - | - | + |
| 7           | CDC | 0 | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|             | British | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 8           | CDC | 10 | 10 | 10 | 10 | 0 | 10 | 0 | 0 | 0 | 0 | 1 | 10 | 1 | 10 |
|             | British | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 9           | CDC | 10 | 10 | 10 | 0 | 5 | 10 | 10 | 10 | 10 | 0 | 0 | 10 | 0 | 10 |
|             | British | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 10          | CDC | 10 | 10 | 10 | 1 | 10 | 10 | 10 | 10 | 10 | 10 | 0 | 0 | 10 | 0 |
|             | British | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 11          | CDC | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
|             | British | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 12          | CDC | 10 | 10 | 10 | 0 | 10 | 10 | 10 | 10 | 10 | 10 | 0 | 0 | 10 | 0 |
|             | British | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 13          | CDC | 0 | 10 | 10 | 1 | 10 | 10 | 10 | 10 | 10 | 10 | 0 | 0 | 0 | 10 |
|             | British | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

* The indicator strain numbers correspond respectively with: S. sonnei 2, 56, 17, 2M, 38, 56/56, 56/98, R1, R6; S. schmitzi M.19 (NCTC8218); S. sonnei 27, 264, 215, R5, and E. coli Row.

† Number of times indicator strains were inhibited when tested 10 times at the Center for Disease Control (CDC).

‡ Reactions reported by British workers.
on colicin typing patterns (Table 2). A slight deterioration of the patterns was noted with the old type 9 strain, but a very serious deterioration in pattern of the type 11 strain occurred. The pattern of the old type 11 resembled the pattern found with type 6 strains.

The colicin typing procedure was used to examine cultures isolated from 33 outbreaks of S. sonnei shigellosis in the United States. We examined from 3 to 60 strains from each epidemic, usually about 15, to determine if all epidemiologically related strains were the same colicin type. A summary of these investigations is shown in Table 3. Type 4 caused one outbreak, type 6 caused two, type 7 caused one, type 8 caused three, and type nine caused 10 outbreaks; 16 outbreaks were caused by untypable strains in that none of the indicator strains were inhibited.

The cultures from these 33 outbreaks included cultures from the eight outbreaks investigated by Reller (16). Our results agreed with Reller's except for two outbreaks, numbered 4 and 8. Reller found that strains from outbreaks 4 and 8 had patterns of inhibition different from previously reported. We found the strains from his outbreak no. 4 to be identical to colicin type 9, and cultures from his outbreak no. 8 to be identical to colicin type 8.

In 4 of the 33 outbreaks, strains appearing to be epidemiologically related were collected which were of a colicin type different from the predominant strains from the outbreaks. The colicin typing results are shown in Table 4 along with the antibiotic resistance patterns. In outbreak 10, there were three isolates which demonstrated a colicin type different from the predominant strain implicated in the epidemic (the unclassifiable strains differed on indicators 10 and 13 from type 9). The same three isolates had a different antibiotic resistance pattern. In outbreak 11 one culture was a different colicin type, but had the same antibiotic resistance pattern. Outbreak 14 represented simultaneous outbreaks in a mental hospital and in the near-by community. The two occurrences were thought to be epidemiologically related. Two colicin types were identified, and the antibiograms of the two types differed. The antibiograms of the type 9 strains were not uniform, but this is not unusual for hospital situations where antibiotic resistances are frequently acquired. Outbreak 18 involved 300 children in a church camp. The camp had been set up temporarily in an abandoned school. Conditions were extremely overcrowded, and subsequent studies revealed that the water supply was grossly contaminated by sewage. Examination of cultures from 45 patients from this epidemic revealed 44 untypable strains, one type 8 and one type 3. Antibiotic susceptibility tests showed the untypable strains and the type 3 strains to be multiply sensitive, but the type 8 strain was resistant to three antibiotics.

The frequency with which the various types were identified from all sources is shown in Table 5. The large number of identical strains from an epidemic situation was included only once in this summary. Sporadic isolates were also counted once. Forty percent of these strains were untypable, 22% were type 9, and the rest were scattered among eight known types and the unclassifiable group. The unclassifiable group includes organisms producing inconsistent or atypical patterns.

### Table 3. Colicin types of epidemic strains of Shigella sonnei from 33 outbreaks

| Type | 1968 | 1969 | 1970 | 1971 | 1972 | Total |
|------|------|------|------|------|------|-------|
| 4    | 1    |      |      |      |      | 1     |
| 6    |      | 2    |      |      |      | 2     |
| 7    |      | 1    |      |      |      | 1     |
| 8    |      | 1    | 2    |      |      | 3     |
| 9    |      | 1    | 3    | 4    | 2    | 10    |
| Un typable | 1 | 3 | 1 | 4 | 7 | 16 |

### Table 2. Change in colicin production due to aged cultures

| Type strain | Culture     | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|-------------|-------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|
| 9           | New strain  | 10*|10 |10 |10 | 0 |10 |10 |10 |10 | 0  | 0  | 0  |10  | 0  |10 |
|             | Old strain  | 10 |10 |10 |10 | 4 |10 |10 |10 |10 | 0  | 0  | 0  | 9  | 1  |10 |
| 11          | New strain  | 0  | 0  | 0  | 0  |0  | 0  |0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
|             | Old strain  | 0  | 8  | 1  | 0  |0  | 3  |6  | 8  | 4  | 0  | 0  | 0  | 0  | 0  | 0  |

* Number of times indicator strains were inhibited when tested 10 times at the Center for Disease Control.

* Culture was held on stock medium for more than 4 years.

* Culture was held on stock medium for more than 3 years.
TABLE 4. Outbreaks yielding multiple colicin types of Shigella sonnei

| Outbreak no. | Mode of spread          | No. of patients from whom isolates were examined | Colicin type | No. of each type | Antibiotic resistances* |
|-------------|-------------------------|-------------------------------------------------|-------------|-----------------|------------------------|
| 10          | Foodborne               | 37                                              | 9           | 34              | Multiplicity sensitive |
|             |                         |                                                 | UC*         | 3               | Te, Su, St             |
| 11          | Person-to-person        | 9                                               | 8           | 8               | Te, Su, St, Ch         |
| 14          | Hospital, person-to-    | 15                                              | 9           | 14              | 2-Ka, Ne, Su, St, Am   |
|             | person                  |                                                 | UT*         | 1               | 1-Ka, Ne, Su, St, Am,  |
|             |                         |                                                 |              |                 | Te, Ce, Ch             |
| 18          | Community, person-to-   | 5                                               | UT          | 1               | Multiplicity sensitive |
|             | person                  |                                                 |              |                 | Su                     |
|             | Water and person-to-    | 45                                              | 9           | 1               | Multiplicity sensitive |
|             | person                  |                                                 | UT          | 44*             | Te, St, Am             |
|             |                         |                                                 | 3           | 1               | Su                     |
|             |                         |                                                 | 8           | 1               | Te, Su, St             |

*The antibiotics are abbreviated as follows: tetracycline (Te); sulfadiazole (Su); chloramphenicol (Ch); kanamycin (Ka); neomycin (Ne); ampicillin (Am); cephalothin (Ce).

* Unclassifiable.

* Untypable.

* One patient yielded both an untypable strain and a type 3 strain.

TABLE 5. Colicin types of Shigella sonnei identified in United States

| Colicin type | Caused epidemic | Sporadic incident | Total | % |
|-------------|-----------------|-------------------|-------|---|
| 2           | 0               | 2                 | 2     | 4 |
| 3           | 0               | 1                 | 1     | 2 |
| 3A          | 0               | 1                 | 1     | 2 |
| 4           | 1               | 2                 | 3     | 6 |
| 6           | 2               | 0                 | 2     | 4 |
| 7           | 1               | 1                 | 2     | 4 |
| 8           | 3               | 1                 | 4     | 8 |
| 9           | 10              | 1                 | 11    | 22|
| 11          | 0               | 2                 | 2     | 4 |
| Untypable   | 16              | 4                 | 20    | 40|
| Unclassified| 0               | 2                 | 2     | 4 |

Because untypable strains constituted 40% of all the strains we examined, we attempted to subtype the untypable group by using the set of indicator strains as the colicin producers and the epidemic strains as the indicator of colicin production, that is, by measuring colicin sensitivity rather than colicin production of the epidemic strain. This method is used in bacteriophage typing of other bacteria and has been used by Aoki et al. (3) in Japan for subtyping S. sonnei. We subtyped the untypable strains from 3 epidemics. Two patterns of sensitivity were noted (Table 6). Some strains from all 3 epidemics gave subtype pattern I, but some strains from 2 of the epidemics also gave subtype pattern II. Multiple isolates from a single individual gave both subtype patterns; therefore, it appears that this may not be a reliable means of subtyping these strains.

DISCUSSION

Most of the colicin type patterns observed with known type strains were reasonably close to those reported by British workers. It appears that some variation in the system does occur between laboratories and from day to day within a laboratory. Slight differences in media, incubation temperature, and incubation time may influence colicin production (6, 14). However, the significance of the slight changes in colicin production patterns is minimized by testing known type strains along with the epidemic strains being examined. Age of the culture may also have an effect on colicin production as was shown by the types 9 and 11 strains in these studies.

With most of the epidemiologically related cultures investigated, a uniform colicin production pattern was noted. Exceptions were observed in four outbreaks, and these discrepancies may be due to three things. (i) Study of any large population may reveal some carriers of a disease or persons with diseases having no epidemiologic relationship to the rest of the group, which could have been the case in outbreak 18. (ii) When epidemics are due to
grossly contaminated situations, more than one strain of the pathogen may be present. This has been noted occasionally in large outbreaks of salmonellosis (4). (iii) The colicin-producing characteristics of an organism may be transferred between bacteria by means of an episome similar to the transfer of antibiotic resistance (8, 9, 15). The latter could explain outbreak 10 (simultaneous episomal transfer of colicin and antibiotic factors) and outbreak 11 (independent transfer of colicin factor). In some epidemic situations, it is impossible to determine which of these factors is involved.

Forty per cent of the strains were untypable, and attempts to subdivide these isolates by testing colicin sensitivity were unsuccessful. The method divides 60% of the strains into 15 established colicin types.

We could not obtain a Gillies type 14 for testing. A Gillies type 15 was found to be identical to Abbott’s type 8 (1) in our studies. Cultures from two outbreaks previously reported to be unclassifiable by Reller (16) were found to be types 8 and 9 in these studies. However, Reller did not have cultures of all known types available for inclusion in his studies, and his indicator strains 11 and 14 differed from those used in these studies. This points up the necessity for including known type strains in each analysis to ensure that the system is working properly.

The colicin typing system appears to be a useful method of differentiating strains. The method has reasonably good reproducibility, but should not be interpreted with the same degree of confidence as some other characteristics such as serotypes. For this reason, known type strains should be included as controls in each run to ensure uniformity of results.

ACKNOWLEDGMENTS

We are grateful to J. D. Abbott and Joan Davies for confirming the colicin typing results of selected cultures. We also thank Carolyn Steele for laboratory assistance and members of the Epidemic Intelligence Service, Center for Disease Control, for providing epidemiological data.

LITERATURE CITED

1. Abbott, J. D., and J. M. Graham. 1961. Colicine typing of Shigella sonnei. Mon. Bull. Min. Health Public Health Lab. Serv. Directed Med. Res. Coun., 20:51-58.
2. Abbott, J. D., and R. Shannon. 1958. A method for typing Shigella sonnei using colicine production as a marker. J. Clin. Pathol. 11:71-77.
3. Aoki, Y., T. Naito, S. Matsuo, N. Fujise, A. Ikeda, K. Miura, and Y. Yakushiji. 1967. Colicine typing of Shigella sonnei. Jap. J. Microbiol. 11:73-85.
4. Armstrong, R. W., T. Fodor, G. T. Culin, A. B. Cohen, G. K. Morris, W. T. Martin, and J. Feldman. 1970. Epidemic Salmonella gastroenteritis due to contaminated imitation ice cream. Amer. J. Epidemiol. 91:300-307.
5. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized disk method. Amer. J. Clin. Pathol. 45:493-496.
6. Blackford, V., L. W. Parr, and M. L. Robbins. 1951. Antibiotic activity of selected enteric organisms. Antibiot. Chemother. 1:392-398.
7. Center for Disease Control. Shigella surveillance report, no. 33, April 1973. Center for Disease Control, Atlanta.
8. Farrall, W. N., and A. J. H. Tomlinson. 1966. Some studies on the epidemiology of Sonne dysentery. Changes in colicine type and antibiotic resistance between 1956 and 1965. J. Hyg. 64:297-303.
9. Fredericq, P. 1957. Colicins. Annu. Rev. Microbiol. 11:7-22.
10. Gillies, R. R. 1964. Colicine production as an epidemiological marker of Shigella sonnei. J. Hyg. 62:1-9.
11. Gillies, R. R., and D. O. Brown. 1966. A new colicine type (type 15) of Shigella sonnei. J. Hyg. 64:305-308.
12. Hettiaratchy, I. G. T., E. M. Cooke, and R. A. Shooter. 1973. Colicine production as an epidemiological marker of Escherichia coli. J. Med. Microbiol. 6:1-11.
13. Morris, G. K., J. A. Koehler, E. J. Gangarosa, and R. G. Sharrar. 1970. Comparison of media for direct isolation and transport of Shigella from fecal specimens. Appl. Microbiol. 19:434-437.
14. Naito, T., M. Kono, N. Fugise, Y. Yakushiji, and Y. Aoki. 1966. Colicine typing of Shigella sonnei. Jap. J. Microbiol. 10:13-22.
15. Ozeki, H., B. A. D. Stocker, and S. M. Smith. 1962. Transmission of colicinegeny between strains of Salmonella typhimurium grown together. J. Gen. Microbiol. 28:671-678.
16. Reller, L. B. 1970. Colicine typing as an epidemiological tool in the investigation of outbreaks of Shigella sonnei. Appl. Microbiol. 21:21-26.