The insertion sequence IS200 fingerprints chromosomal genotypes and epidemiological relationships in *Salmonella heidelberg*

JOHN STANLEY,¹* ANDRÉ BURNENS,² NEVILLE POWELL,¹ NAMOOS CHOWDRY¹ and CLIVE JONES¹

¹NCTC Molecular Genetics Unit, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK

²Swiss National Reference Laboratory for Foodborne Disease, Institute for Veterinary Bacteriology, University of Berne, Langgass-Strasse 122, CH-3012 Berne, Switzerland

(Received 6 April 1992; revised 30 June 1992; accepted 13 July 1992)

In *Salmonella heidelberg* the copy number of the *Salmonella*-specific insertion element IS200 was found to vary from four to six. All strains tested contained at least one common insertion site which was serovar specific, and most strains contained three common sites. Concurrent analysis of plasmids indicated that all insertion sequence copies were chromosomally located, and also supported the equivalence of an IS200 fingerprint and clonality. Seven intra-serovar clonal lines were thereby identified. One of these was associated with human infections, including septicaemias. Another was associated with chicken as a host: all these strains also carried a unique plasmid of 23 MDa, which was typed as a member of the IncX group. The chromosomal fingerprint of a third clone showed it to be a descendant of the chicken line marked by a single IS200 transposition. One or two representatives of four other clonal lines were identified. These lines of *S. heidelberg* could be related by divergent evolution, and the most recent relatives conformed to a continuous branching process model of IS200 transposition. This insertion sequence provided a highly discriminatory molecular marker of the *S. heidelberg* chromosome, and two of the seven clonal lines so identified were associated with distinct clinical/epidemiological contexts.

Introduction

Salmonella heidelberg is a Group B serovar which is consistently among the ten most common causative agents of nontyphoid salmonellosis (Harris *et al.*, 1990; Threlfall *et al.*, 1992). The clinical symptoms associated with *S. heidelberg* range from mild diarrhoea to severe gastrointestinal illness (Choi *et al.*, 1990), and include septicaemic bloodstream invasion in more than 3% of cases (Threlfall *et al.*, 1992). This can vary from a self-limiting asymptomatic infection to meningitis (Ayroza-Galvao *et al.*, 1989) or fatal bacteraemia in children (Rice *et al.*, 1976) and immunocompromised patients (Heal *et al.*, 1987).

For serovars of primary epidemiological or clinical importance, such as *S. typhimurium*, *S. enteritidis* or *S. typhi*, phage typing schemes have been developed which are highly discriminatory at the intra-serovar level (Threlfall & Frost, 1990). *S. heidelberg* is one of a group of important serovars for which there is currently no subtyping methodology, other than profiling of plasmid

content. Although this has often proved a useful epidemiological marker, it is unsatisfactory for genotype analysis, because plasmids are extrachromosomal replicons which are subject to considerations of stability, lateral transfer, and host range. Hence a method for generating discriminatory molecular fingerprints of the chromosome would greatly assist the genetic analysis of evolutionary and epidemiological relationships between strains of *S. heidelberg* (and other serovars of similar importance in human and veterinary disease). The antigen profile of *S. heidelberg* (1,4,5,12:r:1,2), like those of *S. choleraesuis* and *S. typhimurium*, was shown by multilocus enzyme electrophoresis to be confined to a cluster of closely related chromosomal genotypes. Eight such electrophoretic types were identified among 204 isolates (Beltran *et al.*, 1988).

The insertion sequence IS200, specific to *Salmonella* spp. (Lam & Roth, 1983), is the smallest characterized mobile DNA element. This 708 bp element (Gibert *et al.*, 1991) lacks inverted repeats and has only very rarely been observed to transpose in the laboratory (O'Reilly *et al.*, 1990). In theory, the number of insertion sequences and their locations can change rapidly, and the determination of similarity or identity among strains in copy

* Author for correspondence. Tel. (081) 200 4400 ext. 3744; fax (081) 200 7874.

number and position of IS elements is strong evidence of common ancestry (Sawyer *et al.*, 1987). In *Salmonella enteritidis*, IS200 differentiated strains at the intra-serovar level. Both phage type strains and pandemic isolates could be classified in three evolutionary lines defined by chromosomal hybridization profiles of the element (Stanley *et al.*, 1991, 1992b).

We have determined the copy number and profile of IS200 in a collection of *S. heidelberg* isolates. A comparative analysis of plasmid content was made. This and epidemiological background data were correlated

with IS200 fingerprints. We present a genetic analysis of *S. heidelberg*, which is also of potential importance for the molecular epidemiology and molecular phylogenetics of *Salmonella* in general.

Methods

Bacterial strains, culture conditions and genetics. Strains of *Salmonella* used in this study are listed in Table 1. Clinical, food poisoning and veterinary isolates of *S. heidelberg* were from the recent investigative programme of the Swiss National Reference Laboratory for Foodborne

Table 1. *Bacterial strains and probe DNAs*

<i>Bacterial strains</i>			
Strain or isolate number	Source	Epidemiology	Year of isolation
<i>Escherichia coli</i>			
NCTC 50167 (J53.2)			1966
NCTC 50192 (39R861)			
<i>Salmonella typhimurium</i>			
NCTC 12416 (LT2)			1960
<i>Salmonella heidelberg</i>			
1 NCTC 5717	reference strain		1939
2 UB4881	human, faeces	sporadic	1990
3 UB5008	human, faeces	sporadic	1990
4 UB5051	human, faeces	sporadic	1990
5 UB5433	egg pasta	QC, manufacturing process, Yugoslavia	1990
1 UB5445	human, bile	sporadic	1990
2 UB5503	meat	Mexico	1990
3 UB5542	mushrooms	QC, manufacturing process, E. Europe	1990
6 UB5848	human, faeces	sporadic	1990
7 UB6228	human, wound	sporadic	1990
8 UB6333	human, blood	sporadic	1990
9 UB198	chicken, faeces	vet. inv.*	1991
10 UB321	chicken, carcass	vet. inv.*	1991
11 UB378	environment	Indonesia	1991
12 UB462	chicken, carcass	France, imported	1991
13 UB489	chicken, intestine	vet. inv.*	1991
14 UB541	chicken, unspecified	vet. inv.*	1991
15 UB557	chicken, carcass	vet. inv.*	1991
16 UB787	environment, chicken farm	vet. inv.*	1991
17 UB857	chicken parent flock	vet. inv.*	1991
18 UB898	cat, faeces	sporadic	1991
19 UB988	human, wound	persistent infection†	1991
20 UB1146	environment (stream water)	Switzerland	1991
21 UB1631	human, faeces	sporadic	1991
22 UB1764	human, faeces	sporadic	1991
23 UB1960	chicken, slaughterhouse	vet. inv.*	1991
24 UB2016	chicken, carcass	France, imported	1991
25 UB2775	human, faeces	worker, chicken farm	1991
26 UB2868	chicks	vet. inv.*	1991
27 UB3069	human, faeces	sporadic	1991
<i>DNA probes</i>			
Probe	Fragment/size (kbp)	Cloned in:	Reference
IS200	<i>Hind</i> III- <i>Eco</i> RI/0.3	pIZ45	Gibert <i>et al.</i> , 1990
VirA	<i>Eco</i> RI/4.1	pIP1367	Norel <i>et al.</i> , 1989
IncFIC	<i>Eco</i> RI- <i>Hind</i> III/0.97	pULB2440	Couturier <i>et al.</i> , 1988
IncX _{R485}	<i>Eco</i> RI- <i>Taq</i> I/0.59	pDS49	Stalker & Helinski, 1985

* Isolates from veterinary investigation of *Salmonella* carriage in broiler flocks in Switzerland in 1991.

† Isolate from same patient as UB6228; analysed in order to test marker stability *in vivo*.

Disease. Poultry strains were collected for a study by the Swiss Federal Office of Veterinary Public Health of *Salmonella* carriage in broiler flocks. Strains were grown in nutrient broth/agar for DNA isolation, and purity was checked on blood agar plates. Stock cultures were maintained on Dorset-egg agar slopes. Antibiotic resistance markers were determined with susceptibility discs (Oxoid) on Isosensitest (Oxoid) agar. Resistant strains were screened for the ability to transfer antibiotic resistance to *Escherichia coli* J53-2, and transconjugants were screened with respect to the molecular size of acquired plasmid DNA (see below).

DNA techniques. Plasmid DNA of *S. heidelberg* was analysed by the method of Kado & Liu (1981). Plasmid pIZ45 was isolated by alkaline lysis and purified in a CsCl/ethidium bromide gradient according to standard methods (Sambrook *et al.*, 1989). The *Hind*III-*Eco*RI fragment cloned in pIZ45 was isolated by electro-elution from 1.0% agarose (Sigma; medium EEO) and labelled by random-priming (Boehringer Mannheim kit) with ³⁵S-dATP or 16-dUTP-biotin for use as an IS200 probe. Alternatively, the cloned insert fragment was amplified from a 620 bp *Pvu*II fragment of pIZ45 by the polymerase chain reaction (PCR) employing forward and reverse sequencing primers and 16-dUTP-biotin was incorporated during PCR. The biotinylated product was subjected to centrifugal ultrafiltration (Millipore Ultrafree-MC; 30000 NMWL unit).

Genomic DNA was extracted from *S. heidelberg* by the method of Wilson (1987), and 5 µg quantities were digested with one of three enzymes (*Pst*I, *Bgl*II or *Hinc*II) which lack restriction sites within the IS200 sequence (Gibert *et al.*, 1991). Genomic restriction digests or whole plasmid DNAs were electrophoresed in 0.7% agarose, and vacuum-blotted (LKB Vacu-gene apparatus) on to Hybond N nylon membrane (Amersham). Hybridization was by standard methods (Sambrook *et al.*, 1989). Hybridized filters were washed twice with each of the following: 2 × SSC/0.1% SDS for 5 min at 20 °C, 0.2 × SSC/0.1% SDS for 5 min at 20 °C and 0.16 × SSC/0.1% SDS for 30 min at 60 °C. Hybridization reactions were visualized either colourimetrically with the BluGENE (Gibco-BRL) non-radioactive nucleic acid detection system or by autoradiography using standard methods (Sambrook *et al.*, 1989) and Hyperpaper (Amersham).

Results

Analysis of IS200 insertion sites

Genomic Southern blots were made with *Pst*I and *Bgl*II for all *Salmonella* strains listed in Table 1 and hybridized with an IS200 probe as described in Methods. *Pst*I digests provided a clearer resolution of loci than did *Bgl*II and were therefore used for subsequent analysis. Eleven distinct insertion sites of IS200 were detected in *Pst*I digests. Both enzymes confirmed that all *S. heidelberg* isolates contained 4–6 insertion sites, one of which was common to all (*Pst*I, 1.7 kbp, *Bgl*II, 2.3 kbp). The 1.7 kbp *Pst*I IS200 band is likely to be serovar specific for *S. heidelberg*, since it has not been observed in *S. typhimurium*, *S. bovis-morbificans*, *S. paratyphi* or in some twenty other serovars including *S. enteritidis*, *S. berta*, *S. dublin*, *S. panama*, *S. napoli* and *S. miami* (our unpublished results). Data were compared for all strains from genomic Southern blots, such as presented in Fig. 1. The molecular sizes of homologous *Pst*I fragments were

averaged from three blots and varied from 1.7 kbp to over 30 kbp in size.

The IS200 hybridization profile of the type strain of *S. heidelberg*, NCTC 5717, is shown in Fig. 1, track 1. This was provisionally termed IS fingerprint I since the strain isolation date (1939) pre-dated all others in this investigation. It shared a homologous 5.5 kbp *Pst*I fragment with all but three strains (UB4881 or UB5008, and UB378; cf. Fig. 1, tracks 6 and 7).

A similar fragment of about 20 kbp was found in the great majority of strains in the set, i.e. in eighteen human, animal and environmental isolates. Hence all these strains shared three *Pst*I IS200 bands with NCTC 5717. Fifteen of the strains had been isolated from an investigative epidemiological analysis of *Salmonella* infection and contamination during all stages of intensive chicken farming. For example, strains UB857, UB489 and UB198 were veterinary isolates from a parent flock, from chicken gut and chicken faeces; strains UB321, UB462, UB557 and UB2016 were isolates from chicken carcasses, strain UB1960 was from a chicken slaughterhouse and strain UB2775 was isolated from a chicken-processing-plant worker reporting with gastrointestinal illness. The IS200 fingerprint of all these strains exhibited an identical copy number of five and it was termed IS200 fingerprint II. The IS200 profile of three other strains, UB787 (Fig. 1, track 3), UB1146 and UB2868 was termed fingerprint III, and exhibited a copy number of six: five insertion sites were common to the fifteen chicken-associated strains, and the sixth was common only to these three strains which were environmental or veterinary isolates.

Six strains exhibited an IS200 copy number of four and a profile identical to UB5445 (Fig. 1, track 4), which was termed fingerprint IV. In this, the 1.7 kbp and 5.5 kbp fragments were common to NCTC 5717, a third *Pst*I fragment of 8.6 kbp was common to the chicken/environmental group, and the fourth and largest *Pst*I fragment (≈30 kbp) was unique. These strains were human and food isolates, and three were from human septicaemic infections. Strains UB6228 and UB988 were isolates from the same patient, taken four months apart from a persistently infected leg wound. The identity of their IS200 fingerprint confirms its stability as a marker *in vivo* for group IV. A single distinct strain, UB3069 (Fig. 1, track 5) had an IS200 copy number of five, four copies being located in *Pst*I fragments common to the six strains just described. This related profile was termed fingerprint V.

In all blots and with all enzymes UB4881 (Fig. 1, track 6) and UB5008, which were sporadic human gastro-enteritis isolates, yielded identical and novel IS200 profiles. The Indonesian environmental isolate, UB378, exhibited yet another unique hybridization profile (Fig.

Fig. 1. IS200 hybridization to genomic DNA of *S. heidelberg*. Genomic Southern blot made with *Pst*I and hybridized with an internal fragment of IS200 labelled with 16-dUTP-biotin. Tracks 1-7 contained the following strains whose origins are given in parentheses: track 1, strain NCTC 5717 (type strain); 2, UB198 (chicken); 3, UB787 (environment of chicken farm); 4, UB5445 (human, extra-intestinal); 5, UB3069 (human, faecal); 6, UB4881 (human, faecal); 7, UB378 (environment).

1, track 7). A diagrammatic representation of the fingerprints of all *S. heidelberg* strains listed in Table 1 is shown in Fig. 2 (see also assignment of clonal lines, below).

Plasmid analysis

A comparison was made between IS200 fingerprinting and plasmid analysis. Strains were ordered according to their IS200 fingerprint groups, and analysed for plasmids by the method of Kado & Liu (1981). The data are summarized in Table 2. Plasmids of seventeen sizes were detected. Four strains, belonging to three IS fingerprint groups, carried no plasmids. These were NCTC 5717, UB988, UB6228 and UB378 respectively. The plasmid size range varied from 0.8 MDa to 120 MDa.

Ten of the thirty strains exhibited resistance to antibiotics. Only 1/11 chicken strains expressed resistance (UB462: Ap^RSm^RSp^R), while 6/12 human, 2/3 food and one feline isolate did so. All ten strains were resistant to more than one antibiotic. One strain, from Mexico, was resistant to five antibiotics. The feline isolate was resistant to eight antibiotics. Combinations of antibiotic resistance determinants could be associated with large plasmids in UB5008 and UB4881 (human isolates) and UB1146 (environmental isolate), which transferred Km^R Tc^R plasmids of about 120 MDa in genetic crosses to *E. coli* J53-2. Plasmid transfer was detected only at 30 °C and not at 37 °C. Given the high molecular mass, thermosensitivity of transfer and associated tellurite resistance determinant, these plasmids were presumptively assigned to Incompatibility group H. Strain

Fig. 2. Clonal lines of *S. heidelberg* and their IS200 fingerprints: diagrammatic representation of all strains analysed. Strains were analysed as listed in Table 1. The IS200 fingerprints confirmed from at least three genomic Southern blots (*Pst*I) are shown. Molecular size markers shown on the left were *Hind*III fragments of bacteriophage lambda. Assigned clonal lines are indicated in roman numerals above the strain numbers (Sh1 . . . ShVII).

UB5443, a food isolate, transferred a 59 MDa Ap^R plasmid to J53-2 at 37 °C. The five resistance determinants of the Mexican strain, UB5503, were associated with a 120 MDa plasmid, whilst the three markers of UB5848, which shared IS200 fingerprint IV, were associated with a 58 MDa plasmid. The resistance determinants of UB462, the only resistant chicken strain, were associated with a 59 MDa plasmid.

As seen in Table 2, a striking feature of the plasmid analysis is that the eighteen strains assigned to the related IS200 fingerprint classes II and III contained combinations of low-molecular-mass cryptic plasmids not found in the other seven IS200 fingerprint groups: 0.8, 1.0, 1.2, 1.5, 2.0, and 2.5 MDa. Fourteen strains, including ten out of eleven chicken-associated strains, carried a 23 MDa cryptic plasmid (Table 2). Four other strains (UB898, 6333, 1631, and 2775) carried this plasmid and were contemporary isolates from animals and humans presumably infected from a chicken source. Three members of the related IS200 fingerprint groups II/III lacked the 23 MDa plasmid. These strains, UB5433, UB5542 and UB1146, were not associated with chicken, but were food or water isolates. They did, however, contain the aforementioned low-molecular-mass cryptic plasmids. The profiles of cryptic plasmids permitted subdivision of IS200 fingerprint groups II/III.

Hybridization experiments were carried out with replicate filters. No homology with the 23 MDa plasmid of IS200 fingerprint groups II/III, or with any other

plasmid, was found for a VirA probe, the cloned virulence region of the *S. typhimurium* virulence plasmid (see Table 1). In all strains, it was found that this 23 MDa plasmid was homologous (data not shown) to a probe for the basic replicon of Incompatibility group X. An FIC probe hybridized to six plasmids of between 42 and 68 MDa, as shown in Table 2, thereby identifying them as belonging to the F/I complex (Couturier *et al.*, 1989). No hybridization to any *S. heidelberg* plasmid was detected with IS200, including to the transferable R plasmids.

Clonal lines of *S. heidelberg*

Seven unique IS200 fingerprints were detected among the *S. heidelberg* strains analysed. Since no IS200 insertion sites were detected in any plasmid, these were chromosomal fingerprints, subject largely to vertical inheritance. Additional evidence that unique fingerprints correspond to unique genotypes was provided by the distribution of plasmids within the strain set. This occurred along divisions of the chromosomal IS200 fingerprint. For example, strains with fingerprint II and III were all distinguished by the carriage of a 23 MDa plasmid, and/or combinations of small cryptic plasmids not found in strains with other fingerprints, such as group IV. For these reasons, the observed IS200 fingerprints were taken to define clonal lines of *S. heidelberg* (see also Discussion below).

Table 2. Plasmid analysis

Clonal line	Strain number	IS200 copy number	IS200 PstI fragment sizes (kbp)	Antibiotic resistances	Plasmid content (MDa sizes)
ShI	5717	4	2.0 6.3 5.5 1.7		
ShII	5433	5	20	Ap Sm Sp Su	59†, 10, 8, 1.2, 0.8
	5542		10.1	Ap Sm Sp Su	59†, 8, 1.2, 0.8
	6333		8.6		23*, 1.2
	321		5.5		1.2
	462		1.7	Ap Sm Sp Su	59†, 23*, 2
	198				23*, 1.5
	489				23*, 1.5
	541				42†, 23*, 1.5
	557				23*, 2, 1
	898			Ap Cm Km Nm Sm Sp Su Tc Tp	110, 23*, 2, 1
	857				23*, 1.2, 0.8
	1631				23*, 2, 1
	1960				23*, 1.2
	2016				23*, 2, 1
	2775				23*, 2, 1
ShIII	787	6	20		23*, 2, 1
	1146		10.1	Cm Km Nm Sm Sp Su Tc	110, 10, 2, 1.5
	2868		8.6		23*, 2.5, 2, 1
			6.5		
			5.5		
			1.7		
ShIV	5051	4	~30	Sm Su Tc	8, 1.8
	5445		8.6		68†, 3.5
	5503		5.5	Cm Sm Sp Su Tc	120, 4
	5848		1.7	Sm Sp Su	58†
	6228				
	988				
	1764			Sm Su Tc	56, 8, 1.8
ShV	3069	5	~30		56, 4
			8.6		
			6.4		
			5.5		
			1.7		
ShVI	4881	4	~26	Km Nm Su Tc	120
	5008		20	Km Nm Su Tc	120
			8.6		
			1.7		
ShVII	378	4	20		
			9.8		
			5.8		
			1.7		

* Plasmid homologous to IncX_{R485}.

† Plasmid homologous to IncFIC.

Discussion

In the present report we have analysed the copy number and distribution of IS200 in order to differentiate and characterize chromosomal genotypes within *S. heidelberg*. No intraserovar subtyping method, such as phage typing, presently exists for *S. heidelberg*, and IS200 fingerprinting is therefore an appropriate and valuable

epidemiological tool for this serovar. A number of other *Salmonella* serovars, of similar importance to *S. heidelberg* in human salmonellosis, also lack intraserovar subtyping methods. Candidates for a molecular approach to epidemiology by IS200 fingerprinting include *S. hadar*, *S. infantis*, *S. montivideo*, *S. newport*, *S. bredeney*, *S. kedougou* and *S. panama* (Anon, 1991).

The discriminatory power of the method was greater

within *S. heidelberg* (Serogroup B) than was the case for *S. enteritidis* (Serogroup D1). For *S. enteritidis*, the major causative agent of non-typhoid salmonellosis in the UK and many other countries, a discriminatory phage typing scheme has been developed (Ward *et al.*, 1987), and the type strains of this scheme exhibited an IS200 copy number of two and fell into three clonal lines defined by IS200 fingerprint (Stanley *et al.*, 1991). The discriminatory value of IS200 fingerprinting apparently depends in the first instance on the copy number of the element, which varies within specific limits for a given serovar. Among even the present (limited) sample of *S. heidelberg*, where the minimum IS200 copy number was four, seven unique fingerprints were detected.

We conclude that IS200 fingerprints of *S. heidelberg* are stable, vertically inherited, and superior to plasmids for the analysis of genetic relationships. Nonetheless, results presented in this report also suggest that IS200 fingerprinting may be usefully complemented by plasmid analysis. A balanced picture of clonal relationships within a serovar may be gained, and IS200-defined clonal lines may themselves be subtyped by the presence of combinations of plasmids. For example if clonal line ShII (Table 2) is examined for plasmids smaller than and including the 23 MDa plasmid, eight plasmid profiles can be discerned among its fifteen members. If larger plasmids are included, eleven plasmid profiles subdivide ShII.

Some other features of the plasmid analysis were of general significance. A 23 MDa IncX plasmid was exclusively found in chicken and chicken-associated strains of ShII/III, and was absent from non-chicken strains of these clonal lines, such as UB5433, UB5542 or UB1146. It is possible that this IncX plasmid could carry host-specificity determinants for chicken, and in any event, it is a specific marker for strains of chicken origin. Again, it was noteworthy that no homology to *Salmonella* plasmid virulence (Spv) genes encoded by the 4.1 kbp *EcoRI* VirA fragment of the *S. typhimurium* 'virulence plasmid' was detected. Since many strains examined, occurring in two different clonal lines, (predominantly ShIV) were septicaemic, this result may suggest that Spv genes, whose function has been defined by ability to multiply in the reticuloendothelial system of the BalbC mouse, have no relevance for septicaemic invasion in humans. A noticeable finding was that no plasmid copies of IS200 were detected. In their investigation of six *E. coli* insertion sequences, Sawyer *et al.* (1987) found 10% of all IS bands were contained in plasmids. These *E. coli* elements also differed in their occurrence in plasmid loci, from 3% of total insertions for IS4 to 16% for IS5. Although insertions of IS200 were previously observed in *S. enteritidis* plasmids (Stanley *et al.*, 1991), this element shows no preference for *S. heidelberg*

plasmids. This factor, and the consequent lack of lateral distribution, may have influenced the relative copy number stability of IS200 within and between *Salmonella* serovars. Conservation of serovar specific IS200 profiles is a feature of *S. heidelberg* in this analysis, and of *S. enteritidis* and *S. typhimurium* (Stanley *et al.*, 1991, 1992a, b) and reflects intermediate-term evolutionary relationships. Furthermore, IS200 was not found in the large IncH and F/I complex plasmids in this analysis. These plasmids represent the major vectors of transferable antibiotic resistance in *Salmonella*, suggesting that this insertion sequence is not generally disseminated by 'hitchhiking' in transferable plasmids.

We consider that these IS200 fingerprints represent clonal lines of *S. heidelberg* for the following reasons. Firstly, since insertions of IS200 in plasmids could be discounted, the *S. heidelberg* fingerprints differentiated loci which are inherited in accordance with the definition of clonality (Orskov & Orskov, 1983) as being of common chromosomal genetic origin. Genetic mapping showed that the six IS200 insertions in the *S. typhimurium* LT2 chromosome were quite evenly spaced (Lam & Roth, 1983), and there is no reason to believe that this is not the case for these *S. heidelberg* strains. Secondly, the distribution of plasmids in the concurrent analysis suggested that plasmid distribution was subject to host-specific restrictions, and therefore that there were genotypic differences in this respect between clonal lines ShII/III and ShIV/V (and between these and clonal lines ShI, ShVI and ShVII).

When the reference (ECOR) collection of natural isolates of *E. coli* (Selander *et al.*, 1987) was examined by Sawyer *et al.* (1987) for the distribution of six *E. coli* insertion sequences, strains that were electrophoretically identical at 35 enzyme loci shared only 43% of their insertion sequence bands. Strains that differed in one enzyme locus shared 28% of insertion sequence bands, and strains that differed in two or more enzymes shared no insertion sequence bands. The authors therefore hypothesized that insertion sequence numbers and positions change much more rapidly in evolution than do electrophoretic mobilities of proteins, remarking that insertion sequence comparisons might be useful in analysing the phylogenetic history of related strains, and for highly precise strain identification in epidemiology (Sawyer *et al.*, 1987). For *S. heidelberg* and IS200, this indeed appears to be so. A survey of 204 *S. heidelberg* isolates from global sources detected eight genotypes by multilocus enzyme electrophoresis (MLEE) which monitored changes at 23 enzyme loci (Beltran *et al.*, 1988). By contrast, in the present limited sample number, seven genotypes were detected among only 30 strains by fingerprinting a single insertion sequence, IS200.

We hypothesize that IS200 fingerprinting is comp-

lementary to MLEE analysis for phylogenetic purposes, defining short-term as well as intermediate-term evolutionary relationships. For reasons of its greater discriminatory power and relative technical simplicity, it is probably the more suitable of the two methods for epidemiological applications. We suggest that IS200 can provide a general purpose probe for epidemiological and genetic studies of many *Salmonella* serovars. This would avoid the necessity of developing many unique phenotypic subtyping schemes and could be employed in relation to a band matching database of serovars and their (intra-serovar) clonal lines.

The project was initiated with support from the British Council. We thank Dr Morag Timbury for encouraging the development of insertion sequence fingerprinting of current *Salmonella* pathogens. N.P. is a British Industries Research Fund (1991/92) student.

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