EMERGENCE OF *SHIGELLA SONNEI* RESISTANT TO KANAMYCIN AND TO NALIDIXIC ACID, WITHOUT EXPOSURE TO THESE DRUGS

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WHATEVER the mechanism of drug resistance in bacteria its emergence *in vivo* is unexpected, except in response to contact with the relevant or a related anti-bacterial agent. It is therefore of interest that we have observed what appears to be primary or unselected drug resistance in two family outbreaks of shigellosis in Enfield. The resistance was to kanamycin in one family and to nalidixic acid in the other. *Shigella sonnei* of colicine type O was the organism involved in each episode. In the absence of any preceding antibacterial treatment, some but not all of the infected persons in each household consistently excreted apparently pure cultures of a resistant strain.

I. KANAMYCIN RESISTANCE EPISODE

In 1966 a girl of 18 mth and her 6-yr-old brother developed gastro-enteritis, and faeces samples from all five members of the family were examined before treatment. *Sh. sonnei*, colicine type O, was found in every specimen. All the strains were sensitive by paper disk test to chloramphenicol, nalidixic acid, and furazolidone, but resistant to sulphonamide, ampicillin, tetracycline and streptomycin. However, the isolates from four members of this family were also, unexpectedly, resistant to kanamycin and grew well on nutrient agar containing 100 μ g of this drug per ml. The strain from the mother was kanamycin-sensitive and inhibited by 2 μ g per ml in nutrient agar.

Because of this discrepant finding two further faecal specimens were taken from each person in the household, with exactly the same result. Kanamycin-resistant *Sh. sonnei* had not previously been encountered in the Enfield district, or reported elsewhere in Britain, so very detailed enquiries were pursued, but no source of resistant infection could be traced. The family doctor kept careful records and it seems beyond reasonable doubt that kanamycin, neomycin and related drugs had never been administered at home or in hospital to any member of the family.

Bacteriological investigation

Possible sources of resistance factors. The absence of *Sh. sonnei* resistant to kanamycin in the community at that time together with the curious partial distribution of drug resistance within the family, suggested that the shigella

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strains might have accepted R factors from some other enterobacteriaceae present in the bowel, so a search was made for possible donor bacteria. Faecal samples from everyone in the family, taken before treatment, were cultured on MacConkey agar plates with 30- μ g kanamycin disks. The only kanamycin-resistant organisms found were the four strains of *Sh. sonnei* already discovered. Possibly a wider range of media or anaerobic incubation might have disclosed more.

Resistance transfer and loss. All the *Sh. sonnei* strains transferred resistance to *Escherichia coli* K12 in mixed culture (Watanabe and Fukasawa, 1961). At least two R factors were present and were classified as *fi*+ or *fi*- (Watanabe *et al.*, 1964; Lawn *et al.*, 1967). One, *fi*-, was present in all the *Sh. sonnei* strains and gave resistance to ampicillin, streptomycin, tetracycline and sulphonamides. Kanamycin resistance was determined by a second, *fi*+, factor, and the two were transferred independently. The R factor conferring kanamycin resistance was spontaneously lost at a measurable rate from its *Sh. sonnei* host: a single kanamycin-resistant colony was grown in antibiotic-free broth for 4 hr and dilutions were spread on nutrient agar plates to obtain single colonies. When these were tested by replicating to kanamycin-containing agar, four kanamycin-sensitive colonies were identified. They had lost the *fi*+ R factor but retained the *fi*- one, and resembled exactly the kanamycin-sensitive strains isolated from the mother of the family.

Mutation to resistance. Kanamycin-resistant variants were not recovered from serial subcultures of the initial kanamycin-sensitive strain, nor from those which had spontaneously lost kanamycin-resistance in the laboratory.

Growth rates. When the resistant and the sensitive strains were grown in parallel, in broth, no obvious advantage in growth rate was evident for either. When kanamycin-resistant strains were grown in mixed culture with kanamycin-sensitive ones the culture became predominantly kanamycin-resistant, no doubt because the resistance was carried on an infectious R factor.

II. NALIDIXIC ACID RESISTANCE EPISODE

In 1967 all seven children of a family developed dysentery during a weekend. Three of them attended a primary school at which four cases of dysentery due to *Sh. sonnei*, colicine type O, had recently occurred. The strains in these four school infections were sensitive to streptomycin, tetracycline, chloramphenicol, neomycin, kanamycin, nalidixic acid and furazolidone, but resistant to sulphonamide and ampicillin. The patients were treated with furazolidone. *Sh. sonnei* type O with the above antibiotic-sensitivity pattern was isolated from three members of the family, but cultures from another two of the children, though also of type O, differed unexpectedly in being resistant to nalidixic acid. They were not inhibited by disks containing 30 μ g of the drug, and grew well on agar containing 40 μ g per ml. Nalidixic acid-resistant *Sh. sonnei* had not been seen previously. From the remaining two children and the parents no shigellae were isolated.

Two further faecal samples were taken from each member of the family, with identical results. The family had been for many years under the care of a

general practitioner who kept careful records, and a detailed enquiry was made about previous drug treatment. Nalidixic acid had never been previously administered to any member of the family.

At this juncture nalidixic acid was administered to the whole family. *Sh. sonnei* continued to be excreted by the two children with resistant infections, but was not subsequently isolated from the rest of the family.

Bacteriological investigations

Possible sources of resistance factors. The novelty of *Sh. sonnei* strains resistant to nalidixic acid and their discovery in a household where some persons were excreting sensitive strains suggested that the resistance might be determined by an R factor, although resistance to this drug has never yet been found to be transmissible. A search was therefore made for possible donor bacteria. Faeces from every member of the household, taken before treatment, were cultured on MacConkey agar plates with nalidixic acid disks. The only nalidixic acid-resistant organisms found were the *Sh. sonnei* already described, and in one uninfected adult, a faecal streptococcus.

Tests for resistance transfer and for mutation to resistance. All the *Sh. sonnei* strains from this second household carried a transmissible R factor conferring sulphonamide resistance, but no transfer of resistance to ampicillin or nalidixic acid was found. Although nalidixic acid-resistance has never been reported to be transferable, unstable resistance was found in *Shigella flexneri* (Lewis, 1967). No spontaneous loss of nalidixic acid-resistance was observed in our *Sh. sonnei* strains when 1500 colonies were tested by replica plating, but resistant mutants could be recovered from single-colony isolates of the nalidixic acid-sensitive *Sh. sonnei* strains. About 10^9 cells, grown in drug-free broth from a small inoculum (c. 10 cells) of sensitive *Sh. sonnei*, were plated on nutrient agar containing 25 μg nalidixic acid per ml; three resistant colonies developed, which resembled the naturally occurring nalidixic acid-resistant strains from the same household except that they had a slightly lower level of resistance. Sensitive strains were inhibited by 1.25 μg nalidixic acid per ml in nutrient agar plates, but the three selected resistant mutants were inhibited at 40 μg per ml, and the naturally occurring resistant strains only by 80 μg per ml.

Growth rates. When the resistant and sensitive strains were grown in parallel in broth, and their growth measured turbidimetrically, no obvious advantage in growth rate was seen in either. Nalidixic acid-resistant strains were grown in mixed broth cultures with sensitive strains from the same family outbreak. Viable counts were made on nutrient agar with and without incorporated nalidixic acid (25 μg per ml) with repeated subculture of the mixtures through over 100 bacterial generations. The proportion of resistant to total organisms in the mixtures remained almost constant.

DISCUSSION

Primary resistance of *Sh. sonnei* to kanamycin is rare (Davies, Farrant and Tomlinson, 1968*a* and *b*; Aoki, 1968) and resistance to nalidixic acid has not

been reported previously except after treatment with the drug (Couston, 1966; Fernandez, 1966). The circumstances in which resistance to kanamycin and to nalidixic acid were observed in the episodes described are interesting.

In the two household outbreaks of dysentery, each studied before the start of treatment, certain individuals consistently excreted novel resistant strains of *Sh. sonnei*, whereas others excreted sensitive strains of the same colicine type. Neither drug had ever been administered to either family. No other enterobacteriaceae resistant to either drug were found in any of the samples of faeces examined. There was, therefore, no evidence of selection of resistance by exposure to the drug involved, or for the acquisition of transferable resistance factors within the gut, nor was there any ascertainable community reservoir of resistant strains. In fact such strains had not been encountered before. They appeared to arise within the untreated households.

An increase in the proportion of drug-resistant strains of *Sh. sonnei* during the last decade has resulted from the acquisition of R factors (Farrant and Tomlinson, 1966; Davies *et al.*, 1968a); indeed extra resistance genes were shown to be acquired during the course of at least 8.5 per cent. of family outbreaks (Davies *et al.*, 1968b), but the increase has been thought to be due not only to the presence of R factors in the bowel flora but also to the selective effect of exposure to the drugs concerned, particularly sulphonamides, ampicillin, streptomycin and tetracycline. Conversely certain substances, particularly acridine dyes and among them such drugs as mepacrine, are known to "cure" bacteria of such extrachromosomal genetic elements as R factors (Mitsuhashi, Harada and Kameda, 1961; Lebek, 1967; Tomoeda *et al.*, 1968). The possible interactions are complex. In our cases the kanamycin-resistance factor was unstable and spontaneous loss took place in the laboratory. Its origin was not discovered.

The nalidixic acid-resistance found in the second family could not be explained either. Single-step mutation of *Sh. sonnei* cultures to a significant degree of resistance occurs with nalidixic acid, but in the absence of some selective advantage, such as exposure to the drug or an advantage in growth rate, the supplanting of the drug-sensitive parent population would not be expected. Yet no difference in growth rates was found, and neither our patients nor the preceding cases at their school had been treated with nalidixic acid. Variation in genetic qualities acquired from the environment, such as transmissible resistance or colicinogeny factors, have been described (Davies *et al.*, 1968b). This study shows that these and other variations may be encountered even within one household where the infection probably originated from a single source.

SUMMARY

Sh. sonnei resistant to kanamycin in one household and, in another family, to nalidixic acid, is described. Resistance of this organism to these drugs is uncommon. Some individuals within each family excreted sensitive strains of the shigella. There was no history of previous exposure to either drug and the source of the resistant strains is unknown. As far as we know these strains

neither arose from nor spread to the community. Kanamycin-resistance was determined by a transferable factor; no transfer of nalidixic acid-resistance could be demonstrated. The supplanting of the prevailing sensitive strains by new drug-resistant strains of *Sh. sonnei* is unexplained in both families.

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Addendum

In a recently published paper (Smith, H. W., 1969, *Lancet*, **1**, 1174), the author reports the isolation *in vitro* of a nalidixic acid-resistant mutant of the resident *Escherichia coli* of a person, who was then given 1 ml of a broth culture of the mutant by mouth. No nalidixic acid was administered to select for resistance *in vivo*, but the resistant mutant nevertheless colonised the intestine and constituted the bulk of *E. coli* colonies isolated from the person over the next two years, presumably because it had some unexplained selective advantage in the intestine.