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Further studies on the antibiotic resistance of *Shigella sonnei*

I. Transferable antibiotic resistance

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INTRODUCTION

Previous studies on the epidemiology of Sonne dysentery in the London area (Davies, 1954; Farrant & Tomlinson, 1966) described the trend towards an increased frequency of strains of *Shigella sonnei* resistant to sulphonamides and later to streptomycin and tetracycline. It seemed relevant to continue and extend these observations. Recent work on the transfer of antibiotic resistance between organisms of many genera within the family Enterobacteriaceae (Datta, 1962; Watanabe, 1963; Anderson & Lewis, 1965) prompted an attempt to determine how much of the antibiotic resistance of *Sh. sonnei* could be transferred to a suitable recipient strain of *Escherichia coli*.

MATERIALS AND METHODS

Source of cultures

The strains of *Sh. sonnei* examined were isolated in this laboratory from patients in the London area.

We have continued our policy of examining one strain from each incident—an incident being defined as a number of infections that might be expected to have a single source. In practice this has meant that one strain has been examined from each household or family. Day nursery outbreaks and cases in large residential institutions have been excluded from the analysis.

The methods used for the isolation and identification of *Sh. sonnei* were those described previously (Farrant & Tomlinson, 1966).

Antibiotic sensitivity testing

Antibiotic sensitivity was determined by the disk technique using Oxoid disks (sulphatriad 300 μg., streptomycin 25 μg., tetracycline 50 μg., ampicillin 25 μg., neomycin 30 μg., colistin 200 μg.) or disks prepared in the laboratory (chloramphenicol 30 μg.). Tests for sulphonamide sensitivity were carried out on lysed blood agar plates; antibiotic sensitivities were determined on nutrient agar plates. The inoculum for both was a loopful of a 4 hr. peptone water culture.

Minimal inhibitory concentrations (MIC) were determined by incorporating serial dilutions of the antibiotic in nutrient agar over the range 2–2000 μg./ml. and inoculating segments of a plate with a loopful of an overnight broth culture.

Abbreviations

Su = sulphonamide, St = streptomycin, Te = tetracycline, Amp = ampicillin.

Transfer of antibiotic resistance

Transfer experiments were carried out by growing the strain of *Sh. sonnei* in broth with *E. coli* K12 met$^-$ F$^-$ (K12). This mixture was usually incubated overnight but sometimes, to separate progeny of different resistance patterns, shorter incubation periods were used. The *E. coli* recipient was separated from the *Sh. sonnei* donor by plating the mixed culture on medium (minimal agar) which, while supporting the growth of K12, was nutritionally deficient for *Sh. sonnei*. Quantitative estimations of resistance transfer were performed by making serial dilutions of an overnight mixed culture. A loopful of each dilution was spread over an area of a plate of minimal agar with or without appropriate antibiotics (Anderson & Lewis, 1965). Individual colonies were picked from the minimal agar, purified and tested for antibiotic resistance. When the counts on plates containing two different antibiotics were similar and all the progeny picked from each medium were resistant to both antibiotics it was assumed that the resistance determinants were linked. If the counts on the two media were different and progeny resistant only to one antibiotic were isolated from the appropriate medium it was assumed that the resistances travelled independently. For example:

Sh. sonnei resistant to sulphonamide, streptomycin and tetracycline mated with K12 and plated on minimal agar:

Count on minimal agar,	10×10^6 cols./loop.
Count on minimal agar + Su,	5×10^4 cols./loop.
Count on minimal agar + St,	10×10^5 cols./loop.
Count on minimal agar + Te,	10×10^5 cols./loop.
Ten colonies picked from each medium:	
From minimal agar,	1 resistant to St and Te, 9 sensitive,
From minimal agar + Su	2 resistant to Su, St and Te, 8 resistant to Su,
From minimal agar + St,	10 resistant to St and Te,
From minimal agar + Te,	10 resistant to St and Te.

Interpretation: Su resistance transferred to about 0·5% of recipients and travelling separately from a linked (StTe) resistance (approx. 10% of progeny resistant).

Resistance determinants transferred as a single unit are indicated by the use of bracket, e.g. (StTe). Resistance determinants transferred independently are separated by commas, e.g. Su,Te.

Minimal agar was prepared from a double strength solution which could be stored; for use a volume of warmed solution was added to an equal volume of melted double strength agar (24 g. Davis agar/l.). Melting the complete medium proved deleterious. The double strength solution contained NaCl, 10·0 g.; $MgSO_4 7H_2O$, 0·4 g.; $NH_4H_2PO_4$, 2·0 g.; K_2HPO_4, 2·0 g.; lactose, 4·0 g.; dL methionine, 0·01 g.; nutrient broth, 0·4 ml. in a litre of distilled water; this solution was distributed in 100 ml. volumes and sterilized by steaming for 30 min.

Antibiotics, when required, were added to the complete medium just before it was poured into plates to give the following final concentrations; sulphathiazole 5 μg./ml., streptomycin sulphate 10 μg./ml., terramycin hydrochloride 10 μg./ml. and ampicillin 25 μg./ml.

RESULTS

Antibiotic resistance

The fluctuations in the antibiotic resistance of *Sh. sonnei* since this study was started in October 1956 are shown in Table 1. The time interval chosen, including the last quarter of one year with the first three-quarters of next, is appropriate to a disease such as Sonne dysentery in which the incidence gradually increases throughout the winter reaching a maximum in the spring and declining in the summer (Bradley, Richmond, Shaw & Taylor, 1958). The proportion of strains resistant to any particular antibiotic at any one time would be influenced by a large local outbreak producing a large number of strains of a particular pattern. This may account for some minor fluctuations but the strains examined in any season were sufficiently widely distributed in time and space for this not to be a serious source of error.

Table 1. *Antibiotic resistance of* Shigella sonnei 1957–67

Period (quarters)	No. of strains examined	% resistant to		
		Su	St	Te
1956/4–1957/3	556	31	0	1
1957/4–1958/3	1174	63	0	4
1958/4–1959/3	1412	81	0·5	12
1959/4–1960/3	1968	54	6	5·5
1960/4–1961/3	467	58	4	5
1961/4–1962/3	765	80	7·5	14
1962/4–1963/3	2052	90	10	13
1963/4–1964/3	618	92	31	40
1964/4–1965/3	608	95	20	31
1965/4–1966/3	759	93	45	24
1966/4–1967/3	533	82	60	12

It will be seen that most strains examined since the 1961 season have been resistant to sulphonamides. The proportion resistant to streptomycin has been gradually increasing; the proportion resistant to tetracycline has varied from season to season although we have not in recent years observed the low incidence of resistance which existed at the start of our study in 1957.

The sensitivity of the strains to other antibiotics has been tested over various periods.

Ampicillin. Since March 1966 all strains have been examined; some results were reported by Scrimgeour (1966). The proportion of resistant strains has varied from 70% in the third quarter of 1966 to 95% in the third quarter of 1967 (see Table 3).

Chloramphenicol. Strains were examined regularly from October 1962 to November 1963 (over 2000 strains). One strain was found to be resistant. No further testing of resistance to this antibiotic has been carried out.

Neomycin. The sensitivity of all strains has been determined since October 1962. Out of a total of more than 4500 strains only three resistant strains have been found.

Colistin. Since November 1964 all strains have been examined. No resistant strains have been found.

Transferable antibiotic resistance

During the first part of 1966 some preliminary work was carried out on the transferable antibiotic resistance of selected strains, usually those resistant to more than one antibiotic. The results are summarised in Table 2.

Table 2. *The transferable antibiotic resistance of selected strains of* Shigella sonnei

Resistance	No. of strains tested	No. of strains transferring resistance	% of progeny resistant
Su	261	256	0·1–50
St (MIC 800 μg./ml.)	118	0	—
St (MIC 16–256 μg./ml.)	87	81	10–60
Te	100	100	10–60
Amp	66	1	10

Sulphonamide. Most strains tested transferred this resistance to K12. Su resistance was usually transferred independently of other resistances and at a relatively low rate (approx. 0·1–1 % of progeny resistant). A few strains were encountered in which the Su resistance was transferred linked to St resistance (SuSt) or to St and Te resistance (SuStTe). In these strains the transfer of the combined resistance was more efficient, 10–50 % of the recipients receiving the resistance.

Streptomycin. St resistant strains of *Sh. sonnei* could be divided into those with an MIC of 800 μg./ml. or more and those with an MIC in the range 16–256 μg./ml. Those strains with a high level of resistance to St did not transfer this resistance to K12 although many of them had a transferable Su resistance and some a transferable Te resistance. It has been suggested by Watanabe (1966) that this high level resistance is chromosomal. The lower level St resistance was usually transferable and was frequently linked to other transferable resistances, (SuSt), (StTe) or (SuStTe).

Tetracycline. All strains which we examined transferred their tetracycline resistance either separately or linked to St or Su and St. No strain transferred a linked (SuTe) resistance.

Ampicillin. The only strain transferring ampicillin resistance had an MIC of more than 1000 μg./ml. The resistance was transferred linked to (StTe). Strains with non-transferable ampicillin resistance had MICs of about 128 μg./ml.

Since July 1966 all strains resistant to streptomycin or tetracycline and a large number of those sensitive to these antibiotics but resistant to sulphonamides or ampicillin have been tested for their ability to transfer their resistances to K12.

The results are shown in Table 3. On the whole the observations made in the preliminary study on selected strains were found to be generally applicable.

Table 3. *Resistance and resistance transfer of unselected strains of* Shigella sonnei

		% of all strains					
				Transferring			
Quarter	No. of strains tested	Resistant to Su	Resistant to St	St MIC > 800 μg./ml.	St Resistance	Resistant to Te	Resistant to Amp*
1966/3	129	88	58	35	40	48	70 (7)
1966/4	174	60	48	32	16	19	91 (8)
1967/1	106	90	66	54	12	15	85 (8)
1967/2	187	92	64	60	4	5	92 (2)
1967/3	66	97	68	50	17	10	95 (8)

* Figures in brackets = % of all strains tested transferring Amp resistance.

Sulphonamides. Of the 550 strains resistant to sulphonamides, 528 (96 %) were tested for the ability to transfer this resistance to K12. No transfer could be demonstrated with 8 of these strains.

The increased proportion of strains sensitive to sulphonamide in the last quarter of 1966 is accounted for by a large local outbreak in Southwark caused by a strain of *Sh. sonnei* resistant only to ampicillin.

Streptomycin. It is apparent that the high level, chromosomal streptomycin resistance has become more common in this area, whereas the proportion of strains with transferable resistance fluctuates. Only three of the 97 strains with low level (MIC 64–128 μg./ml.) resistance to streptomycin failed to transfer this resistance to K12. In the third quarter of 1966 a strain with unusual characteristics caused a local outbreak of Sonne dysentery in Islington. This strain was resistant to Su, St, Te and Amp and transferred Su and (StTe). The streptomycin MIC was more than 800 μg./ml. and it was therefore an exception to the general rule that high level St resistance does not transfer. However, when the (StTe) resistant K12 progeny were mated with a sensitive strain of *Sh. sonnei* and streptomycin resistant *Sh. sonnei* progeny were examined, it was found that these were resistant only to 64 μg./ml. of streptomycin. It was concluded that the original strain contained two streptomycin resistance determinants, a non-moving chromosomal resistance and transferable streptomycin resistance linked to tetracycline resistance.

Tetracycline. All strains resistant to tetracycline transferred this resistance to K12. The obvious correlation in Table 3 between the percentage of strains with transferable St resistance and those resistant to Te is accounted for by the fact that 78 % of strains resistant to Te had a combined (StTe) transferable resistance.

Ampicillin. Five hundred and five strains resistant to ampicillin were tested. This represents 88 % of all strains resistant to Amp. Most of the strains not tested were those from the outbreak in Southwark referred to previously, from which strains with non-transferable resistance to ampicillin only were isolated. Forty (8 %) of the strains tested were resistant to more than 500 μg./ml. of Amp and

transferred this resistance. The remaining strains had an MIC of less than 500 μg./ml. and this resistance was not transferable. All of the moving Amp resistance was linked either to (StTe) or to St alone.

DISCUSSION

The trend towards increasing antibiotic resistance of *Sh. sonnei* in the London area described in our previous paper (Farrant & Tomlinson, 1966) has continued. In the second and third quarters of 1967 59% of incidents were caused by strains resistant to sulphonamides, streptomycin and ampicillin. Almost all the ampicillin resistance and 88% of the streptomycin resistance was not transferable and presumably chromosomal. If the strains which cause future outbreaks of Sonne dysentery in the area are derived from strains already endemic, the trend towards increasing resistance is likely to continue.

On the other hand resistance to tetracycline is not apparently increasing. All of the tetracycline resistance so far encountered has been transferable and therefore represents a less stable genetic character than mutational resistance. This is confirmed by our previous experience that multiply resistant strains of *Sh. sonnei* appeared to possess only limited powers of spreading and tended to die out in the population. But most of the tetracycline resistance which we encountered was combined with a transferable streptomycin resistance. If it is assumed that strains with transferable antibiotic resistance acquire this from other organisms present in the gut, the transfer process would be favoured by antibiotic therapy. If a patient infected with *Sh. sonnei* had in his gut a donor of combined (StTe) resistance, treatment with either antibiotic might result in the selection of a strain of *Sh. sonnei* resistant to both.

Further characterization of antibiotic resistance, i.e. the determination of the MIC and the ability to transfer, was of value in 'labelling' strains of *Sh. sonnei* in epidemiological studies. Strains from epidemiologically related incidents were found to have the same properties. This was particularly striking in a reception centre (not included in the analysis) where a strain transferring Su and (StTe Amp) was present for over one year. Forty-four strains with these characters were tested during this period. The particular pattern was so uncommon in the rest of the area that it was inconceivable that we were observing the repeated introduction of strains from outside.

Our method of selecting strains, i.e. examining the first strain isolated from an individual or family would enable us to detect transferable resistance only where this was a property of the infecting strain or was acquired early in the course of infection. The extent to which subsequent isolations from the individual or family have transferable resistances not present in the prototype strain is the subject of a separate study.

SUMMARY

The incidence of antibiotic resistant strains of *Sh. sonnei* isolated in the London area is described. Strains were tested for their ability to transfer resistance to *E. coli* K12

Most strains were resistant to sulphonamide and this resistance was transferable.

An increasing proportion of strains was resistant to 800 μg./ml. of streptomycin, but transferable streptomycin resistance was less common and often associated with tetracycline resistance.

The proportion of strains resistant to tetracycline fluctuated but this resistance was always transferred.

Ampicillin resistance was common; only those strains resistant to 500 μg./ml. transferred this resistance.

Resistance to other antibiotics was very rare.

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