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Typhoid fever imported from Mexico to Switzerland. Studies on R factor mediated chloramphenicol resistance

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SUMMARY

A case of typhoid fever caused by *Salmonella typhi* occurred in Geneva. The patient was probably infected in Mexico City. The strain isolated from this patient corresponds with the description of the Mexican *S. typhi* strain, since it is a degraded Vi-strain resistant to chloramphenicol, streptomycin, sulphonamides and tetracyclines. It carried an *fi*$^-$ transferable R factor with a CSSuT resistance pattern. It can be accepted that this case forms part of the Mexican outbreak of chloramphenicol-resistant typhoid fever which has already been observed in visitors to Mexico from England and the United States.

INTRODUCTION

The fact that drug resistance can be transferred between enterobacterial strains by conjugation has important epidemiological and clinical implications. Although resistance transfer apparently occurs less frequently *in vivo* than *in vitro*, it has been demonstrated in man and animals (reviews: Watanabe, 1963; Anderson, 1968). Clinical isolates capable of transferring multiple drug resistance to other strains during mixed cultivation have been described in epidemic shigellosis (Ochiai, Yamanaka, Kimura & Sawada, 1959), in urinary tract infections (Smith & Armour, 1966), in nosocomial infections (Gardner & Smith, 1969) and in *Salmonella* infections (Anderson & Lewis, 1965*a*, *b*; Gill & Hook, 1966).

Recent reports from Mexico have described a protracted outbreak of typhoid fever caused by a strain of *Salmonella typhi* resistant to chloramphenicol because it carries a transferable resistance factor (W.H.O. Weekly Epidemiological Record, 1972). The recent account by Anderson & Smith (1972) of typhoid infection in two British visitors to Mexico, and the occurrence of a similar case at our hospital in a patient returning from Mexico City, prompted us to investigate the resistance pattern of the strain of *S. typhi* isolated from this case and to document its identity with the Mexican strain.

CASE REPORT

A 22-year-old girl was admitted to the Hospital on 6 June 1972 with a 6 days' history of fever, cough, nausea and vomiting. She had been perfectly well until 6 weeks before admission, when she visited South and Central America. While in Mexico City, 3 weeks before her admission to hospital in Switzerland, she suffered nausea, vomiting and diarrhoea of 24 hr. duration, for which she received a short course of chloramphenicol. The symptoms abated until her return to Switzerland. Six days before admission she developed fever up to 40° C., anorexia, nausea and vomiting, followed by an unproductive cough.

On admission the physical signs included a temperature of 40° C., a heart rate of 88/min., a short atypical systolic murmur and a slightly tender abdomen without peritoneal signs.

Routine laboratory findings included a hematocrit reading of 45%, a white blood cell count of 4000/mm.^{3} with a slight shift to the left, a blood urea nitrogen of 14 mg./100 ml., normal electrolytes and normal liver function tests. All blood cultures and two stool cultures taken on the first 2 days after admission yielded *S. typhi* resistant to chloramphenicol but sensitive to ampicillin by the standard disk method.

Treatment was instituted on the 3rd hospital day with 4 g. of ampicillin by mouth, and the patient recovered. Antibiotic treatment was continued for 3 weeks. The TO and TH agglutinin titres were 1/400 and 1/200 respectively on admission; they rose to 1/800 and 1/400 after 1 week and had returned to 1/100 and 1/50 1 month later. However, no significant Vi agglutinin titre was detected during or after the first treatment period.

Because of intermittently positive stool cultures for *S. typhi* during the 2 weeks after completion of the antibiotic treatment, a second 6 weeks' course of ampicillin was given. Careful monitoring of the stools showed all specimens tested to be negative for *S. typhi*; they have remained negative since then.

LABORATORY STUDIES

Bacteriology. The resistance pattern of 9 isolates of *S. typhi* (7 from stools and 2 from blood cultures) was studied, using the standard disk diffusion method. All were resistant to chloramphenicol and belonged to a degraded Vi-strain.

Minimal inhibitory concentrations (MIC). Small inocula (0·1 ml. of an overnight broth culture diluted 10^{-3}) were added to 5 ml. of broth (Bacto Penassay Broth, Difco) containing serial dilutions of drugs. The MIC values chosen were the lowest drug concentrations which inhibited visible growth after overnight incubation at 37° C.

Transfer of drug resistance. The nine chloramphenicol-resistant isolates were examined for the presence of R factors by the methods of Anderson & Lewis (1965*a*, *b*), using *Escherichia coli* K12F$^+$, K12F$^-$ and K12HfrH as recipient strains. Drug-resistant recipient colonies from these mating mixtures were isolated on plates containing the appropriate antibiotic, purified on MacConkey Agar (Difco), and their full resistance pattern was determined by a disk-diffusion method. The

fi (fertility inhibition) character was examined by the method described by Pitton & Anderson (1970), using phages $\mu 2$ and R17 (male-specific) and $\phi 2$ (female-specific). A second series of crosses was carried out, using resistant *E. coli* K12 as donor strains and *S. typhi* phage type A and *S. typhimurium* phage type 36 as recipient strains.

Mechanisms of resistance. The acetylating ability for chloramphenicol was determined by the method of Piffaretti (Piffaretti & Pitton, 1970).

Stability of the plasmid in its original strain of S. typhi. This was determined by the replica plating technique (Lederberg & Lederberg, 1952).

RESULTS

Antibiotic sensitivity tests

The nine isolates were resistant to chloramphenicol (C), streptomycin (S), sulfafurazole (Su) and tetracyclines (T) but sensitive to penicillins and cephalosporins as well as to kanamycin, gentamicin, neomycin and colomycin by the standard disk method. The MIC's of antibiotics for the wild-type strains were 120 μg./ml. for chloramphenicol, more than 200 μg./ml. for streptomycin, 100 μg./ml. for tetracycline, 3 μg./ml. for neomycin and gentamicin, and 6 μg./ml. for kanamycin. The MIC's for chloramphenicol were 160 μg./ml. in the K12 lines, 100 μg./ml. in *S. typhi* A, and 160 μg./ml. in *S. typhimurium*.

Transfer of drug resistance

The nine primary isolates transferred chloramphenicol resistance to *E. coli* K12 at a frequency of about 10^{-4}. All the K12 colonies selected on chloramphenicol showed the stable resistance pattern CSSuT. In the second series of crosses, all resistances were transferred at the same frequency, about 10^{-5}, into the two standard salmonellas, *S. typhi* Vi-type A and *S. typhimurium* type 36 (Anderson, 1966). This CSSuT resistance factor is *fi*$^-$ in K12HFrH lines when tested with the $\mu 2$ and R17 phages. In K12F$^+$, however, the R factor displaces the F factor, giving rise to F$^-$ cells. It thus shows one-sided incompatibility with F, since it is stable in, and seems not to affect the fertility of, Hfr strains, where the F factor is integrated into the chromosome. The same properties have been observed independently in this R factor by E. S. Anderson & H. R. Smith and by D. H. Smith (personal communications). This factor belongs to the 'H group' (Anderson & Smith, 1972; Grindley, Grindley & Anderson, 1972). It does not modify the sensitivity of K12F$^-$ lines towards the $\phi 2$ phage or produce phage restriction in *S. typhi* or *S. typhimurium*.

All the strains studied (the nine wild-type *S. typhi*, the K12F$^+$ and F$^-$ derivatives, and the *S. typhi* and *S. typhimurium* progeny obtained in the second crosses) acetylated chloramphenicol.

The CSSuT complex is probably not very stable in the wild-type strains, since the frequency of spontaneous loss of the resistance was about 3×10^{-3} for C and 10^{-3} for T.

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DISCUSSION

All cultures of the chloramphenical-resistant strain of *S. typhi* isolated from this case have identical properties. They all react with the Vi-typing phages as degraded Vi-strains; and they carry the same *fi*$^-$ transferable R factor with a CSSuT resistance pattern. These results, as well as the clinical and epidemiological history of our patient, establish the identity of these isolates with those implicated in the outbreak of typhoid fever in Mexico (CDC Weekly Report, 1972*a*, *b*). Since Anderson & Smith (1972) have recently studied two C-resistant *S. typhi* cultures involved in this epidemic, it was of interest to determine whether our studies would show that the strain isolated in Switzerland was genetically indistinguishable from that found in Britain. No differences appeared: the Swiss strain belongs to the same Vi-type and carries the same R factor as those isolated in England.

These findings suggest that the Mexican epidemic of typhoid fever was caused by a single strain of *S. typhi* carrying the same R factor throughout. This has the resistance pattern CSSuT, belongs to group H, and displaces the F factor when the latter is in the extrachromosomal state.

An interesting finding in this case is the relatively high rate of spontaneous loss of C and T resistance in our *S. typhi*. This may explain the puzzling fact that one of the stool cultures performed after completion of the first course of antibiotic treatment yielded drug-sensitive *S. typhi*. This sensitive line had the same characteristics – phage type, biochemical and serological properties – as the resistant isolates.

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