

Comparison of four methods of differential typing of isolates of *Shigella sonnei*

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SUMMARY

An epidemiological study of Sonne dysentery in Dundee during the years 1971–6 was made by examining, in respect of 1420 isolates of *Shigella sonnei*, the discriminating power of colicine typing, antibiogram testing, biotyping and resistotyping and the stability of the markers they provided.

Colicine typing identified nine colicine types, including four not previously described. However, because types 4 and 4 var., determined by *col* Ib, and type U, producing no colicines, accounted for 96% of the isolates, discrimination with colicine typing was poor. In antibiotic sensitivity tests, 13 different antibiogram patterns were noted. Less than 1% of the isolates were sensitive to all of the eight antibiotics tested; most were multiply drug-resistant. Resistance to kanamycin, neomycin and paromomycin (KNP) was apparently due to a single resistance determinant, widely distributed in a majority (53%) of the isolates. When definitive times were chosen for reading each biotyping test, only maltose and rhamnose of the 13 'sugars' tested differentiated isolates into prompt- and late-fermenting types. Though the ability to ferment rhamnose was a stable property, it discriminated only 1.5% of the minority, late-fermenting type. Resistotyping with six chemicals discriminated eight epidemiologically valid resistotypes, including three new types. However, 93% of the isolates belonged to only three resistotypes.

Analysis of the data for isolates from 286 epidemiologically distinct episodes showed that the variability of colicine and antibiogram characters, found among isolates within, respectively, 40 and 28% of the episodes, was generally associated with loss or gain of a plasmid ('*col* Ib–KNP') which determined production of colicine Ib and KNP resistance. These characters varied both *in vivo* and *in vitro*. Variability of resistotype characters, on the other hand, was observed in only 28 (9%) episodes, 14 of which possibly represented examples of mixed or sequential infections.

For accurate epidemiological tracing of strains of *Sh. sonnei* in a community, resistotyping, the technique showing the greatest discrimination and least variability of the four tested, should be included as the principal typing method.

INTRODUCTION

In Dundee, as in many other parts of U.K., dysentery caused by *Sh. sonnei* is endemic. Although absent for periods as long as 18 months, large and small outbreaks, often associated with day nurseries, are liable to occur in any season. Nevertheless, apart from a short-term study that followed an explosive water-borne outbreak of Sonne dysentery in Montrose, 30 miles north-east of Dundee, in 1966 (Green *et al.* 1968), information about the clones of *Sh. sonnei* responsible for dysentery in the Dundee area is lacking.

The purposes of this investigation were to characterize the isolates of *Sh. sonnei* present in Dundee and its environs over the extended period from 1971 to 1976 by the use of colicine typing, antibiogram typing, biotyping and resistotyping, to examine the stability of the types so identified and the discriminating power of the different typing methods, and to assess their value for prospective and retrospective analyses.

MATERIALS AND METHODS

Bacteria

Isolates of *Sh. sonnei*, 1420 from 722 patients or symptomless excretors and representing most of those isolated between Jan. 1971 and July 1976, were obtained in the course of routine examination of faecal specimens for intestinal pathogens at the Bacteriology Department, University of Dundee Medical School, Dundee. In addition, duplicate cultures, stored since the time of their original isolation, were available for 128 of these isolates. In this study all stored isolates from an individual patient or members of a family were examined. A further 157 isolates, representing strains present in the Dundee area in the years 1963–9, were also examined.

The 15 standard indicator strains used for colicine typing (Gillies, 1964) were received from Professor R. R. Gillies, Department of Microbiology, The Queen's University of Belfast. Standard indicator strains for the characterization of the colicines secreted by producer strains (Barker, 1980) were supplied by Dr Ruth Barker, Department of Bacteriology, University of Dundee Medical School. Reference strains for standardization of the resistotyping chemicals were strains of *Sh. sonnei* (nos. 56, 71, 106, 147, 201, 233 and 235) obtained from Dr Joan R. Davies, Public Health Laboratory, Guildford, Surrey.

Cultures were maintained on Dorset's egg medium in the dark at ambient temperature.

Identification of Sh. sonnei

Pale, lactose-non-fermenting colonies from deoxycholate citrate agar (Oxoid CM35) cultures were identified as *Sh. sonnei* by standard procedures and confirmed serologically in slide agglutination tests with Sonnei (phase 1 and 2) antiserum (Wellcome Reagents Ltd, Beckenham, Kent).

Colicine typing

The technique of Abbott & Shannon (1958) was used with modifications as described by Gillies (1964, 1978). Isolates were tested for colicine production after

primary incubation for 24 h at 35.5 °C on tryptone soya agar (Oxoid CM131) supplemented with 5% (v/v) horse blood (Oxoid SR50). The patterns of inhibition of the indicator strains were observed, and isolates that gave patterns not conforming to recognized types (Gillies, 1964; Gillies & Brown, 1966; Chan-Teoh *et al.* 1971) were retested with the primary incubation of the producer strains extended to 48 h.

Tests for sensitivity to antibiotics

Antibiotic sensitivity tests were performed on DST agar (Oxoid CM261) with 5% (v/v) lysed horse blood by the method of Stokes (1975), with the antibiotics available on Oxoid Multodisk no. 2861E: ampicillin (2 µg), colistin (10 µg), furazolidone (50 µg), triple sulphonamides (50 µg), kanamycin (5 µg), neomycin (10 µg), paromomycin (10 µg) and streptomycin (10 µg). Resistance to an antibiotic was recorded by a letter assigned to that antibiotic (see Table 3), and sensitivity by its absence.

The level of resistance to streptomycin was measured by the growth of cultures on sensitivity agar containing streptomycin at 10, 50 or 500 µg/ml.

Resistotyping

The technique of Elek, Davies & Miles (1973) was followed except that the resistotyping chemicals A, E, G, I and J (respectively: potassium chromate; acriflavine; potassium cyanate; cupric sulphate; and trisodium orthophosphate), with which other workers had experienced difficulties (Dr Joan R. Davies, personal communication), were omitted. The stock solutions of resistotyping chemicals B (acrylamide), C (boric acid), D (phenyl mercury acetate), F (malachite green), H (magnesium perchlorate) and K (cobaltous chloride) were prepared as recommended by Elek *et al.* (1973) and stored at 4 °C. Fresh stock solutions were prepared at intervals of not more than 2 weeks. Chemicals B, D and H of 'Laboratory' grade and chemicals C and K of 'Analar' grade were from British Drug Houses Ltd (Poole); and chemical F was from George T. Gurr Ltd (London).

Twenty-five test cultures and, for each test chemical, two control cultures, one sensitive and one resistant, were inoculated by means of a Lidwell applicator (Biddulph Ltd, Manchester) to a series of plates of sensitivity agar [Oxoid CM261, buffered at pH 7.6 with tris buffer as recommended by Elek *et al.* (1973)] containing concentrations, increasing stepwise, of one of the six chemicals. This biological titration of each chemical against control strains, sensitive and resistant to each, ensured optimal differentiation of each test culture with respect to each chemical.

Resistance, complete or partial, was indicated by recording the letter of the chemical, sensitivity by omitting it. Because partial resistances were generally not of value for differentiation of this series of isolates, the partial resistance designations of Elek *et al.* (1973) were not used. Thus; the resistotype BCDF-K indicates that the isolate was resistant to chemicals B, C, D, F and K, and sensitive to H.

Biotyping

The ability of isolates to ferment sugars or sugar alcohols was tested in peptone water medium (Oxoid CM9), pH 6.8, with 0.002% (w/v) bromocresol purple as indicator, dispensed in 5-ml amounts in screw-capped ½-oz bottles. The substrates

Table 1. *Inhibition of indicator strains by Shigella sonnei isolates of different colicine types among the Dundee series*

Colicine type	Indicator strain no.*														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	-	+	+	-	-	-	-	-	+	-	-	-	-	-	+
2 var. 1	-	+	(+)	-	-	-	-	-	+	-	-	-	-	-	+
2 var. 2	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+
2 var. 3	-	(+)	-	-	-	-	-	-	+	-	-	-	-	-	+
2 var. 4	-	+	-	-	-	-	-	-	+	-	(+)	-	-	-	+
2 var. 5	-	(+)	-	-	-	-	-	-	+	-	(+)	-	-	-	+
2 var. 6	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
3	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	-	-	+	+	-	+	+	+	-	+
4 var. 1	+	+	+	+	+	(+)	(+)	+	+	-	+	+	+	(+)	+
4 var. 2	+	+	+	+	+	(+)	-	+	+	-	+	+	+	(+)	+
4 var. 3	+	+	+	+	+	-	(+)	+	+	-	+	+	+	-	+
4 var. 4	+	+	+	+	+	-	-	+	+	-	+	+	+	(+)	+
4 var. 5	+	+	0	+	+	-	-	+	+	-	+	+	+	-	+
7	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
11 var. 1	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	-	(+)	(+)	(+)	(+)	+
15	+	-	+	+	+	-	-	+	-	-	+	-	+	+	+
SH1	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+
SH2	+	+	-	+	-	+	+	+	+	-	-	-	+	-	+
SH3	+	+	+	+	(+)	+	+	+	+	-	+	-	+	+	+

* The indicator strains 1-15 correspond, respectively, with: *Sh. sonnei* 2, 56, 17, 2M, 38, 56/56, 56/98, R1, R6; *Sh. dysenteriae* M19 (NCTC 8218); *Sh. sonnei* 2/7, 2/64, 2/15, R5; *E. coli* Row. Inhibition of indicator strains was: + = complete; - = absent; (+) = partial (or doubtful); 0 = weak (or absent).

tested, at a final concentration of 1% (w/v), were: dextrin, dulcitol, inulin, lactose, maltose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose and xylose. Cultures were incubated at 37 °C and examined for acid production daily for up to 21 days.

The ability of isolates to utilize the organic acids *d*-tartrate, *l*-tartrate, *meso*-tartrate and mucate, was assessed by the 'growth stimulation' method of Alfredsson *et al.* (1972).

Episodes

When analysing the results, an 'episode' was defined as an occasion on which two or more isolates of *Sh. sonnei* were recovered from a single patient or from persons from a family group, from 1 to 122 days.

RESULTS

Colicine typing

When primary incubation for colicine production lasted for 24 h, only four recognized colicine types were distinguished among the Dundee isolates: type 3 (1 isolate); type 4 (458); type 7 (22); and type 15 (9). These tests also detected

Table 2. Colicine types of, and colicines produced by, 1420 Dundee isolates of *Shigella sonnei*

Colicine type	No. of isolates of type after primary incubation for		Colicine(s) produced
	24 h	48 h	
U	610	610	None
2	0	12	Ia
3	1	1	K and ?
4	458	600	Ib
7	22	22	?
15	9	9	E-type
2 var. 1-6	20	1†	Ia, or Ib
4 var. 1*	129	153	Ib and B
4 var. 2	2	0	Ib and B
4 var. 3	12	0	Ib and B
4 var. 4	3	0	Ib and B
4 var. 5	62	0	Ib
11 var. 1	7	2‡	B
SH 1*	5	5	E-type
SH 2*	2	2	E-type
SH 3*	3	3	E-type
NRT	75	0	Ib, or Ib and B

* Provisional, new colicine types.

†‡ After primary incubation for 72 h, the inhibition pattern of these strains corresponded to that of colicine type 2 (†) and that of colicine type 4 var. 1 (‡).

?, unidentified colicine(s).

245 isolates (17%) with inhibition patterns that did not conform to recognized types: variants 1-6 of colicine type 2; variants 1-5 of colicine type 4; a variant of colicine type 11; and three apparently new types: SH 1, SH 2 and SH 3 (Table 1). Another 75 isolates (5%), which gave patterns not conforming to those of any recognized types, their variants or the apparent new types, were designated NRT (no recognizable type). Isolates that were untypable (U) because they did not produce detectable colicines, constituted the largest group, namely 43% of all isolates.

By extending primary incubation of the producer strains to 48 h, 178 of the isolates showed inhibition patterns different from those given after primary incubation for 24 h. These differences corresponded to the following changes in colicine types: the type 2 variants to colicine types 2 or 4, the NRT types to types 4 or 4 variant 1, the type 4 variant 5 to type 4, and the variants 2-4 of type 4 and the 11 variant to 4 variant 1 (Table 2). The three isolates that at 48 h had given inhibition patterns of a type 2 variant and a type 11 variant gave inhibition patterns corresponding to, respectively, colicine type 2 and type 4 variant 1 when they were incubated for 72 h before assessment of their inhibition of the indicator strains. The numbers of isolates of each colicine type are shown in Table 2.

The colicines produced by cultures of strains of the different colicine types were identified, in tests with standard colicine indicator strains (Barker, 1980), as follows: type U (no colicines detected), type 4 (Ib), type 4 var. (Ib and B), type

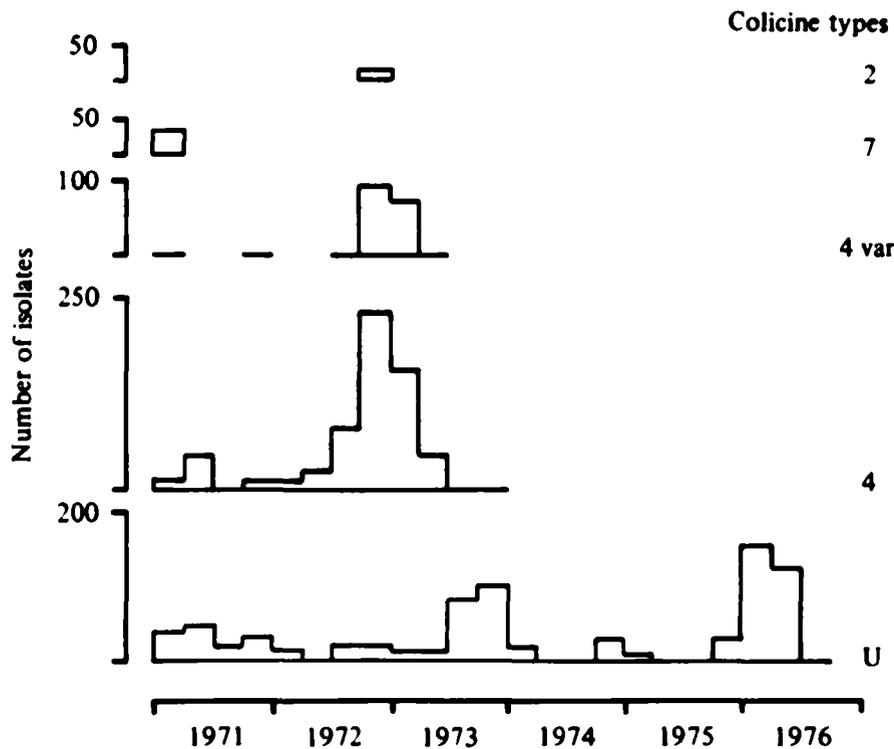


Fig. 1. The yearly distribution of colicine types of *Sh. sonnei*, 1971–6.

2 (Ia), type 3 (K and other, unidentified, colicines), type 11 var. (B) and types 15, SH 1, SH 2 and SH 3 (E-type colicines) (Table 2).

Isolates of type U were isolated throughout 1971, but from occasional patients only between Jan. 1972 and Apr. 1973. Their reappearance in July 1973 was associated with a large outbreak at a time when other types were absent. All but 14 of the 297 isolates from 1974 to 1976 were type U. Isolates of type 4 were recovered intermittently in 1971, predominated in the 17 months from Feb. 1972 to June 1973, and were associated with an outbreak involving more than 100 persons from Oct. to Dec. 1972. Their incidence declined thereafter, and they were not isolated after 1973. Most of the isolates of type 4 var. were recovered from Oct. 1972 to May 1973, i.e. at a time when classical type 4 strains were endemic. All 22 isolates of type 7 were recovered in the first 3 months of 1971. The distribution of the major colicine types over the period studied is shown (Fig. 1).

When the colicine type of the 128 isolates, recovered between Feb. 1971 and Sept. 1972 and which had been stored as duplicate cultures, was determined in 1974, both members of 107 pairs were of the same type. The discrepancies in type between members of the remaining 21 pairs were: types 4 and type U (17 pairs); type 4 var. and type 4 (2); type 4 var. and type U (1); and type 4 and type NRT (1). When 37 single isolates from the years 1963–9 were typed in 1974, 11 of them gave inhibition patterns different from those originally obtained in 1966 (Green *et al.* 1968). Thus, eight isolates originally typed as type 4 were later typed as type 7 (3 strains), or type NRT (3) or type U (2); three isolates, formerly typed as type 2, were later typed as type U (2) or type NRT (1).

Antibiogram testing

Each of the 1420 isolates was sensitive to colistin and furazolidone; only 12 isolates, all recovered between Oct. and Dec. 1972, were sensitive to all eight

Table 3. Drug resistances of 1420 Dundee isolates of *Shigella sonnei*

Year	No. of isolates	No. of isolates resistant to:			
		A	Su	S	KNP
1971	202	77	170	169	63
1972	491	294	350	469	427
1973	430	217	254	387	258
1974	35	7	16	19	0
1975	40	39	38	34	5
1976	222	219	221	179	1
Total	1420	853	1049	1257	754
(%)		(60)	(74)	(88)	(53)

A = ampicillin; Su = sulphonamides; S = streptomycin; KNP = kanamycin, neomycin and paromomycin.

antibiotics tested. The number of isolates resistant to ampicillin (A), sulphonamides (Su), streptomycin (S), kanamycin (K), neomycin (N) or paromomycin (P) are shown, per year, in Table 3. Isolates resistant to K were always resistant to N and P. The majority of the isolates (90%) were resistant to more than one antibiotic.

Isolates of the antibiogram type ASuSKNP were usually of colicine type 4 and formed the largest group (307 isolates) of resistant strains. They were common in Dundee primarily in the 9 months from Aug. 1972 to Apr. 1973. The main outbreaks due to isolates of type SuSKNP (233 isolates, mostly of colicine type 4) occurred during the 14 months from May 1972 to June 1973, particularly at the end of 1972. There were also many isolates of type ASKNP (199) and their distribution was generally similar but not identical with that of type ASuSKNP. Thus, those of type ASuSKNP reached a peak in Oct. 1972 and thereafter declined gradually to Feb. 1973. Those of type ASKNP, on the other hand, reached a peak in Dec. 1972 but thereafter declined rapidly. The distributions of isolates of types ASuS (244) and ASu (79) were similar (Fig. 2), and they contributed to a large outbreak that extended from Oct. 1975 to July 1976. Isolates of type SuS (128) were recovered at irregular intervals during 1971-3 but did not contribute to any major outbreak. Outbreaks due to singly resistant isolates, e.g. S (133) or Su (54), were found mainly in the latter half of 1973 and in 1974, i.e. at times when other antibiogram types were absent (Fig. 2). Thus, isolates of type S were associated with an outbreak in Sept. 1973; simultaneously there was another peak due to isolates of type Su (Fig. 2). The few isolates recovered in 1974 and the first three quarters of 1975 were of types S or Su. A few isolates with other antibiogram types, i.e. ASuKNP (4), AKNP (4), SKNP (7), AS (6) or A (10), were recovered but have been omitted from Fig. 2.

Isolates representative of the different antibiogram and colicine types were tested for their level of resistance to S. Those of types ASuSKNP, SuSKNP and SKNP were resistant to high levels of S (500 µg/ml), as were most of isolates of types ASuS and SuS. Isolates of type ASKNP were resistant to lower levels of S (10-50 µg/ml). When retested in 1974, differences in antibiogram types were observed between members of 26 of the 128 pairs of isolates that had been stored since their original isolation in 1971-2. Thus, loss of resistance was shown by one

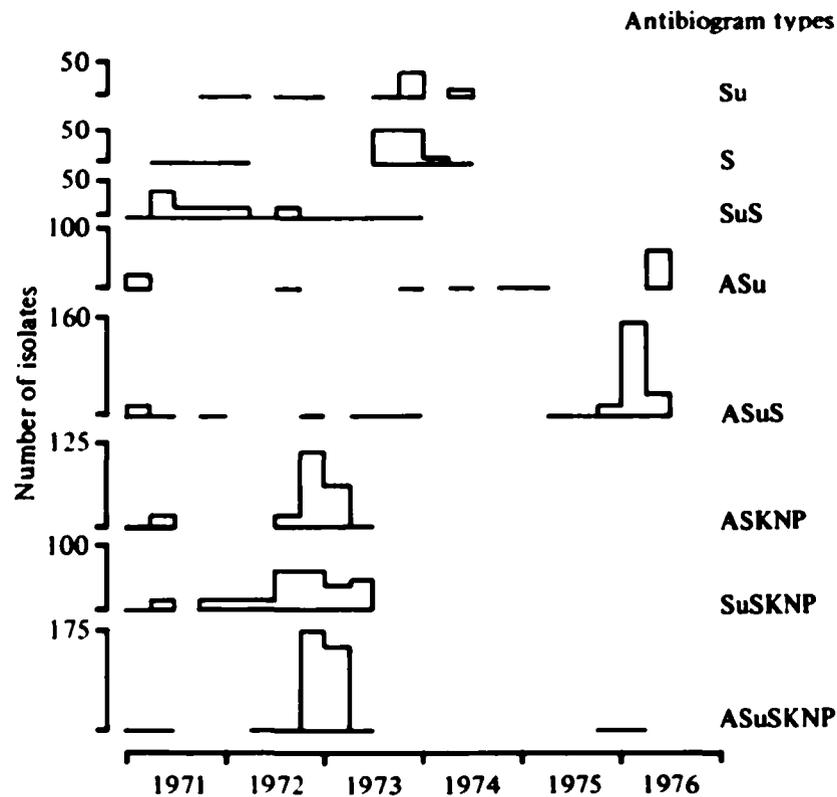


Fig. 2. The yearly distribution of antibiogram types of *Sh. sonnei*, 1971-6.

culture of a pair for: A (2 pairs), Su (14), S (4) and KNP (15). Some had lost resistance to more than one antibiotic. When isolates originally resistant to KNP in 1971 (189 isolates) and 1972 (259) were retested in 1974, 59 and 20, respectively, had lost resistance to KNP.

Correlation between presence of col Ib and KNP resistance

A marked correlation between the presence of *col Ib* and that of determinants for resistance to KNP was demonstrated in studies with duplicate cultures of 20 isolates which at the time of their isolation carried *col Ib* (i.e. colicine types 4 or 4 var.) and were KNP resistant. When retested after storage for 2 to 5 years, changes in either or both of their colicine types or antibiogram types were noted in each pair. The change most regularly observed, in at least one member of 16 of the 20 pairs, was the simultaneous loss of determinants for colicine Ib production and KNP resistance; the independent loss of either was found in the other four pairs (Table 4). Furthermore, when isolates from individual patients or different members of family units were examined, the loss of *col Ib* from strains of types 4 var. or 4 (rendering them, respectively, 11 var. or U after 24 h primary incubation) was frequently associated with the loss of KNP markers (see Table 7, episodes 1, 4, 5, 7, 8, 9, 11, 12 and 19).

Resistotyping

Fifteen resistotypes were observed, four of which accounted for 98% of the 1420 isolates: B-DFHK (634 isolates); B-D-HK (490); --DFHK (199); and --DF-K (42). A further four resistotypes were probably epidemiologically valid types: B-DF-K (22); BCDFHK (7); and BCDF-K (4). The remaining seven resistotypes (Table 5) were represented by a total of 10 isolates recovered from individuals who concomitantly yielded isolates of more common resistotypes.

Table 4. Changes in colicine type or antibiogram type of strains of *Shigella sonnei* associated with loss of colicine Ib or KNP resistance determinants

Colicine type/antibiogram type*		No. of pairs† (of 20 tested) showing change	Character(s) lost
Original	Final		
4/KNP	U/...	14	col Ib, KNP
4/KNP	4/...	1	KNP
4/KNP	U/KNP	2	col Ib
4 var./KNP	U/...	1	col Ib, col B, KNP
4 var./KNP	11 var./...	1	col Ib, KNP
4 var./KNP	4 var./...	1	KNP

* With reference to KNP markers only.

† Duplicate cultures of single isolates.

... Sensitive to KNP.

A higher concentration of chemical F (Helgason, 1977) than that used by Elek *et al.* (1973) was required to detect malachite green sensitivity among the isolates of type B-D-HK; at the lower concentration of Elek *et al.* (1973), 85% of these isolates were resistant to chemical F, i.e. of type B-DFHK. At the higher concentration of F, however, isolates of other malachite green resistant types, including those of type B-DFHK, remained resistant to F. All isolates were partially resistant to cobaltous chloride (Elek *et al.* 1973).

Isolates of type B-DFHK were present throughout almost the whole period of the survey. Those of type B-D-HK were implicated in major outbreaks from Aug. 1972 to Apr. 1973, and from Oct. 1975 to July 1976. Type --DFHK was recovered mainly in the period July 1972 to Apr. 1973. Resistotype --DF-K, which first appeared in June 1976, was responsible for many of the incidents in the final weeks of this survey. Type B-DF-K was recovered only in the first 3 months of 1971 and type -CDFHK only in the period from Oct. to Dec. 1972 (Fig. 3).

Only three of the 128 isolates stored as duplicate pairs showed differences in their resistotypes: the anomalies involved one chemical (F)-, two chemical (F, H)- or four chemical (B, D, F, H)-differences in one of each of the three pairs.

We examined 157 isolates of common and uncommon colicine/antibiogram types representing strains isolated between 1963 and 1969, i.e. in the period prior to that of our survey, and including isolates from the Montrose outbreak (Green *et al.* 1968). Of the 14 resistotypes present (Table 5), one type (B-DFHK) accounted for 75% of the isolates, including those from the Montrose outbreak.

Typing variation within episodes

From a total of 286 episodes, 1230 isolates were cultured; another 190 isolates were recovered from 190 individual patients. Isolates different in one or more of colicine type, antibiogram type or resistotype were found in 129 episodes, representing 47.5% of the isolates. When the primary incubation period of the producer strains was 24 h or 48-72 h, inconsistencies in colicine types among isolates within an episode were found in, respectively, 40 and 20% of the episodes. Inconsistencies in antibiogram types among isolates within an episode were found in 28% of the episodes. The plasmid controlling colicine Ib production and

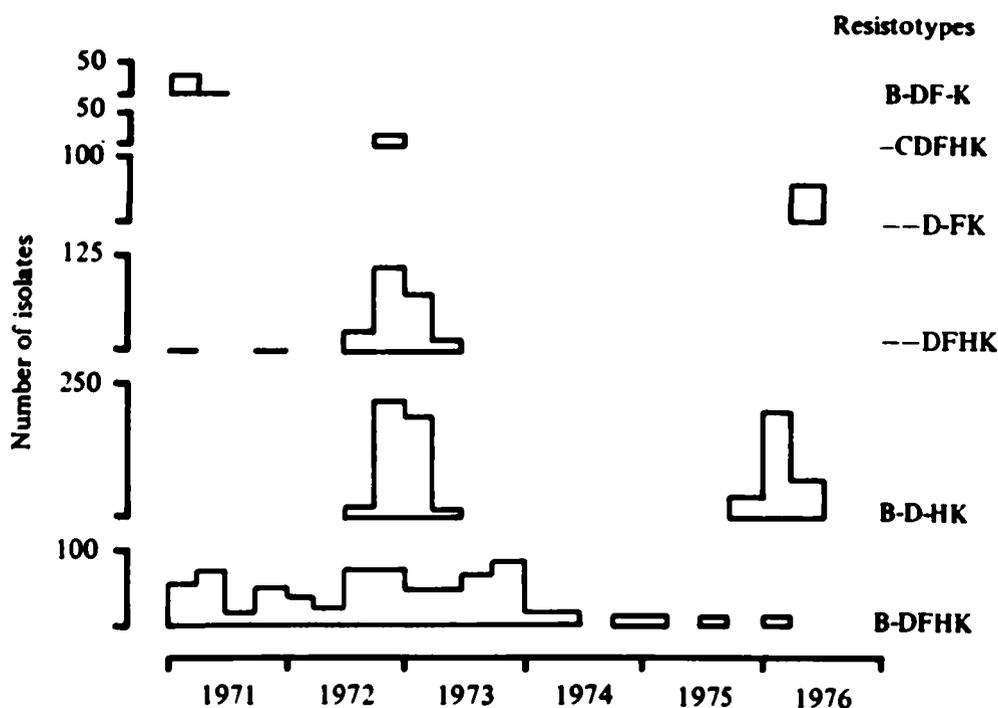


Fig. 3. The yearly distribution of resistotypes of *Sh. sonnei*, 1971-6

Table 5. Resistotypes of Dundee isolates of *Shigella sonnei*

Resistotype	No. of isolates in years	
	1971-6	1963-9
B-DFHK	634	118
B-D-HK	490	1
--DFHK	199	8
--DF-K	42	0
B-DF-K	22	7
-CDFHK	12	1
BCDFHK	7	5
BCDF-K	4	0
Other*†	10*	17†

The other resistotypes (and no. of isolates) were:

* --D-HK (3); B-DFH- (2); B--FHK, B-D--K, B---HK, --DFH- and B--FH- (1 each).

† B-DFH- (4); B----K, --D--K, ---FH- (3 each); BCD---, -CD-HK, BCDFH- and --D-HK (1 each).

resistance to KNP was implicated in, respectively, 75 and 65% of those episodes in which anomalies of colicine type or antibiogram type were noted.

Inconsistencies in resistotype among isolates within an episode occurred in 9% of the episodes. However, because 14 of these were probably either mixed or sequential infections with different types of *Sh. sonnei* (Old, Helgason & Scott, 1981), resistotyping inconsistencies were probably as few as 4%. (For details of episodes, see Helgason, 1977.)

Biotyping

When 111 isolates, representing different colicine, antibiogram and resistotype combinations among the Dundee strains from 1963 to 1969 (41 isolates) and 1971 to 1976 (70) were examined for their ability to ferment 13 sugars or sugar alcohols,

Table 6. Fermentation of seven carbohydrates by 111 strains of *Shigella sonnei*

Day of fermentation	No. of strains that fermented						
	Dextrin	Lactose	Raffinose	Sucrose	Melibiose	Maltose	Rhamnose
1	27	0	0	0	0	54	106
2	6	1	1	0	72	10	5
3	19	9	39	1	21	21	0
4	16	57	12	1	1	15	0
5	16	30	4	2	3	4	0
6	9	13	4	7	0	2	0
7	14	0	3	21	3	4	0
8-14	3	1	24	62	6	1	0
15-21	0	0	8	15	2	0	0
*	1	0	16	2	3	0	0

* No. of strains that had not fermented substrate in 21 days.

all fermented trehalose in 24 h, none fermented dulcitol, inulin, sorbitol or xylose in 21 days, and the only isolate that fermented salicin did so in 20 days. The organic acids *d*-tartrate, *l*-tartrate, *meso*-tartrate and mucate were not utilized in 24 h by any isolate.

The results for the fermentation of the other carbohydrates by these isolates suggested that there was no definitive time that separated isolates into fermenting and non-fermenting types with regard to dextrin, lactose, raffinose or sucrose (Table 6). Furthermore, when the definitive times of reading tests with melibiose were 2 or 3 d, we found, respectively, 72 and 93 isolates that fermented melibiose. However, pairs of otherwise identical isolates gave discrepant results at both of these times; in one experiment a series of 15 isolates of contemporary origin and similar in other typing characters including resistotype, fermented melibiose at different times between 2 and 21 days.

Definitive reading after 1 day separated the 111 isolates into prompt/late fermenters of maltose (54/57) or rhamnose (106/5). Similar testing of all isolates in the larger series confirmed that definitive reading at 1 day gave reproducible results for both maltose and rhamnose fermentation. It was of interest that the only 20 isolates that were late rhamnose fermenters were boric acid resistant (BCDFHK, -CDFHK and BCDF-K). The only other three boric acid resistant isolates recovered were prompt rhamnose fermenters.

DISCUSSION

Colicine typing revealed that 43% of the isolates were untypable, i.e. produced no detectable colicines. Among the typable strains there were nine colicine types, five of which (types 2, 3, 4, 7 and 15) have been previously recognized; the remaining four were new, provisional types (types 4 var., SH1 SH2 and SH3). However, 53% of the isolates were colicine Ib producers and, together with the type U strains, accounted for 96% of all isolates, whereas the other seven types were represented by only 55 isolates (4%). Colicine typing was, thus, of little discriminatory value; this may reflect, in part, the limited geographical area from which our sample originated.

There were other problems associated with the standard methods of colicine typing. Firstly, typable isolates often produced insufficient colicine in 24 h to give a recognizable inhibition pattern with the indicator strains, and about a fifth (22 %) of the colicine-producing isolates required incubation for 48 h or longer before they produced colicine in amounts sufficient to give patterns corresponding to recognized types. This quantitative effect on colicine production was most noticeable among cultures stored for the longest periods before being examined. Similar findings were recognized by Abbott & Shannon (1958) who preferred primary incubation for 4 days to demonstrate the colicine types of cultures stored for more than 6 months. However, on occasion in this study, cultures stored for as little as 4–10 days were weak colicine producers requiring extended primary incubation of 48 h before they gave recognized inhibition patterns. Such isolates gave patterns in which only the most sensitive of the indicators were inhibited, and their colicine types were, accordingly, NRT. Our results suggest that these observed differences in colicine types, which are due to variation in the amount of colicine produced by a culture after either different periods of growth or storage, are so unsatisfactory that they should be avoided by the use of a system that uses specific indicator strains for the identification of the types of colicine produced by colicine-producing cultures, i.e. along the lines recommended by Horák (1975, 1980).

Qualitative differences in observed colicine types were found both among duplicate cultures of the same isolate after storage, and among different isolates of the same strain from episodes involving individual persons or members of the same family (Table 7). Such anomalies were most commonly associated with the loss of the *col* Ib factor from strains of type 4 which, when they carried no other *col* factor, rendered them U (Table 7 episodes 1, 2, 4, 5, 7, 8, 9, 11, 12, 19) or changed them to another colicine type when they carried more than one *col* factor. Thus, when several of the 'Montrose' isolates of type 4 (Green *et al.* 1968) were retested, they gave the inhibition pattern of colicine type 7 strains (unidentified *col* factor). In addition, strains of type 4 var. became type 4 with the loss of the *col* factor B (Table 7, episodes 14, 15, 17) and became U when *col* B and *col* Ib were lost (Table 7, episode 1; Table 4).

Loss of *col* factors was noted particularly among the Dundee cultures stored for the longest periods before examination. Thus, many of the early episodes during 1971 and 1972 yielded mixtures of isolates that were of types 4 or U (Helgason, 1977) and it is probable that many (possibly up to 63 %) of the isolates typed in this study as U were *col* Ib-producers when first isolated. So many of our isolates carried either one or both of the *col* factors Ib and B, and inconsistencies in colicine types within episodes were so frequent among isolates that were otherwise identical as judged by resistotyping (Table 7), that in this study, part of which was retrospective, the information obtained from colicine typing would have been of little value for type differentiation.

Thus, there were both quantitative and qualitative differences in colicine production by isolates, including some stored only briefly, e.g. the loss of colicine production by one culture after storage for 37 days. Results with this series of isolates are in direct contrast to those of other workers who, with a 24-h primary incubation period for colicine production, observed neither quantitative nor qualitative differences in strains stored for many years (Gillies, 1964, 1978; Chan-Teoh

Table 7. Changes in colicine or antibiogram markers of strains of *Shigella sonnei* of uniform resistotype

Episode no.	Patient(s)	Isolation period (days)	No. of isolates	Resistotype	Characters of isolates		
					Colicine type(s) (No. of isolates of type shown)	Drug resistance(s)	
1	a	7	2	B-DFHK(2)	4 var. (1), U(1)	SuSKNP(1), SuS(1)	
2	a	6	6	B-DFHK(6)	4(1), U(5)	S(1), SuS(5)	
3	a	17	5	B-DFHK(5)	4(5)	ASKNP(4), ASuSKNP(1)	
4	a	20	5	B-DFHK(5)	4(3), U(2)	SuSKNP(3), SuS(2)	
5	a, b	8	4	B-DFHK(4)	4(2), U(2)	SKNP(2), SuS(2)	
6	a, b	9	2	B-DFHK(2)	4(2)	ASuSKNP(1), ASKNP(1)	
7	a, b	15	5	B-DFHK(5)	4(1), U(4)	SuSKNP(1), SuS(4)	
8	a, b	26	10	B-DFHK(10)	4(7), U(3)	SuSKNP(7), SuS(3)	
9	a, b, c	23	9	B-DFHK(9)	4(3), U(6)	SuSKNP(1), SKNP(2), SuS(5), S(1)	
10	a, b, c	23	8	B-DFHK(8)	U(8)	ASuS(7), SuS(1)	
11	a, b, c	40	17	B-DFHK(17)	4(12), U(5)	SuSKNP(12), SuS(5)	
12	a, b, c, d	25	14	B-DFHK(14)	4(6), U(8)	ASuSKNP(1), SuSKNP(5), SuS(7), S(1)	
13	a	4	3	--DFHK(3)	4 var. (3)	ASKNP(2), AS(1)	
14	a	4	4	--DFHK(4)	4 var. (3), 4(1)	ASKNP(4)	
15	a	24	6	--DFHK(6)	4 var. (2), 4(4)	ASKNP(6)	
16	a, b	22	10	--DFHK(10)	4 var. (10)	ASKNP(9), A(1)	
17	a, b, c	1	3	--DFHK(3)	4 var. (1), 4(2)	ASKNP(3)	
18	a	7	2	B-D-HK(2)	4(2)	ASuSKNP(1), ASuS(1)	
19	a	32	7	B-D-HK(7)	4(6), U(1)	ASuSKNP(6), ASuS(1)	
20	a, b, c, d	50	7	B-D-HK(7)	4(7)	ASuSKNP(6), ASuS(1)	
21	a	8	3	B-DF-K(3)	7(2), U(1)	ASuS(3)	
22	a	2	3	BCDF-K(3)	'SH3'(3)	ASuS(2), A(1)	

et al. 1971). Because we followed the techniques of Gillies (1964, 1978) we are unable to offer any satisfactory explanation for these observed differences in type stability. Nevertheless, like Abbot & Shannon (1958), our results demonstrated the need for a period of primary incubation longer than 24 h for accurate colicine typing of isolates of *Sh. sonnei*.

The new colicine types SH 1, SH 2 and SH 3, each determined by E-type colicine, should remain provisional until their epidemiological significance has been assessed. Type 4 var., however, was clearly implicated in a major outbreak (Fig. 1). The inhibition pattern of strains of type 4 var. was determined by the presence of colicine B which, when produced in sufficient amounts as after a 48–72 h incubation period, masked the effect of the colicine Ib also produced by type 4 var. strains. With a 24-h primary incubation period, the inhibition pattern obtained with type 4 var. strains was similar to that given by type 4 strains, although the presence of colicine B was indicated by partial inhibition of indicator strains 6, 7 and 14. Furthermore, strains that gave inhibition patterns corresponding to type 11 var. after a 24-h primary incubation period gave the typical type 4 var. pattern (i.e. all indicator strains except 10 completely inhibited) when their incubation was extended to 48–72 h (Plate 1).

The conjoint loss or gain of resistance to K, N and P suggested that their control was by a single gene product, e.g. kanamycin phosphotransferase (Umezawa *et al.* 1967; Ozanne *et al.* 1969), although this has not been proven for these isolates. Furthermore, the finding that loss of the *col* Ib factor was almost invariably associated with the concomitant loss of resistance to KNP (Table 7, episodes 1, 4, 5, 7, 8, 9, 11, 12 and 19), suggested that the colicine Ib and KNP determinants might be associated with an I-like plasmid of the kind described by Dowman & Meynell (1970). Because so many of the isolates of resistotypes B–DFHK, B–D–HK and --DFHK were *col* Ib⁺ and KNP-resistant (Old *et al.* 1981) such a plasmid must have been widely distributed among Dundee isolates until mid-1973. Hence, it was not surprising that among isolates within episodes, inconsistencies in antibiogram typing were found as frequently as those associated with differences in the *col* Ib factor. There was, on the other hand, no correlation between loss of *col* Ib and loss of resistance to other antibiotics such as A, S or Su.

The epidemiologically valid resistotypes among the Dundee isolates were: B–DFHK, B–D–HK, B–DF–K, BCDFHK, BCDF–K, --DF–K, –CDFHK, --DFHK. Each of the eight established types was resistant to phenyl mercury acetate (D) and partially resistant to cobaltous chloride (K); in addition, 98% of the isolates were sensitive to boric acid (C) and 95% were resistant to magnesium perchlorate (H). Thus, these four chemicals afforded little or no discrimination of types among strains in this series. The chemicals of most value for discrimination were, therefore, malachite green (F) and acrylamide (B) which identified, respectively, 65% and 81% of the isolates as resistant. Although the results from both Elek's and the Dundee survey have indicated that cobaltous chloride was poorly discriminatory, further studies with other series are needed before that chemical is excluded from the system.

New resistotypes not detected among the London strains (Elek *et al.* 1973) were: BCDF–K, –CDFHK and --DF–K. Type --DF–K was involved in incidents only in the last month of this survey, at a time when two more common resistotypes

of similar colicine type and antibiogram were present (Old, *et al.* 1981) As with the London strains, resistance to boric acid was a rare character among the Dundee strains, but of value, nevertheless, in discrimination of the types –CDFHK (12 isolates), and BCDF–K (4) (Old *et al.* 1981).

The resistotypes obtained were stable both among isolates within episodes and in cultures after storage. There was no correlation between the age of the stored culture and the origin of the few resistotype variants we noted, and in the majority of episodes in which loss of plasmids resulted in changes in either or both of the colicine and antibiogram types, the resistotypes of isolates remained constant (see, for example, all episodes in Table 7; and Helgason, 1977). The stability of resistotype characters of cultures after storage suggested that resistotyping should prove invaluable in retrospective analyses.

Previous biotyping studies with *Salmonella typhimurium* (Duguid *et al.* 1975) and *Escherichia coli* (Crichton & Old, 1979) have indicated the need to read results of each biotyping test at definitive times that allow the inclusion of all genotypically positive strains and the exclusion of all genotypically negative strains, a requirement not always recognised, however, in biotyping studies. Thus, the only biotyping characters that afforded discrimination among this series of isolates were maltose and rhamnose fermentation. The Dundee isolates were least discrepant with regard to maltose fermentation when the definitive time of reading was one day. We found the 2- and 4-day limits of others (see Hammarström, 1949) highly unreliable because each time of reading included as prompt fermenters many genotypically non-fermenting isolates that produced maltose prompt-fermenting mutants within 2–4 days. Because the majority of Dundee isolates could be accurately defined as prompt or late maltose fermenters, that biotype character proved useful as an ancillary marker for typing *Sh. sonnei* in conjunction with other techniques (Old *et al.* 1981). Occasional difficulties were noted with some types, e.g. isolates of resistotype B–D–HK isolated between Oct. 1975 and July 1976 consistently fermented maltose in 24–30 h, but this consistency was itself a useful marker for these isolates.

Although rhamnose was poorly discriminatory in that there were only 20 non-fermenting isolates, this was a useful biotype character for some important types of *Sh. sonnei* in this series (Old *et al.* 1981). Other workers have found lactose, xylose, raffinose and melibiose useful substrates for strain discrimination (Hammarström, 1949; Tee, 1952; Aoki, 1968; Szturm-Rubinsten, 1968; Chan-Teoh *et al.* 1971) but it is not always clear that other workers separated genotypically fermenting from non-fermenting types at the times chosen for reading.

The finding that strains from the Montrose outbreak were of a colicine/antibiogram type (type 4/ASuSKNP) not previously encountered in that area had suggested that this strain had been introduced to Angus from as far away as Leeds, although epidemiological evidence for that suggestion could not be found (Green *et al.* 1968). In this study the 'Montrose' strain was of resistotype B–DFHK, a type present in the area throughout the years from 1963 to 1976, and strains of resistotype B–DFHK isolated before the Montrose outbreak were U and ASuS (Helgason, 1977). The more likely explanation for the origin of the 'Montrose' strain, therefore, now seems to be that an endemic strain of the type B–DFHK acquired the 'Ib/KNP' plasmid and was thereby converted to the 'Montrose'

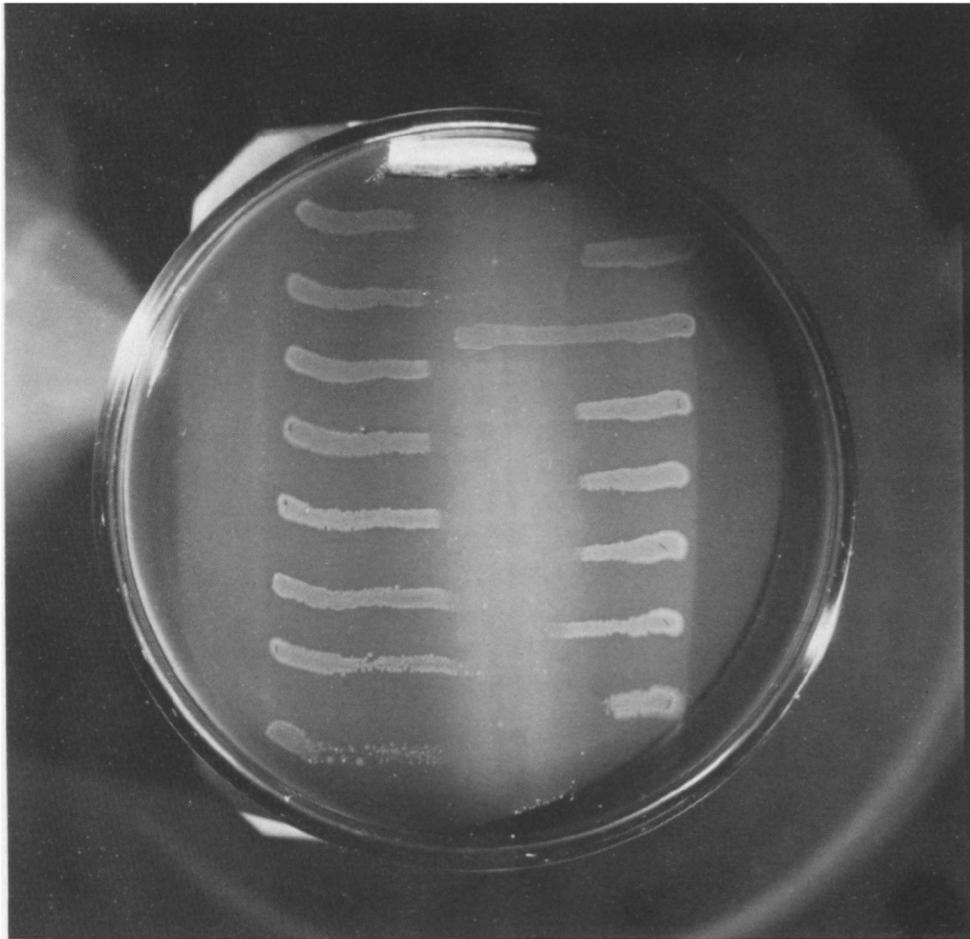
phenotype. Thus, the appearance and subsequent rapid disappearance of the 'Montrose' strain from this area (Green *et al.* 1968) may have been more apparent than real.

Although colicine and antibiogram markers are regularly used in epidemiological studies to establish periods of carriage of *Sh. sonnei* and to identify and monitor different types in a community, these would have been as highly unreliable for such studies among this series of isolates, as they were for other Scottish isolates of *Sh. sonnei* recovered between 1967 and 1971 (Brodie & Porter, 1973). The episodes in Table 7, chosen to demonstrate some examples of changes in colicine and antibiogram types among isolates within only a few episodes, reveal, by contrast, how stable the resistotype characters were. The outstanding conclusion to be drawn from this study is that resistotype characters were subject to less variation *in vivo* and *in vitro* than some markers associated with colicine type or antibiogram. For this reason, resistotyping would seem such a valuable tool that it should probably be used as the principal method of typing in all but short-term epidemiological studies. In a separate paper (Old *et al.* 1981) the great value of resistotyping for the primary identification of different clones of *Sh. sonnei* in the Dundee area is discussed.

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EXPLANATION OF PLATE 1

Inhibition pattern of indicator strains produced by isolate of *Sh. sonnei* of colicine type 4 var. Primary incubation of 48 h at 35.5 °C.