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A new colicine type (type 15) of *Shigella sonnei*

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INTRODUCTION

Colicine typing of *Shigella sonnei* was introduced in this country by Abbott & Shannon (1958); the method depends on the production of various patterns of inhibition on a stock set of indicator or passive strains of other shigellas and interest in the technique has increased rapidly in the last few years. In an earlier publication (Gillies, 1964) modifications of the original technique were described and further evidence was offered of the reliability of colicine type as an epidemiological marker; a hitherto unrecognized type (type 14) of *S. sonnei* was described and evidence was given of its distinctive character.

The present paper furnishes evidence for the existence of another new colicine type (type 15) of *S. sonnei* which was first recognized in Edinburgh in 1965.

MATERIALS AND METHODS

Strains

The twenty-two cultures, from which 181 colonies of the new colicine type of *S. sonnei* were tested, were harvested from faecal specimens in laboratories in Edinburgh and Glasgow and all but three were from residents in Edinburgh. Each colony was identified by its biochemical reactions in tests with composite media (Gillies, 1956) and by agglutination tests with specific antiserum.

The indicator strains were those employed by all British workers and were acquired originally from Dr J. D. Abbott.

Media

Infusion broth was used to culture the indicator strains once weekly or as often as required and was prepared according to Cruickshank (1965); tryptone soya agar (T.S.A.) (Oxoid) was prepared according to the maker's instructions and 5% horse blood added (T.S.B.A.).

Typing technique

This has been detailed previously (Gillies, 1964) and may be summarized as follows. The strain of *S. sonnei* to be typed is inoculated in a diametric streak on a T.S.B.A. plate which is then incubated at 35–36° C. for 24 hr. The macroscopic growth is removed with a sellotape-sheathed glass slide and microscopic remnants of growth are sterilized by pouring 3–5 ml. of $CHCl_3$ into the lid of the Petri dish and replacing the medium-containing portion over the lid; after 15 min. the plate is opened and residual $CHCl_3$ is decanted into a beaker. The medium is exposed to

the air for 3 min. and the fifteen indicator strains are then applied at right-angles to the original line of growth.

The plate is then re-incubated overnight at 37° C. and during this period any colicines produced by the original inoculum will exert their inhibitory activity on the indicator strains. The various patterns of inhibition are then observed (Table 1).

RESULTS

A profuse and almost pure culture of *S. sonnei* was isolated from a faecal specimen submitted from a 7-year old boy on 17 October 1965. Colicine typing of three colonies from the diagnostic plate gave identical patterns of inhibition on the indicator strains (Plate 1), but these differed from the patterns given by any of the sixteen recognized colicine types (Table 1). Fifteen more colonies from the same diagnostic plate were tested and they also gave this new pattern of inhibition; this child was one of a family (S) of eleven and in the next few days five siblings and the father were found to be excreting *S. sonnei*.

Table 1. *Patterns of inhibition given by seventeen colicine-type (producer) strains of* Shigella sonnei *in tests against fifteen standard indicator strains*

Indicator strain no.	Colicine type of producer strain																
	1*a*	1*b*	2	3	3*a*	4	5	6	7	8	9	10	11	12	13	14	15
1	+	+	−	+	+	+	+	−	−	+	+	+	−	+	−	+	+
2	+	+	+	+	+	+	+	+	−	−	+	+	−	+	+	+	−
3	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	+	+
4	−	−	−	−	−	v	v	−	−	−	−	−	−	−	−	v	+
5	−	−	−	+	+	+	+	−	−	+	v	+	−	+	−	+	+
6	+	+	−	+	+	−	+	+	−	−	+	+	−	+	+	+	−
7	+	+	−	+	+	−	+	+	−	−	+	+	−	+	+	+	−
8	−	−	−	+	+	+	+	−	−	+	+	+	−	+	−	+	+
9	−	+	+	+	+	+	+	+	−	−	+	+	−	+	+	+	−
10	+	+	−	+	−	−	+	−	−	−	−	+	−	−	−	−	−
11	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	+	+
12	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−
13	−	−	−	+	+	+	+	−	−	+	+	+	−	+	−	+	+
14	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	+
15	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+

The indicator strain numbers correspond respectively with: *S. sonnei* 2, 56, 17, 2 M, 38, 56/56, 56/98, R 1, R 6; *S. schmitzi* M. 19 (NCTC 8218); *S. sonnei* 2/7, 2/64, 2/15, R 5 and *Escherichia coli* Row.

+ = Inhibition of an indicator strain; v = variable reaction; − = no inhibition of an indicator strain.

Typing of several colonies from each of the diagnostic plates revealed that every strain gave the new inhibitory pattern. During this time another family (L) submitted specimens of faeces and the strains of *S. sonnei* isolated from the index case and the other four members of this household gave the inhibition pattern of the new colicine type.

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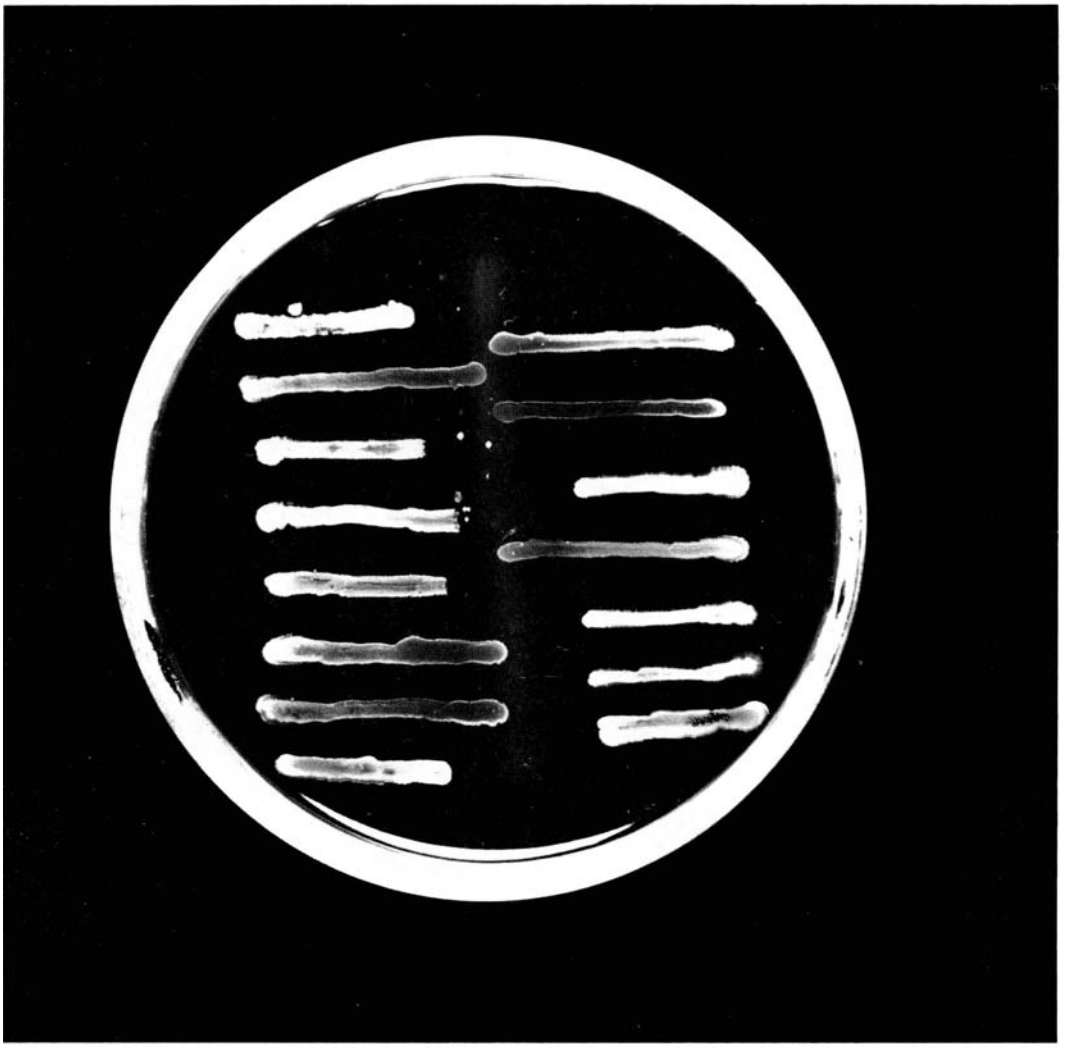

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Inquiry at the Public Health Department revealed that although the two families lived in different municipal wards they regularly visited each other's homes.

DISCUSSION

Our indices of the reliability of colicine type as an epidemiological marker of *Shigella sonnei* are that replicate isolations from an individual should all be of the same type and that there should be uniformity of type in all cases in an epidemic situation; these indices are fulfilled completely in the material presented here.

The number of specimens, the number of positive isolations and the number of colonies typed are summarized in Table 2.

Table 2. *Summary of epidemiological information concerning colicine type* 15 *strains of* Shigella sonnei

	Family S	Family L	Family B	Individuals
No. of persons in family or group	11	5	2	4
No. of specimens	22	8	2	4
No. of persons with *S. sonnei* (type 15) in 1st specimen	7	5	2	4
No. of persons with *S. sonnei* (type 15) in 2nd specimen	4	0	N.T.	N.T.
No. of positive specimens = 22				
No. of colonies tested = 181				
Average number of colonies tested per specimen = 9 (range 1–33)				

N.T. = not tested.

Search of our records since 1959 revealed two further, unrelated patients who were excreting strains of *S. sonnei* that gave inhibition patterns identical with those of the strains from the two families, S and L. These isolations had been made in October 1963 and March 1965 and the strains had been labelled 'unclassifiable' since the pattern was then new and we had no evidence of its significance. The child from whom the isolation was made in March 1965 was, at the time, living in a residential nursery. The possibility was considered that some of the children in family S may have been accommodated in the same nursery at that time, but this was not so.

Similarly no link could be found between families S and L and the adult case in 1963. Eight days after the last isolation from family S a child with bacillary dysentery in another residential nursery was found to be excreting *S. sonnei* of the new colicine type, but no association could be found with any of the previous cases.

The latter child had only recently come from Glasgow and this fact prompted the colicine typing of strains of *S. sonnei* acquired from the City Laboratory in Glasgow. Of 584 strains thus examined three were of the new type; two of these strains had been isolated during August, 1965 from a husband and wife (family B) and the third strain from an unrelated case in November 1965. Search of our records of colicine examinations made on strains received from Glasgow laboratories in previous years did not reveal any instances of the new type.

We considered the possibility that the new type pattern might be an artifact associated with some disturbance of the indicator strains, either by contamination or through variation in their sensitivity to the various colicines. This explanation was unlikely since other producer strains that were being typed at the same time as those of the new type gave various patterns characteristic of established types. Nevertheless, we tested several strains of each established type from our stock of cultures and each gave its typical pattern of inhibition (Table 1).

It will be interesting to note the spread of this new type (type 15) in our locality and we hope that, following this report, its recognition elsewhere will be communicated.

SUMMARY

1. A new colicine type (type 15) of *Shigella sonnei* is described.

2. The epidemiological circumstances associated with its appearance in Edinburgh and Glasgow are summarized.

3. Our indices of reliability as an epidemiological marker are fulfilled by this new type.

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REFERENCES

ABBOTT, J. D. & SHANNON, R. (1958). A method of typing *Shigella sonnei* using colicine production as a marker. *J. clin. Path.* **11**, 71–7.

CRUICKSHANK, R. (1965). *Medical Microbiology*, 11th ed. p. 740. Edinburgh and London: E. and S. Livingstone Ltd.

GILLIES, R. R. (1956). An evaluation of two composite media for preliminary identification of *Shigella* and *Salmonella*. *J. clin. Path.* **9**, 368–71.

GILLIES, R. R. (1964). Colicine production as an epidemiological marker of *Shigella sonnei*. *J. Hyg., Camb.* **62**, 1–9.