Insertion sequence IS200 can differentiate drug-resistant and drug-sensitive *Salmonella typhi* of Vi-phage types E1 and M1

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Summary. The type strains of Vi-phage types E1, M1 and A of *Salmonella typhi*, together with drug-resistant and drug-sensitive strains of phage types E1 and M1 isolated in 1992 from patients associated with India or Pakistan, and a drug-resistant strain of phage type A isolated in South Africa in 1991, were characterised with respect to the presence of plasmids conferring resistance to antimicrobial drugs and their chromosomal insertion sequence IS200 profiles. The three type strains, the drug-sensitive strains of Vi-phage types E1 and M1, and a strain of phage type M1 resistant to ampicillin and trimethoprim but not to chloramphenicol, did not contain plasmids. In contrast, for strains of phage types E1 and M1 resistant to chloramphenicol, ampicillin and trimethoprim, and for the drug-resistant strain of phage type A, the complete spectrum of resistance was encoded by high molecular mass plasmids belonging to the H₁ incompatibility group. Characterisation of IS200 profiles demonstrated that at least 13 IS200 copies were distributed on the chromosome of all strains tested. Although the IS200 profiles of the type strains of Vi-phage types A, E1 and M1 were identical, it was possible to distinguish between drug-sensitive and drug-resistant strains of Vi-phage types E1 and M1 isolated from patients infected in India and Pakistan by this method. It was concluded that although IS200 typing is not as discriminatory as phage typing for the primary subdivision of *S. typhi*, it may be useful for certain epidemiological investigations and, in particular, for investigating the origins of strains with multiple drug resistance.

Introduction

Since 1990, c. 20% of *Salmonella typhi* isolates from patients in the UK have been resistant to chloramphenicol (C), ampicillin (A), trimethoprim (Tm), streptomycin (S), sulphonamides (Su) and tetracyclines (T) (R-type CATmSSuT).^{1–4} Most multi-resistant strains have belonged to Vi-phage types M1 and E1, but a small number of multiresistant *S. typhi* strains of other phage types have also been identified.³ The majority of multiresistant *S. typhi* of Vi-phage type M1 were from travellers with a history of recent return from Pakistan,^{1,3} and those of Vi-phage type E1 from patients infected in India.^{3–5} In such multi-resistant strains, the complete spectrum of resistance (CATmSSuT) is often encoded by plasmids of the H₁ incompatibility group (IncH₁) of c. 120 MDa (180 kb) in size.^{1–5} A few *S. typhi* strains of Vi-phage type M1 and R-type ATmSSu from patients infected in Pakistan have also been identified. In contrast to *S. typhi* strains of Vi-phage type M1 and R-type CATmSSuT, such strains were plasmid-free and re-

sistance to A, Tm, S and Su, previously plasmid-mediated, had become chromosomally integrated.¹ Drug-sensitive strains of *S. typhi* of Vi-phage types E1 and M1 have also been isolated from travellers with a history of recent return from the Indian subcontinent. For example, in 1991, seven (28%) of 25 isolates of Vi-phage type E1 from patients infected in India, and four (16%) of 26 strains of Vi-phage type M1 from patients who had returned from Pakistan, were drug-sensitive (Laboratory of Enteric Pathogens, unpublished observations).

Since 1989, multiresistant *S. typhi* strains have also been isolated in other areas of the world;⁵ however, many of these strains have also been associated with the Indian subcontinent. For example, *S. typhi* strains of Vi-phage types M1 and E1, associated with expatriate workers from the Indian subcontinent, have been isolated in several countries in the Arabian Gulf,⁵ and *S. typhi* strains of Vi-phage type E1, associated with South Asia, have been isolated from patients in Canada.⁶ In contrast, in 1991, an outbreak involving *S. typhi* strains of Vi-phage type A was reported in Natal, South Africa, in which three of six patients died.⁷ This outbreak was not associated with the

Indian subcontinent and the source of the resistant strains was not determined.

IS200 is a *Salmonella*-specific insertion element, first described by Lam and Roth,⁸ which is distributed at conserved loci on the chromosome of many *Salmonella* serotypes.⁹ Unlike enterobacterial insertion elements, such as IS1 and IS5, IS200 transposes rarely under laboratory conditions,¹⁰ and this stability favours its use as a probe for discrimination within some serotypes. Thus, by use of IS200, three chromosomal lineages of *S. enteritidis*,¹¹ two of *S. berta*,¹² and seven of *S. heidelberg*¹³ have been described. The purpose of this investigation was to define IS200 lineages in the type strains of *S. typhi* of Vi-phage types E1 and M1, and to compare these type strains with drug-sensitive and drug-resistant strains of the same phage types isolated from patients infected in the Indian subcontinent since 1991. The IS200 profiles of the type strain of *S. typhi* Vi-phage type A and a multiresistant strain of Vi-phage type A isolated in South Africa in 1991 were also investigated.

Materials and methods

Bacterial strains: phage typing and screening for drug resistance

The origins of the *S. typhi* strains used in this study are listed in the table. Strains were maintained at 18°C on Dorset's egg slopes in the culture collection of the Laboratory of Enteric Pathogens (LEP), phage typed¹⁴ and screened for resistance to antimicrobial drugs.^{15,16}

Transfer of drug resistance and incompatibility grouping of R plasmids

Wild-type drug-resistant strains of Vi-phage types E1, M1 and A were tested¹⁵ for the ability to transfer resistance to a plasmid-free laboratory strain of *Escherichia coli* K12 F⁻ lac⁺ nal^r (strain 14R525). All mating mixtures were incubated at both 28 and 37°C before plating on appropriate selective media containing chloramphenicol, trimethoprim, ampicillin or kanamycin. Nalidixic acid 40 mg/L was used for counter-selection.¹⁵ Transfer frequencies were expressed in terms of the ratio of the number of drug-resistant transconjugants to the number of viable cells of the recipient strain after incubation of the mating mixture for 18 h at 28 or 37°C. For incompatibility testing, plasmids conferring resistance were tested for their ability to co-exist with standard plasmids of defined incompatibility groups; IncH plasmids were further subdivided on the basis of their incompatibility with the F factor of *E. coli* K12.¹⁷

Extraction and analysis of plasmid DNA

Partially purified plasmid DNA was extracted by a modification of the method of Kado and Liu¹⁸ and analysed by electrophoresis on Tris-borate agarose

0.7% w/v gels (H5 Horizontal Gel Apparatus, Gibco-BRL), with the plasmid-containing strain 39R861¹⁹ as a control for size reference plasmids.

Genomic DNA preparation

Genomic DNA from wild-type strains of *S. typhi* was prepared according to the method of Ausubel *et al.*²⁰ The resultant DNA was precipitated by the addition of 0.6 volumes of isopropanol and allowed to stand for 15 min before centrifugation. The DNA was then washed with ethanol 70% v/v, dried and treated with RNAase (final concentration, 50 ng/μl in TE: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) for 30 min at 37°C. DNA preparations were quantified spectrophotometrically. Portions (10 μg) of genomic DNA were digested to completion with *Pst*I (Gibco BRL) and electrophoresed at 30 V for 14 h in agarose 0.7% w/v gels. Gels were then vacuum-blotted (LKB Vacu-gene apparatus) on to Hybond Nylon hybridisation membrane filters (Amersham), and the filters were baked for 2 h at 80°C. Gels containing preparations of plasmid DNA from drug-resistant strains were also vacuum-blotted and baked in the same way.

Preparation and use of IS200 probe

The probe, a multimer of the *Taq*I-*Taq*I fragment of IS200, was amplified by PCR as described by Torre *et al.*²¹ The substrate was plasmid pUA175, which is a pUC19 derivative containing a multimer of the *Taq*I-*Taq*I fragment cloned into the *Eco*RI site. The PCR product was labelled with digoxigenin by random priming according to the manufacturer's instructions (Boehringer-Mannheim).

Hybridisation and detection procedures

Before hybridisation, filters were hydrated in 2 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), and then pre-hybridised for 3 h in a solution containing 5 × SSC, blocking reagent (Boehringer-Mannheim) 1% w/v, laurylsarcosine 0.1% w/v and SDS 0.02% w/v. The pre-hybridisation solution was changed after the first hour of incubation. Filters were then hybridised at 68°C for 16 h in a hybridisation mixture comprising 5 × SSC, blocking reagent 1% w/v, laurylsarcosine 0.1% w/v, SDS 0.02% w/v and labelled probe (final concentration 25 ng/ml). Detection was by the manufacturer's (Boehringer-Mannheim) recommended protocols, and bands were sized in relation to digoxigenin-labelled λ DNA digested with *Hind*III (Boehringer-Mannheim), and *Pst*I-digested *S. typhimurium* LT2 genomic DNA.

Results

Antimicrobial resistance and associated R plasmids

Of the three strains of *S. typhi* Vi-phage type E1, two strains (E1 and P264880) were sensitive to the anti-

Table. Vi-phage types, drug resistance and plasmid content of *S. typhi* strains in the study

Strain no.	Vi-phage type	Isolation		R-type	Plasmids		IS200 pattern†
		year	origin		MW	Inc	
E1*	E1	1918	Russia	sensitive	nd	...	IS200 <i>Sty1</i>
P264880	E1	1992	India	sensitive	nd	...	IS200 <i>Sty2</i>
P268123	E1	1992	India	CATmSSuT	180	H ₁	IS200 <i>Sty1</i>
M1*	M1	1939	Canada	sensitive	nd	...	IS200 <i>Sty1</i>
P269095	M1	1992	Pakistan	sensitive	nd	...	IS200 <i>Sty1</i>
P268309	M1	1992	Pakistan	CATmSSuT	180	H ₁	IS200 <i>Sty3</i>
P211144	M1	1991	Pakistan	ASSuTm	nd	...	IS200 <i>Sty3</i>
A*	A	1937	Canada	sensitive	nd	...	IS200 <i>Sty1</i>
P265187	A	1991	South Africa	CATmKSSuT	180	H ₁	IS200 <i>Sty1</i>

MW, molecular size (kb); Inc, incompatibility group; nd = no plasmid DNA detected.

C, chloramphenicol; A, ampicillin; Tm, trimethoprim; S, streptomycin; Su, sulphonamides; T, tetracyclines; K, kanamycin.

* Type strain.¹⁴

† See text.

Figure. Genomic DNA digested with *psfI* and hybridised with an IS200 probe (A) and diagrammatic representation (B) of IS200 profiles within *S. typhi* phage types. Lane 1, digoxigenin-labelled λ DNA digested with *Hind*III; 2, E1; 3, P264880; 4, P268123; 5, M1; 6, P269095; 7, P268309; 8, P211144; 9, A; 10, P265187; 11, *S. typhimurium* LT2.

biotics tested and did not contain plasmid DNA (table). In contrast, the third strain (P268123) was of R-type CATmSSuT and possessed a plasmid of *c.* 180 kb. Of the four strains of *S. typhi* Vi-phage type M1, two strains (M1 and P269095) were drug-sensitive and two strains (P268309 and P211144) were drug-resistant (R-types CATmSSuT and ASSuTm, respectively). Plasmid DNA was identified only in strain P268309 which, like strain P268123 (phage type E1), carried a plasmid of *c.* 180 kb. Of the two strains of Vi-phage type A, the type strain (A) was drug-sensitive and did not possess plasmid DNA, but strain P265187, isolated in 1991 from the outbreak in South Africa, was of R-type CATmKSSuT (K = kanamycin) and carried a plasmid of *c.* 180 kb which coded for the complete spectrum of resistance.

In conjugation experiments, plasmids from strains P268123, P268309 and P265187 transferred at high frequency ($> 10^{-2}$) when incubated at 28°C but at low frequency ($< 10^{-6}$) at 37°C. These plasmids were all incompatible with standard plasmids of the Inc H

complex and, on the basis of their incompatibility with the F factor of *E. coli* K12, were assigned to the H₁ incompatibility subgroup. Neither transfer nor mobilisation of resistance from strain P211144 (phage type M1, R-type ASSuTm) was detected at either temperature.

IS200 loci: numbers and molecular sizes of pattern fragments

When *Pst*I-digested preparations of genomic DNA from the nine strains of *S. typhi* were hybridised with the digoxigenin-labelled, PCR-amplified IS200 probe, at least 13 IS200 elements were identified in each strain, with IS200 bands ranging from *c.* 22.8 to 1.2 kb in size (figure). Three patterns were identified, provisionally designated IS200*Sty1*, IS200*Sty2* and IS200*Sty3* (table). The type strains of Vi-phage types E1, M1 and A, and the multiresistant strains of Vi-phage types E1 (P268123) and A (P265187) had identical IS200 profiles and were of the pattern designated IS200*Sty1*.

This pattern was characterised by IS200 bands of 22.8, 21.4, 16.4, 10.2, 9.8, 9.2, 5.4, 5.0, 4.6, 3.9, 3.0, 2.3 and 1.2 kb (figure; tracks 2, 4, 5, 6, 9 and 10).

The genotype of the drug-sensitive strain of phage type E1 (P264880) could be differentiated from those of the type strain of Vi-phage type E1 and the drug-resistant strain of this phage type by the presence of an additional IS200 band of 6.0 kb and the absence of a band of 3.0 kb (figure; track 3), and this pattern was provisionally designated IS200*Sty*2. Both multi-resistant strains of Vi-phage type M1 (P268309 and P211144) were identical and could be differentiated from the type strain of phage type M1 and from a drug-sensitive strain of this phage type (P269095) by the presence of an additional IS200 band of 19.0 kb (figure; tracks 7 and 8). This pattern was designated IS200*Sty*3. IS200 loci were not identified on plasmid DNA present in any of the drug-resistant strains.

Discussion

In a previous study⁹ it was reported that the number of IS200 loci in strains of *S. typhi* varied from 10 to 25. However, in contrast to the present study, phage typing was not attempted. In the current investigation, the IS200 profiles of nine strains of *S. typhi* belonging to three Vi-phage types (E1, M1 and A) were characterised, and both multi-resistant and drug-sensitive isolates were included. Hybridisation analysis demonstrated the presence of IS200 elements in the chromosomes of all nine strains investigated. The number of elements varied from 14 (in two strains) to 13 (in seven strains), and three IS200 profile patterns were identified. One pattern, designated IS200*Sty*1, was common to the type strains of Vi-phage types E1, M1 and A, and can, therefore, be regarded as the archetypal *Pst*I-generated IS200 profile of *S. typhi*. This pattern was also identified in a drug-resistant strain of phage type E1 of Indian origin and a drug-resistant strain of phage type A from South Africa. However, a drug-sensitive strain of phage type E1 originating in India could be distinguished from the type strain of phage type E1, and from the drug-resistant strain of this phage type, by the presence of an additional band of 6 kb and the absence of a band of 3.0 kb. This pattern was designated IS200*Sty*2. The observation that strains with the IS200*Sty*1 and IS200*Sty*2 patterns

differ only in the distribution of IS200 elements in their respective chromosomes suggests that the IS200*Sty*2 pattern was derived from IS200*Sty*1 by re-arrangement of an IS200 element in the genome rather than by transposition. The two drug-resistant strains of phage type M1 could also be distinguished from the type strain of Vi-phage type M1, and from the drug-sensitive strain of this phage type, by the presence of an additional IS200 band of 19.0 kb. This pattern was designated IS200*Sty*3 and, in contrast to strains with the IS200*Sty*2 pattern, the presence of an extra IS200 element suggests that this pattern resulted from transposition rather than genomic re-arrangement.

The finding that the IS200 profiles of drug-resistant and drug-sensitive strains of *S. typhi* of Vi-phage type E1 from patients associated with India were different was unexpected. Both multi-resistant and drug-sensitive *S. typhi* of Vi-phage type E1 have caused numerous infections in India in recent years.^{22, 23} These results suggest that drug-resistant strains of *S. typhi* Vi-phage type E1, now endemic throughout India, may have originated elsewhere. However, the differences in IS200 profile were small, and an alternative explanation is that local divergence within the phage type may have taken place. Further studies on strains from patients infected in different areas of India are needed to resolve these hypotheses. Similarly, the observation of difference between the IS200 profiles of drug-resistant and drug-sensitive strains of *S. typhi* of Vi-phage type M1 from patients infected in Pakistan suggests that the multi-resistant strains of Vi-phage type M1 may have originated elsewhere or, alternatively, may be the result of local divergence within the phage type either before or after plasmid acquisition. Again, further studies are required to resolve these hypotheses.

Since the type strains of Vi-phage types E1, M1 and A—three of the most common phage types of *S. typhi*—have identical IS200 profiles, IS200 typing is obviously not as discriminatory as phage typing for the primary subdivision of this serotype. However, the demonstration of differences within some phage types suggests that IS200 typing may be useful for certain epidemiological investigations, and may help in elucidating the origins of the multiresistant strains now endemic in the Indian subcontinent.

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