An epidemiological study of strains of *Shigella sonnei* from two related outbreaks

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SUMMARY

During the winter of 1967-8 Sonne dysentery affected neighbouring North London primary schools. This was not simply due to cross-infection between the two schools, for two different, unusual strains of *Sh. sonnei* were distinguished. One was a novel kanamycin-resistant colicine type 7 strain, and as far as we know this was the first school outbreak due to such a strain to be documented. The other strain was kanamycin-sensitive and of colicine type 0 with a rare specific requirement for aspartic acid. There was some evidence to suggest that the kanamycin-resistant strain was more infective for adults and possibly more pathogenic than the kanamycin-sensitive.

Studies on the transfer of drug resistance and of colicinogeny revealed that the factor determining colicine type 7 was carried on a transmissible plasmid, a new observation. Various drug resistances were also transmissible experimentally, and some were spontaneously unstable. Non-transferable ampicillin-resistance in the colicine type 7 strain and aspartic acid dependence in the colicine type 0 strain enabled all but one of the isolates to be classified into two distinct lines. No common ancestor was found and it was concluded that although occurring together they must have arisen from separate sources.

INTRODUCTION

Strains of *Sh. sonnei* isolated from cases of dysentery at neighbouring primary schools differed initially in drug resistance and colicine type. This was unexpected since many families had children attending both schools. One of the strains was sensitive to kanamycin, while the other was kanamycin-resistant. Neomycin and kanamycin resistance was then rare (Davies, Farrant & Tomlinson, 1968*a*; Thomas & Datta, 1969). We report below our study of the epidemiology and bacteriology of the double outbreak.

EPIDEMIOLOGY

During November 1967 dysentery affected boys at a North London primary school. A kanamycin-resistant strain of *Shigella sonnei* of colicine type 7 was isolated from the cases. The building was old and washbasins were remote from

dirty and awkward playground lavatories. Strains of *Sh. sonnei* cultured from three sites in these toilets were kanamycin-sensitive and colicine type 0. Supervised hand-washing and dipping in 2 % Hycolin after using the toilets and before eating dinner was arranged, and paper towels provided. The lavatories were ultimately replaced. Children who had been ill or absent during the autumn term were excluded from school and only readmitted after negative faeces cultures had been reported. Nevertheless, further cases arose in January. However, nearly all of these were kanamycin-sensitive infections of colicine type 0, and were secondary to infected contacts at a neighbouring mixed primary school. Altogether 28 cases of dysentery and 8 symptomless excretors were found during 3 months among the 200 pupils of the boys' school, 28 of these 36 infections were with kanamycin-resistant, colicine type 7 *Sh. sonnei*.

Early in the course of the boys' school outbreak a few siblings at the nearby mixed primary school for 400 children were infected with colicine type 7 kanamycin-resistant *Sh. sonnei*. In only one instance siblings, one at each school, excreted distinguishable strains, a boy having the kanamycin-resistant strain while his sister, who fell ill a week later, had a kanamycin-sensitive, colicine type 0 infection. During the Christmas holiday seven more children from the mixed school fell ill, also with a kanamycin-sensitive infection resistant only to sulphonamides.

In January this school too was visited and hand-washing and dipping and the prompt exclusion of suspects arranged, but plural cases continued to arise until late in February. A total of 19 cases and 9 excretors was recognized at the mixed school. Fifteen of these 28 infections were with kanamycin-sensitive, colicine type 0 strains and 13 with kanamycin-resistant, colicine type 7 strains. Nine of these 13 had a close contact at the boys' school. A single late case arose at the end of March, but this was probably unrelated since the *Sh. sonnei* strain isolated, although kanamycin-sensitive was of colicine type 7, and had other differences from either of the two outbreak strains.

METHODS

The first confirmed case of Sonne dysentery in any household was listed as the index case. Infected households discovered during the follow-up of contacts were also listed. Households were visited by a health inspector who recorded particulars of each member. Each person was asked to send a faecal sample to the laboratory before the start of treatment. Patients from whom shigellas were isolated were asked to send weekly specimens, starting three or more days after the end of any anti-bacterial treatment, until a negative result had been reported. The rest of the family were then re-examined. Most remained under surveillance until two or three negative specimens had been obtained from infected persons.

Faeces specimens were examined and *Sh. sonnei* identified by conventional methods (see below).

Household cross-infection rates, evidence of severe illness, bacteriological results of drug treatment and the duration of excretion of the two distinguishable strains of *Sh. sonnei* were compared, as far as the small numbers allowed.

RESULTS

Household infection

Information was available to study cross-infection in 46 households, in 31 of which the index case or excretor had a kanamycin-resistant *Sh. sonnei* infection. Altogether 65 % of households and 36 % of contacts were found infected. Table 1 shows that more households were found infected in the group of kanamycin-resistant infections, and that a higher proportion of adult contacts was infected by the kanamycin-resistant than by the kanamycin-sensitive strain. It also shows the usual higher susceptibility of children than of adults to infection.

Severity of illness

Nearly three-quarters of the ascertained *Sh. sonnei* infections among school-children were with symptoms and this was true for both kanamycin-sensitive and kanamycin-resistant strain infections. Naturally symptomless infections tend to escape diagnosis. One child with a kanamycin-resistant infection was admitted to hospital and family doctors in the district said that the kanamycin-resistant type of infection was more severe than the usual dysentery. Cellular stools were seen rather more often in kanamycin-resistant than in kanamycin-sensitive infections.

Treatment

Tetracycline, streptomycin, furazolidine and nalidixic acid were each used. Faeces were bacteriologically positive after treatment in about half the children regardless of the drug used.

Duration of infection

This was taken from the onset of diarrhoea to midway between the last positive and the first negative of a clearance series of faeces specimens. Seventeen children infected with the kanamycin-sensitive strain and 26 with the kanamycin-resistant strain were available for this comparison. No difference in the duration of infection was observed, the median being 24 days for both strains.

BACTERIOLOGY

The two school outbreaks were closely related epidemiologically and the characters by which strains were distinguished, colicinogeny and drug resistance, are known to be determinable by transmissible plasmids. We therefore investigated a sample of the strains in an attempt to find whether the difference between the *Sh. sonnei* found in the two schools could be explained simply by acquisition or loss of plasmids in a single bacterial host, or whether there really were two distinct epidemic strains.

Methods

Faeces were cultured on deoxycholate citrate agar before and after enrichment in selenite-F broth. A 30 μ g. paper disk of kanamycin was placed upon each plate to enable rapid recognition of resistant strains. For sensitivity testing Oxoid 'Multodisks' were used on Oxoid D.S.T. agar with 4 % lysed horse blood. Kana-

Table 1. *Infection among household contacts according to Sh. sonnei strain and age group*

<i>Shigella sonnei</i> strain isolated	Households		Children		Adults 15+ years		Total	
	Exposed	Infected	Exposed	Infected	Exposed	Infected	Exposed	Infected
Kanamycin-sensitive	15	8 (53 %)	33	18 (55 %)	31	3 (9.7 %)*	64	21 (32 %)
Kanamycin-resistant	31	22 (71 %)	56	27 (48 %)	76	23 (40 %)	132	50 (38 %)

* $P < 0.01$.Table 2. *Characteristics of individual strains of Sh. sonnei studied*

Strain from patient no.	Pattern of drug resistance	Colicine type	Aspartic acid dependence	Characters transferred to <i>E. coli</i> K. 12
Boys' school				
B1	A S K Su	7	—	S K Su col
B6	A S K Su	7	—	S K Su col
B11	A S K Su	7	—	S K Su col
B25a	A S K Su T	7	—	S K Su T col
B25b	A Su T	7	—	Su T col
Mixed school				
M1	Su	0	+	Su
M6	Su	0	+	Su
M7	Su	0	+	Su
M20	A Su	7	—	Su col
M29	S Su	0	—	None

NOTE. B25a and b were variants of one isolate. B1, B6 and B11 were three strains studied among 40 of the same colicine type and resistance pattern. M1, M6 and M7 were three strains studied among 23 of the same colicine type and resistance pattern.

mycin (K), ampicillin (A), streptomycin (S), tetracycline (T), Chloramphenicol (C), sulphonamide (Su), nalidixic acid (Nal), and furazolidine (Fx) were tested.

Colicine testing was carried out by the method of Abbot & Graham (1961) and representative strains were sent to the Dysentery Reference Laboratory for confirmation.

Transfer of drug resistance and colicinogeny

Drug-resistant strains of *Sh. sonnei* were grown in mixed culture (Oxoid nutrient broth No. 2) with a nalidixic acid-resistant strain of *Escherichia coli* K 12, J 53-1, which requires proline and methionine for growth. The cultures were incubated overnight and subcultured on minimum salts agar (Clowes & Hayes, 1968) to which was added lactose, proline, methionine, nalidixic acid, and also the particular antibacterial drug for which transfer of resistance was under test. *Sh. sonnei* will not grow on this medium, and *E. coli* K 12 J 53-1 will grow *only* if it has acquired appropriate drug resistance. Colonies growing on these selective plates were purified and identified as *E. coli* K 12 J 53-1 by biochemical tests and their requirement for proline and methionine. They were tested for resistance to drugs listed above and for colicinogeny by using the set of *Sh. sonnei* colicine-typing indicators (Abbot & Graham, 1961).

Nutritional requirements

Strains of *Sh. sonnei* were tested for ability to grow on minimum salts agar with added glucose and nicotinic acid. Cultures unable to grow on this medium were tested on the same medium, further supplemented in turn with each known amino acid and a variety of vitamins, in order to define their nutritional requirements.

RESULTS

Drug resistance

Most strains from the boys' school were resistant to ampicillin (A) streptomycin (S), kanamycin (K) and sulphonamide (Su) and most strains from the mixed school were resistant only to Su. There were some exceptions.

Nutritional requirements

All strains tested showed the normal requirements of *Sh. sonnei* for nicotinic acid. Characteristic strains from the boys' school needed no other supplement and grew well on the minimum salts medium described. Typical strains from the mixed school, however, failed to grow on this medium unless aspartic acid was added and 300 µg./ml. was needed for optimal growth and no other amino acid would substitute for aspartic acid.

Colicinogeny

The characteristic boys' school strains were of colicine type 7, identified by colicinogenic activity against strain No. 17. Typical mixed school strains were of colicine type 0; that is, non-colicinogenic (Table 2). Late in the epidemic a boy excreted a colicine type 7 strain which was resistant to tetracycline (T) as well as to ASKSu, and from this isolate a variant arose spontaneously which was resistant

only to ASuT. Later still a strain of colicine type 7 which was resistant only to ASu was isolated from a girl at the mixed school.

Transfer

Characteristic boys' school strains of *Sh. sonnei* were able to transfer to *E. coli* K 12 resistance to SKSu and colicinogenic activity against indicator strain No. 17. Ampicillin resistance was never transferred. Tetracycline resistance, when it occurred, was transferable, as was sulphonamide resistance from every strain except the very last to be isolated in the outbreak (from M 29). This final strain had non-transmissible resistance to SSu, and unlike the other colicine type 0 strains its growth was independent of aspartic acid.

DISCUSSION

Two unusual strains of *Sh. sonnei* were isolated from cases of dysentery at two primary schools in one district during the same winter terms. Siblings attended both schools. The family doctors involved worked with the Public Health Department and the Public Health Laboratory to investigate and control the epidemic. Later the two strains of *Sh. sonnei* were compared epidemiologically and bacteriologically. A novel kanamycin-resistant colicine type 7 predominated in a boys' primary school, while most of the infections at a neighbouring mixed primary school were with a kanamycin-sensitive aspartic-acid-dependent colicine type 0. The kanamycin-resistant strain appeared to be more infective for adults, and was possibly more pathogenic than the concurrent kanamycin-sensitive strain. No difference was found in the duration of infection by the two strains.

The epidemiological markers differentiating the *Sh. sonnei* strains had practical importance. A strain of colicine type 7 was rife when the boys' school was inspected. Toilet facilities were thoroughly insanitary. Radical improvements were instituted during the autumn term and children who had been ill or absent were excluded until negative faeces cultures had been obtained from them. Nevertheless, dysentery recurred in the school in January, making it appear that control measures were ineffective. It was found, however, that at this stage most strains isolated from the boys' school were colicine type 0 and kanamycin-sensitive; that is, they were of the type prevailing at the mixed school. It seems probable that the control measures against the original outbreak had been effective within the boys' school, but that new introductions of *Sh. sonnei* by close contacts from the continuing outbreak at the mixed school were not prevented.

Our bacteriological studies indicate that the two epidemic strains of *Sh. sonnei* were distinct. Both were unusual, that originating at the boys' school in being kanamycin-resistant, and that arising at the mixed school in having a specific requirement for aspartic acid.

Colicine typing, described by Abbot & Shannon (1958) and Abbot & Graham (1961) is widely used for tracing *Sh. sonnei* epidemiologically, but for many years in Enfield a majority of strains have been non-colicinogenic, that is of type 0. Unusual antibiotic-resistance patterns may reveal a connexion between infections more quickly than colicine typing, which is commonly carried out in batches at

intervals. Davies, Farrant & Tomlinson (1968*b*) have shown that the colicine type and antibiotic resistance pattern of *Sh. sonnei* may change during the course of natural spread of infection in man. In the investigation described here our aim was to find whether it was possible by transfer of plasmids from the colicinogenic multiple-resistant *Sh. sonnei* prevailing at the boys' school, to convert that prevailing at the mixed school into an indistinguishable form.

We were able to transfer colicinogeny, determining colicine type 7, and also resistance to kanamycin, streptomycin and sulphonamides, from the 'boys' *Sh. sonnei* to *E. coli* K12, and thence to typical *Sh. sonnei* strains from the second school. But these transfers still left two 'labels' by which we could distinguish the strains, ampicillin resistance and aspartic acid dependence. The 'boys' strain was ampicillin-resistant, and that resistance was *not* transferable. Scrimgeour (1966) showed that strains of *Sh. sonnei* with a *high* level of ampicillin resistance (> 1000 g./ml.) could regularly transfer resistance, but that the lower level of ampicillin resistance (28–256 g./ml.) now commonly found in *Sh. sonnei* (Davies, Farrant & Uttley, 1970) was not transmissible. Scrimgeour pointed out the epidemiological value of this distinction.

The 'mixed school' strain was dependent upon aspartic acid for growth. Amino acid requirements are unusual in *Sh. sonnei* and when present are useful epidemiological markers (Davies *et al.* 1968*b*). Genetic information for amino acid independence can be carried on transmissible plasmids (Fredericq, 1969; Jacob & Adelberg, 1959), but we did not find aspartic acid dependence to be transferable between our strains of *Sh. sonnei* either directly or by way of *E. coli* K12.

We found that the colicine factor determining type 7 (Abbot & Graham, 1961) was carried on a transmissible plasmid, an observation not previously reported. However, in these outbreaks this colicine appeared to be as stable as was the non-transferable ampicillin resistance or aspartic acid dependence (Table 2). Patient M20 infected in the mixed school with a kanamycin-sensitive colicine type 7 *Sh. sonnei* was probably infected by a 'boys' strain, from which SK resistance had been lost. The atypical primary isolate from the boy (B25) was clearly an example of the acquisition of a transmissible factor determining tetracycline resistance. The subsequent spontaneous appearance in this culture of clones resistant only to ASuT showed that S and K resistance could be unstable. One isolate from a girl (M29) which arose a month after the outbreaks could not be allotted to either prevailing strain and we suggest that it was an unrelated sporadic infection. Using non-transmissible markers alone it was possible to allot all but this one of the strains studied to one or other of two parent lines. Although the first 'mixed school' colicine type 0 kanamycin-sensitive strain to be discovered came from a girl who developed dysentery just 8 days after her brother, who excreted the characteristic 'boys school' colicine type 7 kanamycin-resistant strain, the two strains had different stable epidemiological markers. We were unable to find bacteriological evidence for a common origin. Certainly after the first isolation of the 'mixed school' colicine type 0 strain there appeared to be two independent sources of infection in the double outbreak we have described, and we conclude that the initial differing infection in siblings is likely to have arisen by chance.

REFERENCES

- ABBOT, J. D. & GRAHAM, J. M. (1961). Colicine typing of *Shigella sonnei*. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **20**, 51-8.
- ABBOT, J. D. & SHANNON, R. (1958). A method of typing *Shigella sonnei* using colicine production as a marker. *Journal of Clinical Pathology* **11**, 71-7.
- CLOWES, R. C. & HAYES, W. (1968). *Experiments in Microbial Genetics*, p. 185. Oxford and Edinburgh: Blackwell Scientific Publications.
- DAVIES, J. R., FARRANT, W. N. & TOMLINSON, A. J. H. (1968*a*). Further studies on the antibiotic resistance of *Shigella sonnei*. I. Transferable antibiotic resistance. *Journal of Hygiene* **66**, 471-7.
- DAVIES, J. R., FARRANT, W. N. & TOMLINSON, A. J. H. (1968*b*). Further studies on the antibiotic resistance of *Shigella sonnei*. II. The acquisition of transferable resistance *in vivo*. *Journal of Hygiene* **66**, 479-87.
- DAVIES, J., FARRANT, W. N. & UTTLEY, A. (1970). Antibiotic resistance of *Shigella sonnei*. *Lancet* *ii*, 1157-9.
- FREDERICQ, P. (1969). The recombination of colicinogenic factors with other episomes and plasmids. In *Bacterial Episomes and Plasmids*, pp. 163-74. Ed. G. E. W. Wolstenholme and M. O'Connor. (A Ciba Foundation Symposium.) London: J. & A. Churchill.
- JACOB, F. & ADELBERG, E. A. (1959). Transfert de caractères génétiques par incorporation au facteur sexuel d'*Escherichia coli*. *Comptes Rendues Hebdomadaires des Séances de l'Académie des Sciences* **249**, 189-91.
- SCRIMGEOUR, G. (1966). The ampicillin resistance of *Shigella sonnei*: its epidemiological value. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **25**, 278-81.
- THOMAS, M. E. M. & DATTA, N. (1969). Emergence of *Shigella sonnei* resistant to kanamycin and to nalidixic acid, without exposure to these drugs. *Journal of Medical Microbiology* **2**, 457-61.