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Colicine production as an epidemiological marker for *Shigella sonnei*

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SUMMARY

The reliability of colicine typing of *Shigella sonnei:* as an epidemiological marker has been investigated. Strains from 13 of 110 excretors showed variation in type as determined by typing serial isolates and in 14 of 106 epidemics there was a lack of uniformity in typing results.

The simultaneous presence of two types was found in 11·4 % of patients. *In vitro* variation was seen after 10 days in 6·8 % of isolates from 44 stools; variation had risen to 9 % when isolates were re-examined after one month.

These results suggest that restrictions should be placed on the use of colicine typing as an epidemiologic tool.

INTRODUCTION

Although colicine typing of *Shigella sonnei* has been in use for many years, data on reliability of colicine production as an epidemiological marker are not consistent. Abbott & Shannon (1958) and Gilies (1964), found the method reliable judging by the constancy of type in repeated isolation from individuals and the uniformity of type in epidemics. No changes in colicine production were observed when cultures were re-examined after six months or even years of storage. In contrast it had been reported by Fredericq (1948), that colicinogenic strains may lose the ability to produce colicines after prolonged storage. Recently, Helgason & Old (1981), reported, as very frequent, qualitative differences in observed colicine types among duplicate subcultures of the same isolates after storage and among different isolates of the same strain from episodes involving individual persons or members of the same family. As a result they concluded that the colicine typing method was highly unreliable for epidemiological studies. Green *et al.* (1968) had reported similar findings; when 37 single isolates from the years 1963–9 were retyped in 1974, 11 of them gave inhibition patterns different from those obtained originally.

Since we have used colicine typing for many years, we wish to present our own data concerning the reliability of this method.

MATERIALS AND METHODS

A modified version of the Abbott & Shannon (1958) method was used. The strain of *S. sonnei* to be typed was inoculated across the diameter of a blood agar plate and incubated at 37 °C for 48 h. Growth was killed by placing 1–2 ml of chloroform in the inverted lid of the Petri dish containing filter paper and exposing the culture to chloroform vapour for 1 h. Growth was then scraped to one end and removed together with a small portion of agar. More chloroform was added and the plate was left closed on the bench for a further hour. The filter paper was then removed and the plate left exposed to the air for 3 h with the lid open and the surface of the medium face down. Overnight broth cultures of the indicator organisms were then inoculated at right angles to the position of the original inoculum. The plate was examined after 24 h of incubation.

Nine indicator strains were used for typing – *S. sonnei* 56, 17, 56/56, 2, R6, 2/7, R5, *S. dysenterie* 2 – M19 and *Escherichia coli* Row. Indicator strains were provided by Dysentery Reference Laboratory, London.

The strains of *S. sonnei* typed were obtained from Belgrade Public Health Laboratory and from the Laboratory of the Clinic for Infectious Diseases, Belgrade.

RESULTS

The reliability of colicine typing as an epidemiological marker has been assessed in the usual ways by (*a*) typing strains from serial isolations of one individual during clinical illness and convalescence, (*b*) typing strains epidemiologically related and (*c*) retyping the same strains after storage.

Tables 1 and 2 show the results in relation to uniformity of typing results. Non-uniform results are discussed in the text.

The results presented in Tables 1 and 2 are in agreement with those reported by Abbot & Shannon (1958) and by Gillies (1964).

The recovery of different types from an individual or during an epidemic, could have several explanations – multiple infection, superinfection, *in vivo* or *in vitro* changes in colicine production and, in the case of epidemics, the existence of more than one source of infection.

In order to determine whether more than one colicine type was present at a time in the same person, we typed 217 *S. sonnei* strains isolated from 44 patients. All colonies that differed morphologically (from two to six colonies) from one diagnostic plate were colicine typed. Colicines were produced by 120 (55·3 %) of the 217 strains; they were classified into six different types [types 5 (2·3 %), 6 (20·3 %), 13 (2·8 %), 14 (5·1 %). 15 (17·5 %) and n/c (7·3 %)].

To determine *in vitro* instability, the same strains were typed three times within a 10-day period after isolation and again a month later.

The simultaneous presence of two different types was established in five patients (11·4 %). In all of them one strain belonged to type 6 and the other to one of the following types: 13, 15 u/t and two n/c types.

Changes in inhibition patterns was found in four (1·8 %) of the 217 strains typed three times in 10 days. Two strains belonged originally to type 6. When first retyped one strain became type 13 and did not change any more, the other one lost the ability to produce colicine but a month later was identified again as type 6. Two other strains, both from the same stool specimen, originally n/c types, changed their inhibition pattern twice; at the first retyping, having different n/c patterns and at the last retyping, becoming type 6.

Table 1. *Serial isolation from* 97 *excretors of* Shigella sonnei *who showed uniformity of colicine type**

Colicine type	No. of samples 2	3	4	5	6	7	10	Total no. of strains
u/t	18	11	2	6	2	3	1	150
4	12	6	1	1	1	—	—	57
6	3	3	1	—	—	—	—	19
2	—	4	1	—	—	—	—	16
15	2	—	—	1	1	—	—	15
3	3	3	—	—	—	—	—	15
n/c	6	—	—	—	—	—	—	12
8	—	1	1	—	—	—	—	7
3A	1	1	—	—	—	—	—	5
13	1	—	—	—	—	—	—	2
No. of persons	46	29	6	8	4	3	1	

u/t = untypable strain. n/c = unclassified type.
* Thirteen other individuals showed variation in the type excreted.

Table 2. *Analysis of* 92 *epidemics of* Shigella sonnei *in which there was uniformity of colicine type**

Colicine type	No. of persons in epidemics 2	3	4	5	7	8+	No. of Epidemics	People
u/t	34	4	2	—	2	6†	48	262
4	17	3	—	—	—	3‡	23	82
2	5	—	—	1	—	—	6	15
6	6	—	—	—	—	—	6	12
13	—	2	—	—	—	—	2	6
n/c	2	1	—	—	—	—	3	7
14	—	—	—	—	—	1§	1	14
8	—	—	—	—	1	—	1	7
3	1	—	—	—	—	—	1	2
15	1	—	—	—	—	—	1	2
Total	66	10	2	1	3	10	92	409

u/t = untypable strain. n/c = unclassified type.
* In 14 other epidemics there was lack of uniformity of type.
† 12, 15, 17, 25, 44 and 47 cases respectively in each epidemic.
‡ 8, 10 and 21 cases.
§ 14 cases.

Retyping of the same strains a month after isolation revealed changes in ten strains (4·6 %). Four strains, of which three came from the same stool specimen, were originally n/c types and then type 6. Five strains, all from the same specimen, belonged first to type 4 and then became type 14. One strain, originally type 6, changed to type 15.

When the frequency of type changes was considered in relation to the original 44-stool specimens, changes in type were observed in strains from three out of 44 specimens (6·8 %) at 10 days and from four out of 44 specimens (9 %) at one month.

Most of the variable strains changed once in type, but in three strains changes were observed two and three times.

DISCUSSION

It seems possible that observation of differing frequencies of variable strains could depend on the type prevalent in the investigation; that is, that colicine types could differ in their instability. In our study, variations in inhibition patterns were found in strains type 4, type 6 and n/c types. Typing conditions could also influence the results. Abbott and Graham (1961) pointed out the importance of having a standard medium as 'the activity of certain colicines such as those produced by type 6 and 7, is affected by small changes in the composition of the medium'. Helgason & Old (1981) showed that changes in the time of incubation of typing strains has a similar effect.

The majority of unstable strains were isolated from stool specimens where two different types were found simultaneously. In one of five such specimens, changes in inhibition patterns appeared in a 10-day period. In three specimens, changes were recognized a month after isolation. As a result of changes, all colonies in these four specimens became uniform in type. In that way at the end of the investigation two different types were present only in one patient – type 6 and a colicine non-producer strain were found in his stool.

As suggested previously finding more than one type in an individual at the same time could be the result of multiple infection or superinfection, but could also be the result of *in vivo* variation. The fact that in four of five patients originally possessing two different types, type differences between strains were later lost, could be most probably explained as a result of *in vivo* variation.

The presence of different types of *S. sonnei* in one individual, as well as *in vitro* instability of some colicinogenic types, both confirmed in this investigation, requires that this method be used only as supplementary proof of epidemiologic data concerning ways of transmission and source of infection. As storage of strains can influence colicine production, it seems advisable to perform typing immediately after isolation, particularly as typing of strains after long storage is not, independently of type variation, of any practical use.

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