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Discrimination by multiple typing of isolates of *Shigella sonnei* in Dundee (1971–6)

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SUMMARY

Different strains of *Shigella sonnei* present in Dundee from 1971 to 1976 were identified by a 'multiple typing' method in which resistotyping, used as the main method of differentiation, was supplemented by colicine typing, antibiogram testing and biotyping. At least 19 different 'multiple types' (MTs) were identified by combining information from the four typing techniques. The relation of the different types and their possible derivation from each other are discussed.

The practical value of multiple typing was demonstrated in a study of 247 isolates, of three distinct MTs, recovered from 178 persons involved in an extended outbreak centred primarily on day nurseries.

A few episodes that yielded isolates of different resistotypes were analysed to determine whether the cultures were: isolates of the same strain different in resistotype as a result of *in vivo* or *in vitro* variation of resistotype characters, or isolates of distinct strains of different resistotypes.

The multiple typing approach clarified the way in which different MTs emerged, persisted, disappeared or co-existed in the community during the 6 years of the study.

INTRODUCTION

Different types of *Shigella sonnei*, identified by colicine and antibiogram typing, may differ in their ability to spread in a community and the disappearance of apparently well-established types is often followed by the appearance of other 'new' types that become established in turn (Barrow & Ellis, 1962, 1967; Cook & Daines, 1964; Farrant & Tomlinson, 1966; Gillies, 1965).

The question remains, however, as to how 'new' strains within a community can be recognized as truly different from endemic strains, and as to whether the appearance, spread and subsequent disappearance of new clones within a population follow the chance acquisition or loss of characters conferring the ability to spread (Farrant & Tomlinson, 1966), or are dependent on herd immunity of the population (Gillies, 1965).

The present investigation was directed towards answering these questions by identifying the major and minor types of *Sh. sonnei* responsible for outbreaks of Sonne dysentery in the Dundee area from 1971 to 1976 by typing the isolates by four different methods in conjunction, 'a multiple typing approach', to accumulate the maximum of information about the different types and their distribution.

MATERIALS AND METHODS

Bacteria

Isolates (1420) of *Sh. sonnei* were recovered from 722 patients or symptomless excreters in the Dundee area between January 1971 and July 1976 (Helgason & Old, 1981).

Colicine typing

The method of Abbott & Shannon (1958) was used. A primary incubation period of 48–72 h was often required for colicine-producing cultures to produce sufficient colicine to give recognized patterns of inhibition corresponding to known colicine types. In addition, four new colicine types – 4 var, SH1, SH2 and SH3 – were encountered (Helgason & Old, 1981).

Antibiogram testing

Antibiotic sensitivity tests were performed (Helgason & Old, 1981) with the following antibiotics (symbols): (A), ampicillin; colistin; furazolidone; (Su), triple sulphonamides; (K), kanamycin; (N), neomycin; (P), paromomycin and (S), streptomycin. Resistance to an antibiotic was indicated by recording its corresponding symbol and sensitivity by omitting it. Thus, isolates of type ASuS were resistant to ampicillin, sulphonamide and streptomycin and sensitive to the other five antibiotics tested.

Resistotyping

The technique of Elek, Davies & Miles (1973) was used with the following six resistotyping chemicals: (B), acrylamide; (C), boric acid; (D), phenyl mercury acetate; (F), malachite green; (H), magnesium perchlorate and (K), cobaltous chloride (Helgason & Old, 1981). Resistance was indicated by recording the letter of the chemical and sensitivity by its absence. Thus, resistotype BCDF–K indicated that the isolate was resistant to chemicals B, C, D, F and K and sensitive to chemical H.

Biotyping

The ability of isolates to ferment maltose or rhamnose in peptone water medium at 1-day definitive times of reading was assessed (Helgason & Old, 1981).

Multiple typing

Each multiple type (MT) of *Sh. sonnei* was defined by combining the resistotype/colicine/antibiogram characters; thus, strains of resistotype B–DFHK of colicine type 4 and antibiogram type SuSKNP were designated the multiple type (MT) B–DFHK/4/SuSKNP. If among strains of such a type, a minority were colicine untypable variants, the lesser type was designated parenthetically: B–DFHK/4(U)/SuSKNP. When relevant, supplementary information relating to biotype characters and level of streptomycin resistance was used to aid strain discrimination.

RESULTS AND DISCUSSION

When the results of colicine typing, antibiogram testing, resistotyping and biotyping were considered together, the 1420 isolates included at least 19 MTs of *Sh. sonnei* (Table 1). Not all types however, were represented by large numbers of isolates.

Table 1. *Multiple types of* Shigella sonnei *in Dundee*, 1971–6

Multiple type no.	Resisto-type	Colicine type(s)	Antibiogram type(s)	Strepto-mycin*	Fermentation of†	
					maltose	rhamnose
MT1	B–DFHK	4(U)	SuS(KNP)	H	L	P
MT2	B–D–HK	4	ASuSKNP	H	L	P
MT3	–-DFHK	4 var (4)	ASKNP	L	P	P
MT4	B–DF–K	4, 7(U)	ASu(S)	S(L)	P	P
MT5	B–DFHK	U	ASuS	H	P	P
MT6	B–DFHK	7(4)	ASu	S	P	P
MT7	B–DFHK	3, 15	ASu	S	P	P
MT8	B–DFHK	SH1	Su(ASu, ASuS)	S(L)	P	P
MT9	B–DFHK	U	S(AS, ASuS)	H	L	P
MT10	B–DFHK	U	SuS	H	P	P
MT11	B–DFHK	U	ASuS	L	P	P
MT12	B–DFHK	U	Su(ASu)	S	P	P
MT13	B–D–HK	U(15)	ASuS	L	P′	P
MT14	–-DF–K	U	ASu	S	P	P
MT15	BCDFHK	U(SH2)	ASuS	S(L)	P	L
MT16	BCDF–K	SH2, SH3	A(ASuS)	S	P	L
MT17	–CDFHK	U, 2	...	S	P	L
MT18	BCDFHK	U, 2	Su	S	P	L
MT19	BCDFHK	U(15)	S(SuS)	S	P	P

* S, streptomycin sensitive; L, H, resistant to, respectively, low or high levels of streptomycin (Helgason & Old, 1981).

† Fermentation of carbohydrate was: P, prompt (1 day); P′, prompt (30 h); L, late (2–21 days).

..., sensitive to eight antibiotics (Helgason & Old, 1981).

Identification of multiple types

Types MT1–MT3, 1971–3

The predominant strains in Dundee from 1971 to mid-1973 were of types MT1, MT2 and MT3, comprising *c.* 92% of the 961 isolates recovered in that period (Helgason & Old, 1981). Two of the three major resistotypes (Helgason & Old, 1981) were composed of strains most of which were of colicine type 4: B–DFHK/4 and B–D–HK/4. Subdivision of the colicine type 4 strains into these two resistotypes was supported by their different antibiograms; thus, the former was generally sensitive and the latter generally resistant to A. These isolates, therefore, formed the two MT types MT1 and MT2 with the characters B–DFHK/4(U)/SuSKNP and B–D–HK/4/ASuSKNP, respectively (Table 1). The importance of the use of malachite green in differentiating F-resistant isolates of strain MT1 from F-sensitive isolates of type MT2 has been discussed (Helgason & Old, 1981). Apart from differences in sensitivity to malachite green and ampicillin, isolates of these two

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Table 2. *Changes in typing characters of strains of* Shigella sonnei *from patients in episodes due to strains of uniform resistotype*

Episode no.	Patient(s) involved	No. of isolates	Day(s) of isolation	Strain characters: Resisto-type	Colicine type	Antibiogram type
1	a	4	1–7	--DFHK	4	ASKNP
	a	2	11–25	--DFHK	4 var	ASKNP
2	a	1	1	B–DFHK	15	ASu
	b	1	1	B–DFHK	3	A
3	a, b, c	3	1–4	B–DFHK	U	Su
	d	1	24	B–DFHK	SH1	ASuS
	a	1	38	B–DFHK	SH1	ASu
4	a, c	7	1–25	B–DFHK	U	ASuS
	b	1	10	B–DFHK	U	SuS
5	a	1	1	B–DFHK	U	Su
	a	2	4–9	B–DFHK	U	ASuS
6	a	1	1*	BCDFHK	U	ASuS
7	a	1	1*	BCDFHK	SH2	ASuS
8	a	1	1*	BCDF–K	SH2	ASuS
9	a	1	1*	BCDF–K	SH3	A
	a	2	2–3	BCDF–K	SH3	ASuS

* The index strains in episodes 6, 7, 8 and 9 were isolated in 1975 on respectively; 29 April, 18 August, 21 July, 4 September.

types were otherwise similar (Table 1); their distributions however, were dissimilar: whereas isolates (400) of MT1 were present almost continuously for a 2·5-year period from January 1971, those (286) of type MT2 were isolated only during the nine months from August 1972 to April 1973. Type MT2 may represent a malachite green-sensitive variant of type MT1 that arose in the course of the epidemic spread of the latter strain, and persisted for almost a year in the community as a new epidemiologically distinct strain. Although of stable resistotype, considerable instability in colicine and antibiogram markers, associated generally with the loss of the 'Ib–KNP' plasmid, was noted among isolates of type MT1 (Helgason & Old, 1981).

Isolates (197) of type MT3 belonged to the third major resistotype, --DFHK, and most of them were of colicine type 4 var (i.e. carried both *col* Ib and *col* B) and had an antibiogram pattern (ASKNP), associated with low level resistance to S, different from those of types MT1 and MT2. The occasional recovery in the period August to October 1972 of isolates of --DFHK of different colicine types from different individuals (see, for example, episode 1, Table 2), followed thereafter by the almost uniform isolation of colicine type 4 var isolates of --DFHK, suggested that the index isolate associated with the outbreak was *col* Ib and that it subsequently acquired *col* B. The uncommon character of acrylamide (B) sensitivity, the general carriage of *col* B, the low level of resistance to S and prompt fermentation of maltose were properties that indicated that type MT3 was

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probably a distinct type that could not have readily been derived from either type MT1 or MT2. Type MT3 (--DFHK/4 var (4)/ASKNP) and type MT2 were commonly isolated during almost the same period.

Types MT4–MT8, 1971–3

At the time that types MT1–MT3 predominated in the community, five other types, MT4–MT8, were also present from 1971 to mid-1973, together accounting for *c.* 6% (56) of the isolates found. For example, a clone responsible for small outbreaks in the early part of this study was type MT4. Most of the 22 isolates of this type were of colicine type 7 and of antibiogram type ASu(S). A minority, carrying the *col* Ib–KNP factor, were of colicine type 4 and ASuSKNP. All fermented maltose promptly (Table 1). In February and March 1971, there were a few isolates of type MT5 that, although possessing some characters, including high level S resistance, in common with isolates of type MT1, differed from it in being prompt fermenters of maltose (Table 1). It is unlikely that these two types are related unless, for example, type MT5 was derived from type MT1 by a series of simultaneous genetic events including mutation to maltose-fermenting ability, acquisition of resistance to A and loss of the '*col* Ib–KNP' determinants.

The few isolates of type MT6 recovered in early 1971 were sensitive to S and fermented maltose promptly, characters differentiating them from isolates of both type MT1 and type MT5 (Table 1). Isolates of type MT6 of colicine type 4 carried a *col* Ib factor which masked the effect of a second *col* factor (the determinant of colicine type 7). Strains of the latter type, i.e. carrying both *col* factors, were associated with the 'Montrose' outbreak (Whyte, 1968) and were of resistotype B–DFHK (Helgason & Old, 1981). Thus, type MT6 might have been derived from the 'Montrose' strain (Green *et al.* 1968). There were only two isolates of type MT7, recovered in July 1972 from members of the same family (episode 2, Table 2). Apart from differences in their colicine types (*viz.* colicine type 3 carried *col* K and two further unidentified *col* factors; colicine type 15 carried a type-E *col* factor), these isolates were similar. In 1973, four members of another family yielded isolates of another B–DFHK line MT8 that fermented maltose promptly and were of different antibiogram types (episode 3, Table 2); some were of the new, provisional colicine type SH1, determined by an E-type *col* factor. Apart from the carriage of different *col* factors, however, the isolates of types MT6–MT8 were similar to each other and to the non-colicinogenic type MT12 (*vide infra*), and these four types may have been related.

The sudden disappearance in April 1973 of types MT2 and MT3, which had been isolated in large numbers since August 1972, was followed in June 1973 by the disappearance of type MT1, the third of the dominant clones present between 1971 and mid-1973. Several weeks later, an apparently new colicine untypable (U) strain of *Sh. sonnei* emerged in the community. In three years from mid-1973 to mid-1976, 95% of the 469 isolates recovered were colicine untypable; these included three distinct resistotypes: B–DFHK, B–D–HK and --DF–K.

Types MT9–MT12, 1973–5

The 200 colicine-untypable isolates of resistotype B–DFHK recovered between July 1973 and August 1975 were of six different antibiogram types, none associated

with KNP resistance: ASuS, ASu, AS, SuS, Su and S (MTs 9–12, Table 1). Differences in other typing characters suggested that these isolates were heterogeneous and also unlikely to be closely related to type MT1. Thus, among the U isolates of types MT1, and those of MT9 and MT10 with different antibiogram patterns (*viz.* S, AS, ASuS and SuS) but similar in their high level S resistance, only types MT1 and MT9 fermented maltose late. Prompt fermentation of maltose, a character common to types MT5–8 and MT10–12, suggested a closer relationship of these latter types to each other than of any of them to types MT1 and MT9; thus, there may have been two distinct B–DFHK lines, one maltose prompt, the other maltose late-fermenting. Differences were also observed in streptomycin sensitivity but classification on the basis of sensitivity or resistance to antibiotics was not helpful in view of the observed instability of antibiotic resistances even among isolates within episodes (e.g. episodes 4 and 5, Table 2). In the absence of further discriminating tests, it was, therefore, impossible to establish the actual relationships among the nine different MTs of resistotype B–DFHK. That difficulty was probably due in part to the fact that B–DFHK is a common resistotype among British strains of *Sh. sonnei* (Elek, Davies & Miles, 1973; Helgason & Old, 1981). The use in resistotyping of the additional chemicals, potassium cyanate and sodium orthophosphate (not used in this study) had previously suggested the existence of sub-types within that resistotype (Elek *et al.* 1973).

Types MT13 and MT14, 1975–6

Isolates (204) of resistotype B–D–HK were recovered in the 10 months from October 1975, after an absence of 2·5 years. It is unlikely that isolates of type MT13 (B–D–HK/U (15)/ASuS) were derived from type MT2, also of resistotype B–D–HK, so common in Dundee in 1972 and 1973. The latter fermented maltose late (3–9 days) and had high level resistance to S whereas the former fermented maltose promptly (in 30 h) and showed low level resistance to S. Type MT13 is discussed more fully below.

Towards the end of this study members (41) of a third resistotype, ––DF–K (MT14, Table 1), that had not been previously recovered in Dundee were detected among the colicine U strains. Thus, among the colicine untypable strains present between 1973 and 1976, three resistotypes were present: B–DFHK (MTs 9–12), B–D–HK (MT13) and ––DF–K (MT14).

Types MT15–MT19 (boric acid resistant)

The other MTs detected, accounting for only 1·6% of all isolates, were those associated with boric acid resistance, C (Table 1).

Despite the fact that resistotyping generally gave more stable results than other typing methods, there were some problems in interpreting partial resistance to some chemicals in resistotyping. Such a problem was demonstrated with types MT15 (two isolates) and type MT16 (four). The index isolate (April 1975) was U, but the other five (July–September 1975) had acquired the different E-type *col* factors associated with the new, provisional colicine types SH2 and SH3 (episodes 6–9, Table 2); one of the six isolates had lost the resistance determinants Su and S. Each isolate, however, fermented maltose promptly and rhamnose late, the latter being a rare character among the Dundee strains. Repeated testing of these

six isolates, including tests on the same sets of plates, showed that the observed resistotype differences were not due to technical variability in the system. Types MT15 and MT16 were, therefore, probably related and the likeliest explanation for the difference in resistotype is that type MT16 (sensitive to H) was derived from type MT15 (partially resistant to H) as a result of *in vivo* variation of a resistotype character. Similar *in vivo* variation of resistotype characters in *Escherichia coli* has been reported but is rare (Crichton, 1980; Crichton & Old, 1980; Old *et al.* 1980).

This and a previous report (Helgason & Old, 1981) support the view of Elek *et al.* (1973) that strains of a single type resistant to a particular chemical give too many partially resistant variants for partial resistance *per se* to be of general value. However, the new resistotype –CDFHK described by Helgason & Old (1981) was characterized by partial resistance to magnesium perchlorate, H. The 12 isolates of that type were recovered between October and December 1972 in three separate episodes involving four persons in Dundee and Perth. Each isolate was partially resistant to H, fully sensitive to the eight antibiotics tested and fermented rhamnose late. In addition, the final seven isolates had acquired *col* I a (i.e. were of colicine type 2). Because each of these characters was rare among the Dundee strains, their combined presence in this group of isolates indicated that despite the observed differences in the carriage of the rare *col* I a (i.e. rare for this series), they were probably closely related members of the same type MT17.

There were only two isolates of type MT18 (BCDFHK). Like those of type MT17 (–CDFHK), these were both partially resistant to H, and fermented rhamnose late; one also carried *col* I a (Table 1). Furthermore, they were isolated from geographical areas in which MT17 was present at the same time. It is possible, therefore, that the type MT18 isolates were variants of type MT17 that had acquired *in vivo* resistance to acrylamide (B). In such rare cases, even the application of the multiple typing technique does not allow ready interpretation of the data.

The only isolates of resistotype BCDFHK that were not variants of other resistotypes (*viz.* BCDF–K and –CDFHK) were three found in three separate incidents in Dundee between December 1971 and August 1973. These isolates, fully resistant to H, were the only three in the series of 1420 with the unusual combination of properties: boric acid resistance and prompt rhamnose fermentation. Despite a lack of evidence of their epidemiological relatedness and differences in colicine and antibiogram types, these may have been three members of the same rare type MT19.

Resistotype differences within episodes

An earlier study has shown that the colicine type and antibiotic resistance markers of sequential isolates of *Sh. sonnei* from an individual or family within an episode often varied (see, for example, table 7; Helgason & Old, 1981), but on the other hand, isolates from at least 91 % of 286 identified episodes were uniform in resistotype (Helgason & Old, 1981). It is, however, important to determine whether the resistotype differences observed in the remaining 9% of episodes represent variation of resistotype characters among different isolates of the same strain, or reflect the presence of different strains of truly distinct resistotypes responsible for mixed or sequential infections in a patient or a family. The results of that enquiry are shown in Table 3.

Thus, in one family (episode 10, Table 3) two patients excreted isolates of type

Table 3. *Episodes in which resistotyping indicated the presence of different strains of* Shigella sonnei

Episode no.	Patient(s) involved	No. of isolates	Day(s) of isolation	Strain characters: Resisto-type	Colicine type	Antibiogram type
10	a, b	2	1–6	B–D–HK	4	ASuSKNP
	a	6	21–63	–CDFHK	2	...
11	a	1	1	B–D–HK	U	ASuS
		1	10	--DF–K	U	ASu
12	a	1	1	--DF–K	U	ASu
		1	7	B–D–HK	U	ASuS
13	a	1	1	B–D–HK	4	ASuSKNP
		1	29	--DFHK	4 var	ASKNP
14	a, b, c, d	12	1–17	B–DFHK	U	ASuS
	a	1	6	B–DF–K	U	ASuS
15	a	2	1–7	B–DFHK	4 var	ASKNP
		1	8	B–DF–K	4	ASKNP
16	a	3	1–13	B–DFHK	4	ASKNP
		1	10	B–DF–K	4	ASKNP
17	a	1	1	--DFHK	4	ASuSKNP
		2	2–8	B–DFHK	4	ASuSKNP
18	a	1	1	B–DFHK	4	ASuSKNP
		3	2–3	B–D–HK	4	ASuSKNP
19	a	1	1	B–DFHK	4	ASuSKNP
		2	1–2	B–D–HK	4	ASuSKNP
20	a	1	1	B–DFHK	4	ASuSKNP
		1	2	B–D–HK	4	SKNP
21	a	1	1	B–DFHK	4	ASuSKNP
		2	15–18	B–D–HK	4	ASuSKNP
22	a	1	1	B–DFHK	4	ASuSKNP
		1	8	B–D–HK	4	ASuSKNP
23	a	2	1–12	--DFHK	4	ASKNP
	b	1	11	B–DFHK	4	ASKNP
	a	1	19	BCDFHK	2	Su

..., sensitive to eight antibiotics tested (Helgason & Old, 1981).

MT2 (days 1–6), and thereafter (days 21–63) type MT17 was excreted by one of them; these two types were different by all typing methods. Moreover, different types of *Sh. sonnei* were excreted by another three patients in episodes 11 and 12 (MT13 and MT14) and episode 13 (MT2 and MT3). In these four episodes, the isolates probably represented different strains, the resistotypes of which showed differences in respectively, three (episodes 10–12) and two (episode 13) resistotype characters.

There were another nine episodes in which patients excreted apparently different types of *Sh. sonnei* of different major resistotypes. Thus, in 1971, isolates of resistotypes B–DFHK and B–DF–K were recovered from three episodes (14–16,

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Table 3) and B–DFHK and --DFHK from another (episode 17). In 1972–3, five episodes (18–22) in which cultures of B–DFHK and B–D–HK were present were identified. In each of these nine examples, involving four of the five most common resistotypes, the difference between type B–DFHK and each of the types B–DF–K, --DFHK and B–D–HK was in resistance to one chemical only, so that analysis of these episodes was difficult. It is possible that in each episode (14–22) a single isolate was either mistyped or showed *in vivo* variation in one resistotype character. However, neither possibility would account for the detection of apparently 'mixed' infections only at times when strains of the implicated resistotypes were present simultaneously in the community (Helgason & Old, 1981). In each of these nine episodes, the other typing data afforded no additional strain discrimination (Table 3); thus it was impossible to decide whether the isolated cultures were those of the same strain showing variation in a single resistotype character or those of distinct strains of resistotypes that differed by one resistotype character only. Finally, if neither of the cultures of types B–DFHK or --DFHK was mistyped in a single character, three types of *Sh. sonnei* were involved in episode 23, one of them distinguishable from the others by all typing methods (Table 3).

From each of the other 12 episodes that yielded isolates of different resistotypes, we recovered single isolates of minority resistotypes (see footnote *, table 5; Helgason & Old, 1981). These 12 isolates of uncommon resistotype probably represented variants that had arisen, *in vivo* or *in vitro*, from strains of more common resistotypes, *viz.* B–DFHK (8 episodes), B–D–HK (3) and --DFHK (1).

Day nurseries outbreak

Relatively few isolates (50) of *Sh. sonnei* were recovered in the Dundee area during 1974 and the first three quarters of 1975 (Helgason & Old, 1981). Thereafter, however, between October 1975 and July 1976, there was an extensive outbreak, in the course of which 247 isolates of *Sh. sonnei* were recovered from 178 dysentery patients or their contacts. Re-emergence of Sonne dysentery in Dundee was marked by the appearance in October 1975 of strains of resistotype B–D–HK which were, however, of a type (MT13 and variants, Table 4) which could be readily discriminated from strains of the same resistotype of type MT2 (Table 1), previously isolated in Dundee in April 1973. During 87 days from the start of the outbreak, isolates of type MT13 or its variants were recovered on 26 occasions, but only from six patients, five in one family. By mid-January 1976, however, it was apparent that Sonne dysentery had been introduced to day nursery A and seven of the 18 children there excreted type MT13. The first two bacteriologically proven cases were children of an itinerant family newly arrived in Dundee from the north of England after temporary residence there, and originally suspected to have introduced the infection into day nursery A. However, multiple typing showed that, despite any known contact with the earlier community cases, they had acquired MT13, the then recently established type present in Dundee.

By early February 1976, Sonne dysentery was well-established in a second day nursery (B) into which it had probably been introduced by two children who attended both nurseries (A and B). All the isolates recovered from 39 children, staff or family contacts were of type MT13. Although another child had attended both day nursery A and a third nursery (C), the latter remained free from infection.

Table 4. *Characters of* Shigella sonnei *isolated in Dundee from October* 1975 *to July* 1976

		MT characters of strains:			
	Isolates (no.)	Resistotype /	Colicine type /	Antibiogram	Patients (no.)
	185	B–D–HK /	U /	ASuS (MT13)	138
	6	B–D–HK /	U /	ASuSKNP	2
	3	B–D–HK /	U /	SuS	2
	3	B–D–HK /	U /	ASu	3
	7	B–D–HK /	15 /	ASuS	5
	39	--DF-K /	U /	ASu (MT14)	28
	1	--DF-K /	U /	A	1
	1	--DF-K /	U /	ASuS	1
	2	B–DFHK /	U /	ASuS (MT5)	1
Total	247				181*

* Three patients yielded cultures of different types.

The first two confirmed cases in a fourth day nursery (D), in which dysentery was established by mid-February, were children who resided near confirmed cases; either might have been responsible for the introduction of infection into day nursery D. In the succeeding 4 weeks, another 40 persons associated with that latter nursery yielded isolates of type MT13, and there were further isolations of that type in the community. On 11 March, a child in an area in which type MT13 was prevalent yielded two colicine-untypable cultures that had the same antibiogram pattern as type MT13; these were, however, of resistotype B–DFHK (i.e. type MT5).

By the end of March a fifth day nursery (E) had become involved in the outbreak and most of the early cases there yielded strains of type MT13. However, in the first 2 weeks of June, one child from day nursery E and a contact (episodes 11 and 12, Table 3) produced a mixture of types of *Sh. sonnei* that differed in three resistotype characters, *viz.* MT13 and MT14, the latter a type hitherto not recognized in Dundee. Thereafter, in June and July, we isolated from 32 persons associated with day nursery E, strains of type MT14 and its variant types (Table 4). The closure of the nurseries at that time for the annual summer holiday marked a temporary subsidence of the outbreak and the end of the present survey. The distribution of the different types recovered from this extended outbreak is shown in Table 4.

For this group of strains, colicine typing would have provided poor strain discrimination since most of them were colicine untypable, and would have delineated only the seven isolates of colicine type 15 which, as has been demonstrated, were variants of MT13 that had acquired an E-type *col* factor. Similarly, reliance on antibiotic resistance markers would have included in the same ASuS class members of three distinct resistotypes. Changes in antibiogram patterns accounted for most of the observed variant types (Table 4). Resistotyping, on the other hand, indicated the presence of three distinct types (MT5, MT13 and MT14)

and showed that the many minority types were variants of these. Furthermore, resistotyping allowed the identification of those episodes in which different types were present.

Combined typing methods

Because an epidemic strain in the course of its spread *in vivo* may show variation even in those generally stable characters used for its monitoring, it is difficult, when a single typing technique has been employed, to know whether observed minority types are closely related to the majority, are derived from them by *in vivo* variation of typing characters, or are distinct. Such questions are more readily resolved by the use of combined typing techniques (a multiple typing approach) in which the data from different, reliable typing systems are used in conjuction. For example, in studies on the epidemiology of *Salmonella typhimurium* infections, biotyping and phagetyping were more valuable than either alone (Anderson *et al.* 1978); for *Escherichia coli*, biotyping and resistotyping, supplemented on occasion with one or other of: haemagglutinin typing, serotyping, colicine typing or antibiogram typing provided more useful information than any single method alone (Crichton & Old, 1980). In these studies multiple typing not only afforded better strain discrimination but also detected occasional variation of character of epidemic strains *in vivo* (Barker, Old & Sharp, 1980; Old *et al.* 1980).

Although epidemiological studies of *Sh. sonnei* are generally based on data from antibiogram and colicine typing, it should be remembered that: many of these characters are plasmid-determined and transferable (Davies, Farrant & Tomlinson, 1968*a*; Morris & Wells, 1974); a single factor may determine both colicine and antibiogram markers (Elek *et al.* 1973; Helgason & Old, 1981); the ability to produce these characters may be lost *in vitro* (Abbott & Shannon, 1958; Helgason & Old, 1981); and characters may be acquired *in vivo* by strains from *c.* 5% of patients (Davies, Farrant & Tomlinson, 1968*b*). In the present survey, each of these problems made it difficult to identify strains as similar or dissimilar, even those from outbreaks limited in time and place.

The original suggestion that resistotyping might prove useful in the study of *Sh. sonnei* (Elek *et al.* 1973) has not been widely tested. This study with the Dundee isolates of *Sh. sonnei* has confirmed its value for type discrimination. Indeed, the success of a multiple typing approach for analysis of the epidemiology of *Sh. sonnei* was limited only by the absence of an ancillary typing method as reliable and sensitive as resistotyping.

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