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Colicine production as an epidemiological marker of *Shigella sonnei*

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INTRODUCTION

For at least 20 years Sonne's dysentery bacillus has been responsible for most cases of diarrhoea in which a causal agent has been incriminated. Several attempts have been made to establish a method of differentiating strains of this organism, which, among the Shigellae, is unique in being a serological entity; biochemical reactions (Bojlén, 1934; Tee, 1952) allow only subdivision into strains which do or do not ferment xylose. The value of such a marker is further reduced since only a minority of strains ferment this substrate.

Bacteriophage typing, introduced in Sweden (Hammarström, 1947; 1949), has been assessed in Britain by Mayr-Harting (1952) and Tee (1955); although at least thirteen types could thus be identified evidence of type instability *in vivo* discouraged the further use of this method for epidemiological purposes.

Davies (1954) reported that the determination of sensitivity of *Shigella sonnei* to sulphonamides may yield useful information regarding the spread of infection; similarly, she noted that occasional strains were exacting in regard to tryptophan or histidine with the synthetic medium employed for sensitivity testing. Such information may be useful on a short term basis but it is of restricted application, since in many areas the majority of Sonne strains are now resistant to members of the sulphonamide group.

In 1958, Abbott & Shannon investigated the susceptibility of *S. sonnei* to colicines produced by strains of *Escherichia coli*; the variation in sensitivity to the active coli strains of Sonne cultures which were epidemiologically related led these authors to conclude that 'it was unlikely, therefore, that the sensitivity patterns of Sonne strains to colicines could be used as a basis for a typing method'. Conversely, they noted that epidemiologically significant types could be established if colicine production by the Sonne strains was used as a marker; thus colicine types of *S. sonnei* are identified by the patterns of inhibition produced on selected indicator or passive strains of other Shigellae.

Subsequently Abbott & Graham (1961) detailed the type incidence of 1247 strains of *S. sonnei* in England (mostly from Manchester) and extended the number of recognizable types from 7 to 15; Barrow & Ellis (1962) recorded the type incidence of 896 strains in the Bradford area and added further evidence of the reliability of colicine typing as an epidemiological marker.

The present paper describes modifications to the technique used by previous workers and records the validity of the method as an epidemiological tool.

MATERIALS AND METHODS

Strains. Indicator strains and colicine-type strains of *S. sonnei* were obtained from Dr J. D. Abbott and have been maintained on Dorset's egg medium at 4° C. with subculture every 3–4 months. Strains of *S. sonnei* to be typed were acquired from Ruchill Hospital, Glasgow, during 1959–60 and from Leeds since 1960, as well as strains from our own diagnostic laboratories since 1959; all strains were checked biochemically using composite media (Gillies, 1956) and by agglutination tests with specific antiserum before being tested for colicine production and determination of sensitivity to sulphonamides, tetracyclines, chloramphenicol, streptomycin, 'Humatin' and 'Colomycin' using the disc diffusion technique.

Media. Infusion broth was used to culture the indicator strains once weekly or as often as required and was prepared according to Cruickshank (1960, p. 190).

Tryptone Soya Agar (T.S.A.) (Oxoid) was prepared according to the maker's instructions and various concentrations of horse blood added (T.S.B.A.).

Dorset's egg medium was prepared according to Cruickshank (1960, p. 214).

RESULTS

Modifications of the typing technique

Abbott & Shannon's technique requires 3 days preliminary incubation of the strain to be typed (producer strain) and thereafter 5 hr. processing before the indicator (passive) strains are applied. Several preliminary experiments were undertaken with the aim of reducing these times and otherwise streamlining the technique so that it would remain accurate but become more acceptable for 'routine' use. The results of these experiments are summarized in the following paragraphs and detailed protocols are available on request.

(1) The producer strain can be removed from the medium before exposure to $CHCl_3$ and this is done with a microscope slide; the end of the slide used to clear the growth is sheathed with a layer of sellotape. This allows complete removal of the macroscopic growth very cleanly and without requiring the removal of a portion of the medium; any danger of laboratory infection from this manoeuvre is reduced by jettisoning the material on to cellulose wadding soaked with $CHCl_3$.

(2) Effective sterilization of the surface of the medium before applying the indicator strains requires not more than 15 min. exposure to $CHCl_3$ vapour.

(3) Residual traces of $CHCl_3$ are removed by only a few minutes exposure to the natural atmosphere, whether the $CHCl_3$ has acted as a vapour or in liquid contact with the medium ($CHCl_3$ is not adsorbed by solid media).

(4) There is no significant difference in size of zone of inhibition of the indicator strains with various blood concentrations or depths of medium; these facts were established by varying the concentration of blood ($2\frac{1}{2}$, 5, 10 %) and also the depth of the medium by using small and large Petri dishes (diameters internally of $3\frac{1}{2}$ in. and 4 in. respectively) and in each case using 10, 15 and 20 ml. volumes of medium so that six different depths of medium were investigated. At this time all plates were still poured in two layers as recommended by Abbott & Shannon (1958), the basal layer of plain T.S.A. and the top layer incorporating the

concentrations of blood as indicated—the medium volumes in any one plate were equally divided between the layers.

(5) No advantage was found in layered plates except perhaps the saving in blood but this is offset since for routine use 5 % blood is used in place of 10 % as used by Abbott & Shannon.

Thus the procedure which has been in use for more than 4 years can be summarized as follows: the Sonne strain to be typed is inoculated in a diametric streak on a T.S.B.A. plate (15 ml. of medium regardless of whether small or large size plates are in use); incubation is continued for 24 hr. at 35–36° C. Thereafter the macroscopic growth is removed with a sellotape-sheathed slide, 3–5 ml. of $CHCl_3$ is placed in the lid of the Petri dish and the medium containing portion replaced for 15 min.; the plate is then opened and the residual $CHCl_3$ decanted into a beaker and retrieved for further use by filtering through filter paper.

The medium is exposed to the air for a few minutes and the fifteen indicator strains applied at right-angles to the original line of growth; eight strains are placed on the left and the other seven on the right half of the plate. This number of strains can be accommodated without difficulty even on a small size Petri dish and the resultant zones of inhibition, although only half the width of those obtained by full application across the plate, are easily noted (Pl. 1, figs. 1, 2).

Finally 8 or more hours incubation at 37° C. allows growth of the indicator strains and recognition of patterns of inhibition.

Validity of the technique

More than 5000 strains have been typed by the modified method described; the first 480 strains were examined in parallel by Abbott & Shannon's method and gave identical results. Occasional batches of strains have been thus examined in duplicate over the last 3 years and no discrepancy in type identification noted.

The reliability of colicine typing as an epidemiological marker has been assessed by two indices; first, by noting the constancy of type excreted by any one individual in serial isolations during clinical illness and convalescence (Table 1).

In addition to the 521 individuals who showed uniformity of colicine type of *S. sonnei* on two or more occasions, there were twenty-eight who showed variation in the type excreted.

The second index of reliability was that employed by Abbott & Shannon, namely the uniformity of type in any one epidemic; an epidemic is defined as two or more isolations of *S. sonnei* within a 4-week period from different persons in a family or institution. The epidemics in which there was uniformity of colicine type are summarized in Table 2.

Of these 534 epidemics 472 were in private households and sixty-two in residential or day-care institutions (the majority being children's nurseries); additionally there were twenty-three epidemics where more than one colicine type was encountered.

In Table 3 is given the type distribution for Edinburgh cases between 1959 and 1961; only the first isolation from any individual case or any epidemic is included and instances of mixed type infection have been excluded.

Details of the breakdown of this distribution seasonally and in different wards of Edinburgh will be incorporated in a later publication, but it should be noted that even in the brief period of 3 years there was evidence of the originally predominant Type 7 strain being ousted by other types and particularly that for brief periods untypable strains accounted for almost all isolations.

Table 1. *Serial isolations from* 521 *excretors of* Shigella sonnei *who showed uniformity of colicine type**

Colicine Type	No. of repeat isolations (excluding original) 1	2	3	4	5	6	7	Total no. of strains
7	227	68	17	7	4	.	2	476
u/t	29	18	8	5	3	.	.	124
4	28	8	8	1	.	.	.	72
6	16	3	1	1	.	.	.	29
14	7	4	2	.	.	.	.	21
2	28	3	.	.	.	.	.	34
11	9	4	.	.	.	.	.	17
1*a*	3	1	.	.	.	.	.	5
3*a*	1	1	.	.	.	.	.	3
8	2	.	.	.	.	.	.	2
9	1	.	.	.	.	.	.	1
3	1	.	.	.	.	.	.	1
No. of persons	352	110	36	14	7	.	2	785

* 28 (5·37 %) other individuals showed variation in the type excreted.
u/t = untypable strains.

Table 2. 534 *epidemics of* Shigella sonnei *in which there was uniformity of colicine type**

Colicine Type	No. of persons in epidemic 2	3	4	5	6	7	8	> 8†	No. of Epidemics	People
7	146	78	31	15	9	3	5	6‡	293	1006
u/t	48	22	15	6	2	.	2	10§	105	533
2	26	15	8	1	.	.	1	$2^{15,22}$	53	179
4	26	4	3	4	1	1	.	$2^{10,15}$	41	134
11	6	2	2	2	.	.	1	.	13	44
6	3	.	3	.	1	.	.	1^{22}	8	46
1*a*	4	.	2	.	.	.	.	1^{14}	7	30
14	2	.	1	1	.	.	.	1^{31}	5	44
3	3	1	.	1	.	.	.	.	5	14
8	1	1	.	.	.	.	.	.	2	5
3*a*	2	.	.	.	.	.	.	.	2	4
Total	267	123	65	30	13	4	9	23	534	2039

* In twenty-three (4·31%) other epidemics there was lack of uniformity of type.
u/t = untypable strains.
† Superscribed figures indicate the number of cases in such epidemics.
‡ 13, 16, 19, 21, 37 and 60 cases.
§ 9, 10, 12, 15, 15, 23, 24, 35, 43 and 67 cases.

Table 3. *Frequency distribution of colicine types of* Shigella sonnei *Edinburgh* 1959–61*

Colicine Type	No. of strains	As percentage of All strains	As percentage of Typable strains
7	620	58·7	63·9
2	127	12·02	13·1
4	91	8·62	9·4
u/t	86	8·14	—
11	51	4·83	5·3
6	38	3·59	3·9
14	12	1·14	1·2
3	10	0·95	1·0
8	9	0·85	0·9
3*a*	6	0·57	0·6
1*a*	4	0·38	0·4
9	1	0·09	0·1
12	1	0·09	0·1
Total	1056	.	.

* Only the first strain isolated from any individual or epidemic is included; instances of mixed type infection are excluded.

Table 4. *Patterns of inhibition of* Shigella sonnei *on indicator strains*

Indicator strain no.	Colicine type 1*a*	1*b*	2	3	3a	4	5	6	7	8	9	10	11	12	13	14
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, 2M, 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 *Bact. coli* Row.

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

Identity of Type 14 *strains*

The pattern of inhibition given by Sonne strains of this hitherto undescribed type is given in Table 4 along with the characteristic patterns for the types previously described by Abbott and his colleagues.

Although Type 14 strains have occurred infrequently the evidence for its identity and epidemiological significance is clear cut; strains of this type appeared in June

and July 1959 in a Borders village with four household epidemics and several isolated cases occurring and was the only type isolated in the area.

Its next appearance was in February 1960 in a private school in a small village 20 miles north and west of the earlier episode; in the school outbreak with thirty-one excretors of Type 14 *S. sonnei* it was noted that the only symptomless excretor was one of the domestic staff who had suffered clinical illness with this type when living in the Borders village the previous year. One further case occurred in May 1960 in Edinburgh which could not be related epidemiologically to any of the previous excretors.

It will be seen from Table 4 that Type 14 differs from Types 3 and 3*a* in the variability of its activity against indicator strain no. 4 and in lacking activity against indicator strain no. 14; it differs further from Type 3 strains by lacking inhibitory activity on indicator strain no. 10.

DISCUSSION

Colicine typing of *Shigella sonnei* can be performed more rapidly and economically and without loss of accuracy by the modified technique described; type identification can be made within 36 hr. of isolation using only one plate.

Considering the reliability of colicine typing for epidemiological purposes it should be noted that Abbott & Shannon give no detail regarding the first index used in the present investigation other than that 'repeated isolations from the same patient were tested on a number of occasions, with identical results'.

The results given in Table 1 show that 521 (94·9 %) of 549 individuals showed uniformity of colicine type on two or more occasions. In fourteen of the twenty-eight cases with lack of uniformity in the type of *S. sonnei* excreted serially, only one colony was tested on each occasion; the results are shown abstractly where X and Y represent different colicine types:

	Isolation	
No. of cases	1st 2nd 3rd	
13	$X \rightarrow Y \rightarrow Y$	(replacement)
1	$X \rightarrow Y \rightarrow X$	(replacement with reversion)

In the remaining fourteen patients, multiple colonies (3–15) were tested from each isolation and eight patients showed excretion similar to the thirteen in the above group, i.e. on second or subsequent isolations there was apparent replacement of one type by another. In the six other cases the patterns were as outlined:

	Isolation		
No. of cases	1st	2nd 3rd	
3	X and Y	$X \rightarrow X$ or $Y \rightarrow Y$	(elimination)
3	$X \rightarrow X$ and Y	$\rightarrow X$ and Y or X or Y	(acquisition: with maintenance, with reversion, with replacement)

This latter situation was also seen on two occasions in household epidemics each involving two siblings of school age in whom a second type was acquired and with one pair of children was maintained along with the original type over five more isolations; in the second pair the freshly acquired strain was maintained for two further isolations when the original type could not be discovered in spite of multiple colonies being examined from each diagnostic plate.

It is not surprising that an individual may harbour more than one colicine type at any one time when Sonne dysentery is so ubiquitous; it is accepted that a person may excrete two separate and distinct types of salmonella and instances of combined excretion of a shigella and salmonella occur in diagnostic laboratories. Such findings do not invalidate serological typing techniques and at present there is no evidence that the presence of two colicine types of *S. sonnei* in one individual is associated with type instability *in vivo* which would of course invalidate the technique for epidemiological purposes; there are, on the other hand, incidents which lend weight to *in vivo* stability and show that excretion of two types is a real occurrence; two children are known to have excreted Type 4 *S. sonnei* more or less regularly for more than 3 years without any change of type (neither child has ever had a recognizable attack of dysentery). Another child participated in two epidemics in March and July 1959 and on the first occasion she and the other cases were excreting Type 2 strains, whereas on the second occasion all of the cases were of Type 7 except this child who was excreting Types 2 and 7 and continued to do so for five further examinations over 3 weeks.

Further evidence of such occurrences must be sought by follow-up studies so that *in vivo* instability of colicine production can be conclusively eliminated. With regard to *in vitro* instability, there is no evidence of change in colicine production either qualitatively or quantitatively when cultures have been checked at 6- to 12-month intervals over the last 4 years.

The second index of reliability revealed, in the present study, that 534 (95·9 %) of the 557 epidemics were homogeneous as regards colicine type. Regarding the twenty-three epidemics which were exceptional in showing lack of uniformity of colicine type it is noteworthy that eight were in households and fifteen in institutions, whereas uniformity was seen in 472 households and sixty-two institutional outbreaks; this high proportion of mixed type epidemics in institutions is statistically significant. One institutional outbreak of mixed types has been summarized above and in the few other instances when epidemiological data were available it was apparent that mixed type epidemics were real and most unlikely to be due to type instability *in vivo*; in all such mixed epidemics the majority of cases were of one type and frequently only one patient deviated from the common type.

The incidence of colicine types in Edinburgh during 1959–61 differs appreciably from that reported in Manchester and Bradford over approximately the same period; this is particularly obvious in regard to untypable strains with an incidence of 8·1, 16·9 and 43 % respectively in these cities. In Manchester Types 1*a* and 7 account for 60 % of typable strains, and in Bradford Types 2 and 4 for 83 %; in Edinburgh, however, 63·9 % of typable strains are Type 7 and types 2 and 4 account respectively for 13·1 and 9·4 % whilst Type 1*a* has rarely been encountered.

Type 6 strains amounted to 3·9 % of typable strains in Edinburgh but this type is notable for its rarity in the other two centres.

Abbott & Graham considered that on the basis of epidemiological evidence Types 1*a*, 1*b*, 2, 4 and 7 could be regarded as distinct types; we have insufficient experience of either of the Type 1 strains but can confirm their view on the other three types and in addition consider that Types 6, 11 and the new Type 14 are distinctive from evidence gathered during this investigation.

These differences in type distribution in the three centres should excite renewed interest in Sonne dysentery and it is hoped that other workers will report their experience with this recently acquired epidemiological tool; further inter-regional differences and annual variations in type incidence within certain regions have been studied and along with other epidemiological data are being prepared for publication.

SUMMARY

1. Modifications of the technique of colicine typing are reported which allow more rapid and economical identification of *Shigella sonnei* for epidemiological purposes without loss of accuracy.

2. The technique is epidemiologically valid as judged by the constancy of type in repeated isolations from individuals and the uniformity of type in epidemics; it is considered that the few instances of lack of uniformity in these indices are probably examples of true mixed type infection. There is no evidence of type instability *in vitro*.

3. Type incidence differs significantly from that reported in two other centres; a new colicine type (Type 14) is described which brings the total of recognizable types to 16. It is considered that in addition to the five types which were previously thought distinctive, three other Types, 6, 11 and 14, can now be so regarded.

4. There is a need for continued studies to resolve the few discrepancies associated with infection by more than one type either in the individual or in an epidemic; it is hoped that colicine typing will reawaken interest in Sonne dysentery.

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REFERENCES

ABBOTT, J. D. & GRAHAM, J. M. (1961). Colicine typing of *Shigella sonnei*. *Mon. Bull. Minist. Hlth Lab. Serv.* **20**, 51–8.

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

BARROW, G. I. & ELLIS, C. (1962). Colicine typing of *Shigella sonnei* by replicate multiple-slide inoculation of indicator organisms. *Mon. Bull. Minist. Hlth Lab. Serv.* **21**, 141–7.

BOJLÉN, K. (1934). Thesis: Dysentery in Denmark, Copenhagen.

CRUICKSHANK, R. (1960). *Mackie and McCartney's Hand-book of Bacteriology*, 10th ed. Edinburgh and London: E. and S. Livingstone Ltd.

DAVIES, J. R. (1954). A simple method of determining the sulphonamide sensitivity of *Shigella sonnei*. *Mon. Bull. Minist. Hlth Lab. Serv.* **13**, 114–17.

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

HAMMARSTRÖM, E. (1947). Bacteriophage classification of *Shigella sonnei*. *Lancet*, i, 102–3.

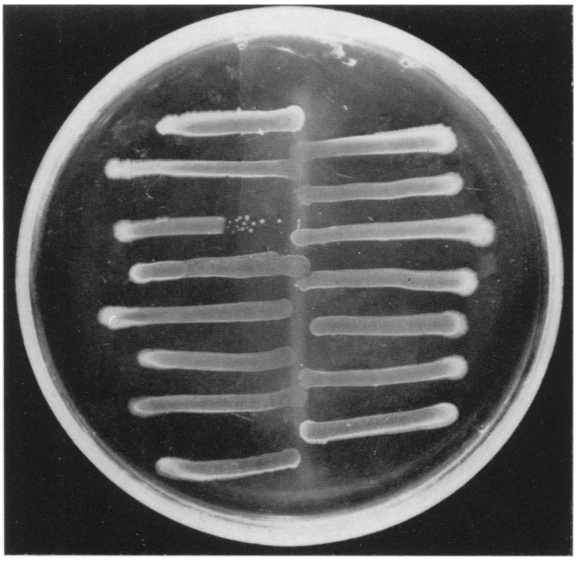

Fig. 1

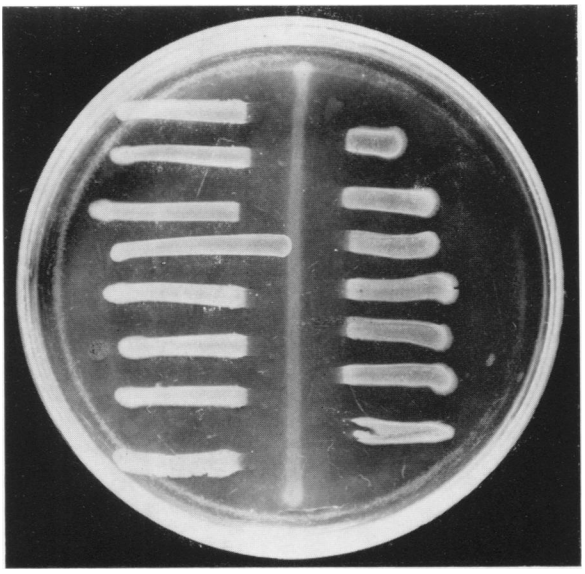

Fig. 2

HAMMARSTRÖM, E. (1949). Phage-typing of *Shigella sonnei*. *Acta med. scand.* **133** (Suppl. no. 223).

MAYR-HARTING, A. (1952). The phage typing of *Shigella sonnei*, and the limits of type stability. *J. gen. Microbiol.* **7**, 382–96.

TEE, G. H. (1952). Xylose fermentation by *Shigella sonnei*. *Mon. Bull. Minist. Hlth Lab. Serv.* **11**, 68–77.

TEE, G. H. (1955). Bacteriophage typing of *Shigella sonnei* and its limitations in epidemiological investigation. *J. Hyg., Camb.*, **53**, 54–62.

EXPLANATION OF PLATE 1

Fig. 1. Pattern of inhibition given by *S. sonnei* colicine type 7.

Fig. 2. Pattern of inhibition given by *S. sonnei* colicine type 3.