RESISTOTYPING OF *SHIGELLA SONNEI*

S. D. ELEK*, JOAN R. DAVIES† AND ROBERTA MILES*

*Department of Medical Microbiology, St George's Hospital Medical School, London, S.W.1**
and Public Health Laboratory, Guildford, Surrey†

RESISTOTYPING is based on the selective toxicity of randomly chosen chemicals. By the use of a suitable range of selective substances, at critical concentrations, a pattern of resistance can be obtained that is characteristic of a strain. This approach was first described by Elek and Higney (1970) and illustrated by its application to *Escherichia coli* in relation to serology. The general principle can, however, be applied to any commonly occurring pathogenic bacterium for which a system of typing is required for epidemiological purposes.

Shigella sonnei is a single species, and cannot be subdivided by serology. It is also the commonest and only endemic dysentery bacillus at present in England. Small- or medium-sized outbreaks occur fairly frequently and colicine typing, considered together with the antibiogram, provides at present the only tool for the tracing of this organism within a community. Eleven substances were chosen as the basis of a resistogram system for *S. sonnei*. The results of typing were compared with the colicine types and antibiograms, and were examined in the light of the known epidemiological background of the isolates.

MATERIALS AND METHODS

Strains. Nineteen strains of *S. sonnei* were selected to establish the resistogram system. They all came from patients in the London area, and were isolated in the first half of 1969. On the basis of colicine type, antibiogram and geographical distribution they were believed to be epidemiologically unrelated.

Culture media. Difco Nutrient Broth, at final pH 6.8, was used throughout for liquid cultures. The solid medium was Difco Antibiotic Medium no. 2, made up in tris-buffer {Tris(hydroxymethyl)methylamine, BDH no. 27119} at pH 7.6, but in a concentration 10 per cent. higher than recommended by the manufacturers, to allow for the dilution caused by the addition of the antibacterial agent.

Screening of antibacterial compounds. This was done by the gradient plate method (Szybalski, 1952). A variety of organic and inorganic compounds, as well as dyes, were tested, and those that showed selective inhibition of the standard strains were further investigated. The bottom wedge contained the chemical (1 ml in solution to 9 ml of agar) and the top wedge was agar (10 ml). After drying for 20 min. at 37°C, the plates were inoculated by streaking suspensions of the standard strains along the gradient from the low to the high concentration. Each plate accommodated six or seven strains. The inoculum was a loopful of an overnight broth culture diluted 1 in 10 in sterile water. The plates were read after 18 hours' incubation at 37°C. Compounds that showed very little or no selective toxicity for the standard strains gave much the same length of growth for all the organisms. Potentially useful selective toxicity was shown by lines of growth of different length. In all, 157 compounds were so tested and 24 showed selectivity on gradient plates. Each of

Received 16 Nov. 1972; accepted 1 Jan. 1973.

these compounds was further investigated by preparing a series of plates containing different concentrations of the compound around the value that appeared discriminatory on the wedge plate. This was done by adding 3-ml volumes of each concentration of the compound to 27 ml of agar for each plate poured.

The whole range of plates was next inoculated with suspensions of the reference organisms by means of a multiple-inoculating machine. This provided 32 inoculation sites per plate, and the inoculum was adjusted to contain about 3000 organisms. That concentration of the compound that gave the most clear-cut discrimination was selected. Of the 24 compounds chosen on the basis of the gradient plate, only 11 proved satisfactory after this titration, because the remainder gave too many intermediate results or were unstable on storage.

The 11 compounds chosen are shown in table I, and the resistance patterns obtained with reference strains are shown in table II. Chemicals referred to as A, C, I and K were

TABLE I
Compounds chosen for resistotyping of Shigella sonnei

Reference letter	Compound	Concentration (per cent. w/v) in	
		master solution	working solution*
A	Potassium chromate	1.0	0.07
B	Acrylamide	6.0	6.0
C	Boric acid	3.0	3.0
D	Phenyl mercuric acetate	0.01	0.0004
E	Acriflavine	0.1	0.1
F	Malachite green	0.01	0.0025
G	Potassium cyanate	0.3	0.3
H	Magnesium perchlorate	13.0	13.0
I	Cupric sulphate	0.25	0.25
J	tri-Sodium orthophosphate	10.0	10.0
K	Cobaltous chloride	0.35	0.35

* Three ml of working solution is added to 27 ml of nutrient agar.

obtained from British Drug Houses Chemicals Limited, Poole, Dorset, in Analar grade. B, D, E, G, H and J were also supplied by the same firm, but were laboratory-grade reagents. Chemical F was obtained from Edward Gurr Limited, 42 Upper Richmond Road, London, S.W. 14. All solutions were prepared in glass-distilled water; chemical D was first dissolved in 2 ml of ethanol to which 98 ml of water were then added.

Although in theory plates could be poured with the exact concentrations shown in table I, in practice the selective toxicity has such fine margins that a biological titration of each freshly made master solution is required for resistogram typing. This was carried out as follows. The working dilutions were freshly prepared from the master solution as shown in table I; 2.6 ml, 2.8 ml, 3.0 ml, 3.2 ml, and 3.4 ml of the working dilution were each added to 27 ml of nutrient agar to produce five plates differing slightly around the optimal concentration. For chemicals H and J an even finer range was required, with intervals of 0.1 ml. The range of plates was then tested with the reference strains. Generally, but not always, the optimal concentration was that obtained when 3 ml of the working dilution was added to 27 ml of nutrient agar.

This titration had to be carried out each time a new master solution was prepared. The master solutions were made up in 100-ml quantities and kept in hard glass containers with plastic caps at 4°C. It was found that all the master solutions could be stored for at least 1 wk, and some for longer periods, without repeating the biological titration.

The technique is so sensitive that even traces of material leached out of soft glass, or of

rubber washers, may affect the results. It was therefore necessary to use hard glass containers with special white plastic caps without washers (Sterilin Ltd). All glassware, except the pipettes, was washed by a technique normally used in tissue culture work. We did this in the Miele G19 automatic washing machine, using programme no. 24. The pipettes were acid-washed in the usual way. Disposable plastic petri dishes were used throughout.

Technique of resistotyping. Three plates were prepared with each chemical: the optimal concentration as determined earlier by the biological titration and at the concentrations immediately above and below this. A control plate, of nutrient agar alone, was included. The plates were dried at 37°C for 20 min. and then inoculated by means of an inoculating machine with solid stainless steel cylinders of 3-mm diameter. The inocula consisted of about 3×10^3 organisms. This was achieved by growing the bacteria for about 18 hr in 1-ml broth cultures at 37°C. The cultures were next placed at 4°C for 3 hr and then diluted by the addition of 9 ml of fresh broth. They were reincubated for 2½ hr at 37°C in a water-bath. These cultures were then diluted 1 in 10 in sterile distilled water and a single loopful of a Takatsy loop was transferred into 0.25 ml of distilled water contained in one of the glass cups of the inoculating machine. All the plates, starting with the control, were inoculated without delay, by means of the machine, and incubated at 37°C overnight. The reference strains were included in every batch of tests.

Unless confluent growth was obtained on the control plate the results could not be accepted. The growth on the plate containing the optimal concentration given in the biological titration of each chemical was then recorded. The plates containing slightly more and slightly less than the optimal concentration were referred to if a doubtful reading was obtained.

Strains showing confluent growth were considered resistant and this result was recorded by the letter assigned to the chemical (see table II). Strains showing no growth were considered sensitive and this was recorded by a dash in place of the letter. Two other forms of resistance were encountered. One consisted of confluent fine gonidial colonies and was denoted by a small "f" over the letter of the chemical. The other was less than confluent growth, but more than ten colonies per inoculum. This was recorded by brackets around the code letter. Although both these forms of resistance were recorded, they represent partial resistance and such strains were classified as resistant for typing purposes.

The resistotype of a strain is the profile of the organism as described by its resistance pattern to the 11 chemicals, each used at the chosen concentration. In this way, letter combinations were obtained, e.g., a strain might be recorded as AB-DE(F)GHIJK^f. For purposes of typing, such a strain would be regarded as AB-DEFGHIJK, and any other strain showing the same basic pattern would be regarded as probably identical. Strains differing by one or more letters would be considered different. The strains were tested in batches of 30; each batch consisted of an unknown mixture of strains derived from various outbreaks.

Colicine typing. This was carried out with nine indicator strains as described by Abbott and Graham (1961).

Antibiograms. The techniques for antibiotic sensitivity testing and resistance transfer were those described by Davies, Farrant and Tomlinson (1968*a, b*). Strains were inoculated onto special media containing selected concentrations of the antibiotics. A modified phage-typing machine was used to inoculate the plates. Strains were tested for sensitivity to sulphonamide (20 µg per ml), streptomycin (10 µg per ml), tetracycline (5 µg per ml), ampicillin (5 µg per ml) and neomycin (10 µg per ml). Transfer of resistance was investigated by mating the strains with an antibiotic-sensitive strain of *E. coli*, strain K12. The chromosomal resistance to streptomycin was demonstrated by growing the strains on nutrient agar containing 800 µg per ml of streptomycin (Davies *et al.*, 1968*a*).

RESULTS

Two hundred and seventeen strains of *S. sonnei* isolated in south-east England between 1968 and 1972 were resistotyped. Another 30 strains

TABLE II
Standard patterns obtained with 19 reference strains of *Shigella sonnei*

Strain number	Colicine type	Resistance patterns obtained with compound											Resistotype
		A	B	C	D	E	F	G	H	I	J	K	
1	8	+	+	-	+	+	+	-	+	+	+	+	K' J
2	6/11	+	+	+	+	+	+	+	+	+	+	+	K' -
3	7	+	+	+	+	+	+	+	+	+	+	+	K' J
4	7	+	+	+	+	+	+	+	+	+	+	+	K' J
5	0	+	+	+	+	+	+	+	+	+	+	+	K' J
6	0	+	+	+	+	+	+	+	+	+	+	+	K' J
7	0	+	+	+	+	+	+	+	+	+	+	+	K' J
8	4	+	+	+	+	+	+	+	+	+	+	+	K' -
9	4	+	+	+	+	+	+	+	+	+	+	+	K' -
10	7	+	+	+	+	+	+	+	+	+	+	+	K' J
11	0	+	+	+	+	+	+	+	+	+	+	+	K' J
12	7	+	+	+	+	+	+	+	+	+	+	+	K' J
13	0	+	+	+	+	+	+	+	+	+	+	+	K' J
14	0	+	+	+	+	+	+	+	+	+	+	+	K' J
15	8	+	+	+	+	+	+	+	+	+	+	+	K' J
16	0	+	+	+	+	+	+	+	+	+	+	+	K' J
17	0	+	+	+	+	+	+	+	+	+	+	+	K' J
18	9	+	+	+	+	+	+	+	+	+	+	+	K' J
19	0	+	+	+	+	+	+	+	+	+	+	+	K' J

+ = Confluent growth; (+) = growth less than confluent, but more than ten colonies; +f = heavy growth of fine gonidial colonies; - = no growth.

* = Occasional variation from partial resistance to no growth and vice versa.

TABLE III
Summary of five family outbreaks

Episode number	Location (and year)	Number of strains examined	Resistance to antibiotic*					Colicine type	Resistotype†											Number of strains with minor variation‡ deviation§	
			Su	St	Te	Am	Ne		K'	J	I	H	G	F	E	D	C	B	A		
1F	Tower Hamlets (1971)	5	S	S	S	S	S	6	(A)	B	C	D	E	F	G	H	I	J	K'	0	0
2F	Bexley (1971)	5	S	R	R	S	R	4	A	B	-	D	E	F	(G)	H	I	J	K'	0	0
3F	Lambeth (1971)	9	S	R	R	R	S	7	A	B	-	D	E	(F)	(G)	H	I	J	K'	1	0
4F	Abinger (1971)	3 3	R R	R R	S R	S R	S S	0	A	B	-	D	E	F	(G)	H	I	J	K'	4	0
5F	Maidstone (1971)	5	R	R	S	R	R	7	A	B	-	D	E	F	(G)	H	I	J	K'	1	0

* Su = Sulphonamide; St = streptomycin; Te = tetracycline; Am = ampicillin; Ne = neomycin; R = resistant; S = sensitive.

† Brackets relating to resistotype markers denote partial resistance; f = numerous fine colonies.

‡ Minor variation means difference in degree of resistance only (bracket or f).

§ Major deviation means a difference of at least one marker in the resistotype.

obtained from other areas were similarly tested. The resistotyping was done blindly, i.e., the person carrying it out did not know the origin, colicine type or antibiogram of the organism. This information was supplied subsequently, and the results were analysed in the light of the known epidemiological findings. We attempted to examine two distinct problems: to decide whether resistotyping is a valid approach in principle for epidemiology, and to determine whether the system is sufficiently accurate in its present form.

Typing of epidemiologically related groups of strains

The most compact form of an outbreak in time and space is that occurring in a family; there is a high probability that all members of the family will have been infected with the same strain. Table III shows the findings in five family outbreaks comprising 30 strains. Within each family all the strains were of the same resistotype, although some strains showed minor variations in the degree of resistance. In episode 4F, three members of the family were excreting a strain with acquired resistance to tetracycline and ampicillin but this was not associated with a change in resistotype. The minor variations in this episode were not correlated with the acquired drug resistance.

Next, we considered outbreaks occurring in schools and day nurseries (table IV); here again there is a reasonably high probability that all the contemporary cases will be infected with the same strain. In nine of the 11 outbreaks, comprising 69 strains, only minor variations in resistotype were observed. These outbreaks included six strains that had acquired or lost antibiotic resistance and one that had a different colicine type from the other strains isolated in the same school. However, all these six strains retained the resistotype of the episode with which they were associated. In two of the outbreaks there were strains of a different resistotype from the other strains in the episode; two of nine strains in episode 4S and one of seven strains in episode 7S were different.

The remaining 102 strains we examined were from 13 outbreaks occurring in various boroughs or other geographically defined areas of London (table V). Such an outbreak was defined as a number of cases of Sonne dysentery occurring in an area in a 3-mth period from which strains of the same antibiotic resistance pattern and colicine type were isolated. These cases were not so closely related epidemiologically as those in the family and school outbreaks. From seven of the borough outbreaks a total of 48 strains gave consistent results on resistotyping. In the six other outbreaks 12 out of 54 strains showed a major difference in resistotype from other strains with which they were associated. It will be seen that five of these 12 discrepant results occurred in a single episode (3B), and it is worth considering this episode in detail since it demonstrates a number of practical points about resistotyping.

Episode 3B. The nine strains in this episode were isolated from adjoining areas in the boroughs of Islington and Hackney in the last quarter of 1968. They were all of colicine-type 7 and were capable of transferring resistance to sulphonamide and to streptomycin and tetracycline; the latter two resistance determinants were always transferred together. Strains with these characteristics were very uncommon elsewhere in London at that time.

TABLE IV
Summary of 11 school and day-nursery outbreaks

Episode number	Location (and year)	Number of strains examined	Resistance to antibiotic					Colicine type	Resistotype										Number of strains with		
			Su	St	Te	Am	Ne												minor variation	major deviation	
1S	Islington (1971)	11	R	R	S	S	S	6	A	B	C	D	E	F	G	H	I	J	K'	1	0
2S	Reading (1972)	6	S	R	R	R	S	7	A	B	-	D	E	(F)	(G)	H	I	J	K'	1	0
3S	Guildford (1971)	10	R	R	R	R	S	0	A	B	-	D	E	F	G	H	I	J	K'	4	0
4S	Hammersmith (1968)	9	S	R	R	R	S	7	A	B	-	D	E	(F)	-	H	I	J	K'	2	2
5S	Islington (1968)	5 2	R S	R R	R R	R R	S S	0	A	B	-	D	E	F	G	H	I	J	K'	1	0
6S	Hackney (1968)	4 1	R R	R R	S R	R R	S S	0 4	A	B	-	D	E	F	(G)	H	I	-	K'	1	0
7S	Southwark (1968)	7	R	R	S	R	S	9	A	B	-	-	(E)	(F)	(G)	H	I	J	K'	1	1
8S	Hackney (1968)	6 1	R R	R R	S R	R R	S R	9	A	B	-	D	(E)	(F)	(G)	-	I	J	K'	6	0
9S	Camden (1968)	5	R	R	S	R	S	9	A	B	-	D	(E)	F	-	H	I	J	K'	2	0
10S	Bexley (1968)	7	S	S	S	R	S	0	A	B	-	D	E	F	(G)	H	I	J	K'	4	0
11S	Lambeth (1968)	9 1 1	R S R	R R R	S S R	R R S	S S S	0	A	B	-	D	E	(F)	(G)	H	I	J	K'	3	0

Symbols as in table III.

By chance, these strains had been tested in three different batches with the following results:

Batch 1	Strain 1	A B - D E - - - I - K ^f
	Strain 2	(A) B - (D) E - - - I - K ^f
	Strain 3	(A) B - (D)(E) - - - I - K ^f
Batch 2	Strain 4	A B - D (E) - - (H) I J K ^f
	Strain 5	A B - (D)(E) - - (H) I J K ^f
	Strain 6	A B - (D)(E) - - (H) I J K ^f
	Strain 7	A B - (D)(E) - - (H) I J K ^f
Batch 3	Strain 8	A B - (D)(E) - - (H) I - K ^f
	Strain 9	(A) B - D E - - (H) I - K ^f

Disregarding minor variations with chemicals A, D and E, it will be seen that these strains had in common sensitivity to chemicals C, F and G; they were the only strains in our series to show this pattern. It seems reasonable to assume that this outbreak represents the spread of a single strain in an area and that the discrepant results obtained with chemicals H and J are due to technical variability. Our biological titrations have shown that the concentration of these chemicals in the medium is extremely critical and very small differences can affect the results. When these nine strains were re-tested on the same plates they were all sensitive to H and J. Thus the correct resistotype of episode 3B should have been AB - DE - - - I - K^f. This underlines the need to test, or re-test, all strains from a suspected outbreak on the same set of plates.

All strains reported so far, including the reference strains, had been isolated in south-east England, most of them in the London area. We examined a further 30 strains from other geographical areas and the results are shown in Table VI. The British strains were believed to have come from discrete episodes, the Polish strains were from a defined area, but we have no information about their precise relationship.

Relationship between antibiotic resistance, colicine type and resistotype markers

To assess whether resistance to the chemicals was independent of antibiotic resistance, we analysed the results for each chemical against each antibiotic. For example, with chemical G and sulphonamide, 123 strains were resistant to both, 8 strains were sensitive to both, 43 strains were sensitive to G and resistant to sulphonamide and 43 strains were resistant to G and sensitive to sulphonamide. Likewise with the other antibiotics there appeared to be no correlation between antibiotic resistance and any of the resistotype markers.

The relationship between colicine type and resistotype is shown in table VII. Of the strains responsible for 29 episodes of infection, 14 belonged to a single resistotype, characterised by sensitivity to compound C. Of the 13 episodes which were colicine-type 0, there were three of a different and distinctive resistotype; of the remaining 16 episodes in which the strain was a colicine producer, 12 were of a distinctive resistotype. As we have already seen, apparent change of colicine type *in vivo* did not appear to alter the resistotype (see episodes 6S and 9B).

It was also necessary to show that the genetic determinants for resistance to our chemicals were not carried on the same episome as antibiotic resistance determinants. Ten of the strains included in the outbreaks had gained or lost

TABLE V
Summary of 13 area outbreaks in London

Episode number	Location (and year)	Number of strains examined	Resistance to antibiotic					Colicine type	Resistotype	Number of strains with	
			Su	St	Te	Am	Ne			minor variation	major deviation
1B	Islington (1968)	10	R	R	R	R	S	0	A B - D E (F) G H I J K'	2	0
2B	Islington (1968)	6	R	R	S	R	S	0	A B - D E F G - I J K'	3	0
3B	Islington and Hackney (1968)	9	R	R	R	R	S	7	A B - (D) (E) - - (H) I J K'	1	5
4B	Islington (1968)	9	R	R	S	R	S	0	A - - D E (F) G H I J K'	4	2
5B	Southwark (1968)	18	R	R	S	R	S	9	A B - D (E) F - H I J K'	6	1
6B	Camden (1968)	5	R	R	S	R	S	9	A B - D (E) (F) - H I J K'	1	1
7B	Southwark (1968)	5	R	S	S	S	S	0	A B - D E F (G) H I J K'	0	0
8B	Lambeth (1968)	9	R	S	S	R	S	7	(A) (B) - D (E) - (G) H I J K'	3	0
9B	East London (1972)	1 6	S S	R R	R R	R R	R R	4 3a	A - - D (E) (F) (G) H I J K'	3	0
10B	Lambeth (1968)	6	R	R	R	R	S	0	A B - D E (F) (G) H I J K'	1	0
11B	Hammersmith (1968)	5	R	S	S	R	S	0	A B - D E (F) (G) H I J K'	2	0
12B	Hackney (1968)	8	R	R	S	R	S	7	A B - D E F - H I J K'	3	0
13B	Bexley (1968)	4 1	R R	S S	R S	R S	S S	0	A (B) - D E F (G) H I J K'	2	1

Symbols as in table III.

an R factor without change in resistotype. We next investigated the effect on resistotype of antibiotic resistance transfer *in vitro*. Two strains of *S. sonnei*,

TABLE VI
Strains from regions other than south-east England

Location	Number of strains examined	Colicine type	Resistotype											Number of strains with	
														minor variations	major deviations
Aberdeen	7	4	(A)	B	-	(D)	E	F	-	H	I	J	-	0	1
Poland	4	2	(A)	B	-	D	E	F	G	H	I	J	K'	4	0
	2	2	(A)	B	C	D	E	F	G	H	I	J	K'	0	0
Poland	6	4	(A)	B	-	D	E	F	G	H	I	J	K'	5	0
Cardiff	6	0	(A)	B	-	D	E	(F)	-	H	I	J	K'	5	0
Birmingham	2	0	A	B	-	D	E	F	-	H	I	J	K'	1	0
	3	0	A	B	-	(D)	E	F	G	H	I	J	K'	0	0

Symbols as in table III.

TABLE VII
Relationship between colicine type and resistotype markers in 29 episodes comprising 217 strains

Colicine type	Resistotype*											
Number of episodes												
0	10	A	B	-	D	E	F	G	H	I	J	K'
	1	A	-	-	D	E	F	G	H	I	J	K'
	1	A	B	-	D	E	F	G	-	I	J	K'
	1	A	B	-	D	E	F	G	H	I	-	K'
7	3	A	B	-	D	E	F	G	H	I	J	K'
	1	A	B	-	D	E	-	G	H	I	J	K'
	1	A	B	-	D	E	-	-	-	I	-	K'
	2	A	B	-	D	E	F	-	H	I	J	K'
9	1	A	B	-	-	E	F	G	H	I	J	K
	3	A	B	-	D	E	F	-	H	I	J	K
	1	A	B	-	D	E	F	G	-	I	J	K'
4	1	A	B	-	D	E	F	G	H	I	J	K'
6	2	A	B	C	D	E	F	G	H	I	J	K'
3A	1	A	-	-	D	E	F	G	H	I	J	K'

* The degree of resistance is not recorded in brackets; f = numerous fine colonies.

A and B (table VIII) known to transfer antibiotic resistance were mated with *E. coli* K12. Resistant progeny were selected and mated with two sensitive strains of *S. sonnei*, C and D. Resistant progeny of the second matings were selected and resistotyped. The transfer of resistance to the *S. sonnei* recipients

was done in two stages for the following reasons. We wished to use as recipients naturally occurring strains rather than resistant mutants, and we wanted to select twice for the antibiotic-resistance determinant so that the chance of coincident transfer of resistance to a chemical would be reduced. It is not our contention that chemical resistance is not episomally determined, we merely wanted to show that such determinants were not generally carried on the same episome as the R factors. Donor A transferred resistance to sulphonamide and tetracycline independently. Recipient C accepted the sulphonamide resistance of A, but not the tetracycline resistance; recipient D accepted both resistance determinants. Donor B transferred resistance to sulphonamide and also resistance to streptomycin, tetracycline and neomycin. The last three drug resistances were transferred at a high rate and were accompanied by the colicine factor determining colicine-type 4. Both recipients accepted the R factors of donor B.

TABLE VIII
Characteristics of donor and recipient strains of Shigella sonnei

Donor strain	Recipient strain	Resistotype	Colicine type	Resistance to antibiotic				
				Su	St	Te	Am	Ne
A	...	A B - D E F (G) H I J K ^r	0	R	S	R	R	S
B	...	A B - D (E) (F) (G) ^r H I J K ^r	4	R	R	R	S	R
...	C	A B - - E (F) (G) H I J K ^r	0	S	S	S	R	S
...	D	A - - D (E) - - H I J K ^r	4	S	S	S	R	S

Symbols as in table III.

Table IX shows the results obtained in the mating experiments. It is noteworthy that the resistotype of the progeny was always that of the recipient, although the donor had successfully transferred various antibiotic resistances and in some instances a change in the colicine type.

DISCUSSION

Evaluation of resistotype markers

We reviewed our findings for each chemical, considering whether the results were consistent within an episode and also whether the chemical was useful in distinguishing strains from different episodes. Thus, of the 217 strains from south-east England, 214 were fully or partially resistant to chemical A. The three sensitive strains occurred in two different episodes, rendering the resistotype different from the rest of the strains within the episodes. With chemical B, however, 201 strains were resistant, and the 16 strains that were sensitive were confined to two episodes (4B and 9B) and all the strains in these episodes shared this character. On this basis, chemical B proved more consistent and gave a more useful discrimination than chemical A. Applying this

TABLE IX
Results of *in-vitro* transfer of drug resistance in *Shigella sonnei*

Donor strain	Recipient strain	Antibiotic resistance selected in progeny	Resistotype of progeny										Colicine type of progeny	Resistance of progeny to antibiotic						
			Su	A	B	-	-	E	F	(G)	H	I		J	K'	Su	St	Te	Am	Ne
A	C	Su	A	B	-	-	E	F	(G)	H	I	J	K'	0	R	S	S	S	R	S
B	C	Su	A	B	-	-	E	(F)	(G)	H	I	J	K'	4	R	R	R	R	R	R
B	C	St	A	B	-	-	E	(F)	(G)	H	I	J	K'	4	S	R	R	R	R	R
B	C	Te	A	B	-	-	E	(F)	(G)	H	I	J	K'	4	S	R	R	R	R	R
B	C	Ne	A	B	-	-	E	(F)	(G)	H	I	J	K'	4	S	R	R	R	R	R
A	D	Su	A	-	-	D	(E)	-	-	H	I	J	K'	4	R	S	S	S	R	S
A	D	Te	A	-	-	D	(E)	-	-	H	I	J	K'	4	S	S	R	R	R	S
B	D	Su	A	-	-	D	(E)	-	-	H	I	J	K'	4	R	S	S	S	R	S
B	D	St	A	-	-	D	(E)	-	-	H	I	J	K'	4	S	R	R	R	R	R
B	D	Te	A	-	-	D	(E)	-	-	H	I	J	K'	4	S	R	R	R	R	R
B	D	Ne	A	-	-	D	(E)	-	-	(H)	I	J	K'	4	S	R	R	R	R	R

Symbols as in table III.

reasoning to the others we concluded that chemicals B, C, D, F, G, H and J were "good" markers but that chemicals A, E, I and K were of little value. It must be stressed, however, that, for each chemical, resistant and sensitive strains of *S. sonnei* exist, as shown in the list of reference strains (table II). Thus, strains from other areas may give different resistotypes from those we encountered in south-east England. For example, the strains from Aberdeen (table VI), were all sensitive to chemical K. Final assessment of the value of chemicals A, E and I will be possible only when more strains from other areas have been investigated.

We have ignored, for purposes of resistotyping, differences in the degree of resistance, shown by brackets or f readings. In most instances strains from the same episode gave identical readings, even in degree of resistance, if they were all tested in one batch. In practice, a critical evaluation of strains would always be done by comparing them with each other and with control strains in the same test. Some of the compounds used were more prone than others to batch variation and discrepancies on re-testing.

Epidemiological evaluation of resistotyping

The principal objection to using the antibiogram to characterise a strain for epidemiological purposes is that many of the antibiotic resistance determinants are episomal. During the course of infection about 5 per cent. of patients can be shown to excrete strains of *S. sonnei* with newly acquired antibiotic resistance (Davies *et al.*, 1968*b*). Determinants for colicinogeny may be on the same episome as antibiotic-resistance determinants, and some strains change their colicine type when they acquire resistance within an outbreak (see episode 6S). Others may show a change in colicine type without concomitant change in antibiotic resistance. This means that two strains that differ only by a single episome (which may code for resistance to several antibiotics, for colicinogeny or for both) cannot necessarily be regarded as different epidemiologically. We deliberately included in our series some strains that were known to have acquired or lost antibiotic resistance. These changes were not accompanied by changes in resistotype, nor could we demonstrate any change in resistotype accompanying antibiotic-resistance transfer *in vitro*.

The 29 episodes included some thought to be related. Episodes 4F and 3S both occurred in Surrey late in 1971. The strains in and around Guildford at that time were resistant to sulphonamide and streptomycin and were colicine-type 0. This pattern was shown by the strains from three members of family 4. The strains from the remaining three members of this family and also from school 3 had additional resistances to tetracycline and ampicillin and these resistances were transferred together. The resistotype of the strains from the two episodes was the same and it seems likely that they were both derived from the local endemic strain. Episodes 5S and 1B were also related to each other; school 5 was in the same area of Islington as the cases in episode 1B, and the strains in both these episodes were of the same resistotype.

The strains from five episodes (7S, 8S, 9S, 5B and 6B) were all colicine-type 9 with transferable resistance to sulphonamide and chromosomal resistance to streptomycin and ampicillin. Strains with these characteristics were very common in London in 1968. The details of these episodes are shown in table X. From the resistotypes it appears that the strains endemic in Southwark and Camden were similar, in that they were all sensitive to chemicals C and G. The strains in Hackney were different. The strains from Tower Bridge School, Southwark (episode 7S), are particularly interesting. The first strain isolated from this episode was of resistotype AB – D(E)(F) – HIJK[†], which corresponds to the Southwark endemic strain. The six other strains in the episode were isolated 2 wk later. These were all of resistotype AB – – (E)(F)(G)HIJK[†]. It is possible that this represents a change in resistotype occurring early in the outbreak, followed by spread of the “new” strain.

To understand the epidemiology of Sonne dysentery it is important to be able to decide whether a number of cases occurring in a school or nursery over a period of time represent a continuous spread within the establishment or the repeated introduction of strains from the community at large; resistotyping may help to determine this.

The strains from only two episodes (1F and 1S) were resistant to chemical C. All these strains were of colicine-type 6 and ampicillin sensitive. These characteristics are uncommon in indigenous strains and suggest that the strains may have originated abroad. Two of the strains from Poland and two of the reference strains were also resistant to chemical C. The Polish strains belonged to colicine-type 2; of the reference strains, one was of colicine-type 6, and the other was of colicine-type 0 and sensitive to all the antibiotics—a finding very uncommon in London. This suggests that the usefulness of resistotyping might not only be in the characterisation of strains in a local outbreak, but also in giving some indication of the origin of strains.

In this work we applied a somewhat rigorous test for the evaluation of resistotyping. Most of our strains came from a limited area over a short period of time. Many were selected because they were difficult to differentiate by existing methods. The strains were typed in batches, but strains from an episode were not necessarily in the same batch.

Fifteen of the 217 strains gave discrepant results on resistotyping. Five of these discrepancies were traced to technical variability (episode 3B). Of the remaining discrepancies, seven were from “borough” outbreaks; although they appear as failures of the method, some of the strains were possibly not connected with the episode to which they were attributed.

Eleven compounds would theoretically allow for the recognition of 2048 different resistotypes. Among the 19 reference strains, only 14 resistotypes were found. Three strains were AB – DEFGHIJK[†], a further three were AB – DEF – HIJK[†] and two were AB – DEFGHI – K[†]; the remaining types occurred singly. Fourteen of the outbreaks were due to strains of type AB – DEFGHIJK[†] and five of type AB – DEF – HIJK[†]; the remaining ten comprised seven other types. It is perhaps not surprising that a relatively small number of types were represented in our material, because the reference strains

TABLE X
Outbreaks due to Shigella sonnei, colicine-type 9, in London

Episode	Location (and month)	Number of strains examined	Resistotype	Number of strains with		Resistotype of strains showing major deviation
				minor variation	major deviation	
5B	Southwark (March-April)	7	A B - D (E) F - H I J K'	3	0	...
5B	Southwark (June)	6	A B - D (E) F - H I J K'	1	0	...
5B	Southwark (July)	5	A B - D (E) F - H I J K'	2	1	AB--(E)(F)-HIJK'
7S	Southwark (May)	7	A B - - (E) (F) (G) H I J K'	1	1	AB-D(E)(F)-HIJK'
6B	Camden (March)	5	A B - D (E) (F) - H I J K'	1	1	AB-D(E)(F)(G)-IJK'
9S	Camden (July)	5	A B - D (E) F - H I J K'	1	0	...
8S	Hackney (August)	7	A B - D (E) (F) (G) - I J K'	5	0	...

All 42 strains were resistant to sulphonamide, streptomycin and ampicillin, and sensitive to tetracycline and neomycin. Symbols as in table III.

were isolated in the early months of 1969—the period immediately following that in which most of the outbreaks occurred. However, strains from other parts of Great Britain and from Poland (table VI) also included examples of types AB – DEFGHIJK¹ and AB – DEF – HIJK¹. The use of other compounds in further investigations might permit these common types to be subdivided.

The aims and limitations of typing systems

The aim of typing in epidemiology is to trace a strain as it passes from one individual to another. In a sense the word “typing” is a misnomer, because all that is required is to be able to say with reasonable certainty whether two strains are the same or different. According to our present concepts the species is a genetic unit consisting of a large intercommunicating gene pool. The individual is merely a temporary vessel holding a small portion of the gene pool for a short period of time (Mayr, 1963). This definition carries in itself the main limitations of typing, as we understand it in medical microbiology. All the methods employed in typing bacteria hold essentially only for tight little outbreaks, i.e., for short periods of time and within a relatively small area.

Thus, for *S. sonnei*, the antibiogram combined with colicine type is often an adequate characterisation. However, as we have seen, a single R factor can change the colicine type or antibiogram or both. The resistotype may reveal the fact that an R factor is operating because, as we have seen, antibiotic resistance or colicine type may pass from one strain to another without a change in resistotype. Furthermore, when many strains have the same colicine type and antibiogram, resistotyping may be used to subdivide them.

Resistotyping, once its technical difficulties are overcome, provides an additional system of typing. Our results suggest that it is possible to subdivide strains that belong to the same colicine type and have the same antibiogram in an epidemiologically meaningful way. Likewise, it is possible to relate strains to each other although they have different colicine types or antibiograms or both. It is now recognised as regards staphylococci, that the phage typing pattern is not *per se* an indicator of identity between two or more cultures and may often need independent epidemiological evidence to relate them to one another. Clearly this represents a general truth applicable to all forms of typing. The purpose is to characterise strains in such a manner that the rest of the epidemiological information is either confirmed or negated.

SUMMARY

Resistotyping consists of testing bacterial strains against a set of arbitrarily chosen chemicals that show selective toxicity at a critical concentration. The pattern of resistances, which defines the resistotype, is believed to describe isolates for epidemiological purposes.

Such a system was devised for *Shigella sonnei* and 247 strains obtained from various outbreaks were tested. Thirty-four outbreaks in families, schools and localities were originally defined by their timing and geographical incidence,

together with the colicine type and antibiotic resistance pattern of the strains isolated.

In general, strains from an outbreak belonged to the same resistotype. This was not altered in those strains that showed a change in antibiogram or colicine type. In some cases, strains from different sources but of the same colicine type could be differentiated into separate resistotypes, which corresponded to geographical outbreaks. In other instances, however, strains of the same resistotype were subdivided by their colicine type into different outbreaks.

In-vivo findings and in-vitro transfer of plasmids indicated that the resistotype markers were independent of the colicine type and antibiotic resistance markers. Thus, although the resistotype markers may also be episomally located, they are apparently not on the same episomes as the determinants of colicine type and antibiotic resistance.

All typing systems ultimately depend on the genetic stability of their markers, and it is theoretically impossible to devise a perfect system for epidemiology. This statement applies equally to phage and colicine type and to antibiogram and resistotype. In the case of *S. sonnei*, the method of resistotyping appears to be a useful adjunct for detailed epidemiology.

We wish to express our thanks to the Endowment Fund of St George's Hospital for the grant which made this investigation possible.

REFERENCES

- ABBOTT, J. D., AND GRAHAM, J. M. 1961. Colicine typing of *Shigella sonnei*. *Mon. Bull. Minist. Hlth*, **20**, 51.
- DAVIES, JOAN R., FARRANT, W. N., AND (the late) TOMLINSON, A. J. H. 1968a. Further studies on the antibiotic resistance of *Shigella sonnei*. I Transferable antibiotic resistance. *J. Hyg., Camb.*, **66**, 471.
- DAVIES, JOAN R., FARRANT, W. N., AND (the late) TOMLINSON, A. J. H. 1968b. Further studies on the antibiotic resistance of *Shigella sonnei*. II The acquisition of transferable antibiotic resistance *in vivo*. *J. Hyg., Camb.*, **66**, 479.
- ELEK, S. D., AND HIGNEY, LEONIE 1970. Resistogram typing—a new epidemiological tool: application to *Escherichia coli*. *J. Med. Microbiol.*, **3**, 103.
- MAYR, E. 1963. *Animal Species and Evolution*, Oxford, p. 11.
- SZYBALKI, W. 1952. Gradient plates for the study of microbial resistance to antibiotics. *Bact. Proc.*, p. 36.